adult treatment panel III [18] and international conference of low blood cholesterol [4]. The Japan Atherosclerotic Association also defined 5.69 mmol/L (220 mg/dl) or greater as a threshold criterion for hypercholesterolemia; [19] on the other hand, the manual of Health and Medical Service Law in Japan recommends medication for hypercholesterolemia when serum TC is 6.71 mmol/L (260 mg/dl) or greater [20]. Finally, we added "4.66 mmol/L (180 mg/dl)" as an additional cut-off point because there was a large number of participants with TC levels between 4.14 and 5.17 mmol/L. Consequently, the relationship between serum TC and mortality was determined in the following seven groups with 0.51 mmol/L (20 mg/dl) increments: <4.14 mmol/L (<160 mg/dl), 4.14–4.65 (160–179), 4.66–5.17 (180–199), 5.18–5.68 (200–219), 5.69–6.20 (220–240), 6.21–6.70 (240–259) and ≥6.71 mmol/L (≥260). We used the participants with TC levels between 4.14 and 4.65 mmol/L as a reference group because this was the largest of the seven TC groups. We also used quintiles of serum TC to group the participants (<4.16, 4.16–4.59, 4.60–5.03, 5.04–5.60, ≥5.61 mmol/L).

Age-adjusted mean values and the prevalence of baseline characteristics were estimated using analysis of covariance or the chi-square test. The multivariable adjusted hazard ratio (HR) for all-cause or cause-specific mortality was calculated using a Cox's proportional hazards model adjusted for age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking and alcohol intake. We used three dummy variables to classify subjects based on their smoking habit (never-smoked; ex-smoker; current smoker ≤20 and >20 cigarettes/day, with never-smoked being defined as the reference group) and their alcohol intake (never-drank; ex-drinker; occasional drinker and daily drinker, with never-drank being defined as the reference group). Gender-specific analyses were also carried out.

The analyses were repeated excluding all-cause deaths within the first 5 years of follow-up and/or deaths due to liver disease during the entire follow-up period.

All confidence intervals were estimated at the 95% level and significance was assumed at a P-value of <0.05. The Statistical Package for the Social Sciences (SPSS Japan Inc. version 13.0J, Tokyo, Japan) was used for all the analyses.

3. Results

The mean age in our entire study population was 50.0 ± 13.2 years (mean ± S.D.), 49.7 ± 13.1 years in men and 50.1 ± 13.3 years in women. The mean serum TC was 4.88 ± 0.87 mmol/L (188.6 ± 33.6 mg/dl), 4.81 ± 0.85 mmol/L (186.0 ± 32.9 mg/dl) in men and 4.93 ± 0.88 mmol/L (190.7 ± 34.0 mg/dl) in women.

Table 1 shows the age-adjusted means and prevalence of the baseline characteristics of all the participants in each

Table 1
Age and age-adjusted mean value and prevalences of baseline characteristics stratified by cholesterol level at the baseline survey in 1980, NIPPON DATA80

Risk characteristics	Baseline serum total cholesterol level (mmol/L)							P-values[a]
	<4.14	4.14–4.65	4.66–5.17	5.18–5.68	5.69–6.20	6.21–6.70	6.71-	
Men								
TC, stratum mean (mmol/L)	3.74	4.39	4.91	5.41	5.90	6.41	7.30	
No. of persons	851	1000	937	648	354	167	78	
Age (years)	51.0 (14.0)	50.0 (13.4)	49.3 (13.1)	48.8 (12.6)	48.9 (11.8)	50.2 (12.2)	49.3 (11.0)	<0.001
Albumin (g/L)	43.0 (0.10)	44.1 (0.09)	44.4 (0.09)	45.0 (0.10)	45.4 (0.13)	45.6 (0.22)	45.7 (0.31)	<0.001
BMI (kg/m^2)	21.7 (0.13)	22.0 (0.09)	22.6 (0.09)	23.2 (0.11)	23.5 (0.14)	23.9 (0.21)	24.1 (0.29)	0.030
Hypertension (%)	44.9	46.3	50.3	50.3	55.4	57.5	62.8	<0.001
Diabetes (%)	1.2	0.8	1.2	1.7	1.2	0.6	2.6	0.603
Daily drinker (%)	46.7	49.5	48.9	49.4	48.3	36.5	50	0.081
Current smoker (%)	66.9	66.4	63.9	56.3	59.3	57.5	53.8	<0.001
Heavy smoker (>20 cigarettes day^{-1}) (%)	21.2	24.6	25.1	23.3	30.5	29.3	28.2	0.017
Women								
TC, stratum mean (mmol/L)	3.78	4.40	4.91	5.40	5.91	6.40	7.20	
No. of persons	952	1183	1142	925	528	275	176	
Age (years)	44.7 (12.9)	47.3 (13.0)	50.6 (12.8)	53.1 (12.9)	54.8 (12.0)	56.3 (11.5)	56.9 (11.6)	<0.001
Albumin (g/L)	43.1 (0.08)	43.3 (0.07)	43.6 (0.07)	43.9 (0.08)	44.0 (0.10)	44.0 (0.17)	44.3 (0.19)	<0.001
BMI (kg/m^2)	22.1 (0.10)	22.4 (0.10)	22.8 (0.10)	23.2 (0.11)	23.6 (0.15)	23.8 (0.20)	24.4 (0.30)	<0.001
Hypertension (%)	27.0	32.7	37.5	46.8	54.0	56.4	58.5	<0.001
Diabetes (%)	0.2	0.6	0.6	1.2	1.5	2.2	3.4	<0.001
Daily drinker (%)	3.2	2.6	3.0	2.7	3.2	1.8	2.8	0.920
Current smoker (%)	7.9	7.9	10.1	9.2	11.0	5.5	9.1	0.068
Heavy smoker (>20 cigarettes day^{-1}) (%)	0.7	0.3	0.8	0.6	1.5	0.7	0.6	0.291

[a] Analysis of covariance for continuous variables, chi-square test for categorical variables. The null hypothesis is that each mean or prevalence among all TC categories was equal. Numbers in parentheses are standard deviations for age and standard errors for other variables.

cholesterol category. The mean age in each TC group was similar in men although analysis of covariance showed statistical significance. For women, there was a trend of increasing age with increasing cholesterol levels. There were significant differences in the mean values for albumin and BMI, with these being greatest in the higher cholesterol group in both genders. There were also significant differences in the prevalence of hypertension in both genders and in the prevalence of diabetes in women. In men, the highest TC group had the highest prevalence of diabetes and current drinker; however, they did not reach statistical significance by chi-square test because of small sample size of the highest TC group. The lowest TC group had the highest prevalence of smoking in men.

The total person-years studied were 159,293 with a mean follow-up period of 17.3 years. During the follow-up period there were 1841 deaths (992 males and 849 females). Of these, 36% ($N=666$) were due to cardiovascular disease that included 128 coronary heart disease deaths and 306 stroke deaths (intra-cerebral hemorrhage, $n=65$; cerebral infarction, $n=174$; others, $n=67$).

Among the total deaths, 30% ($n=558$) were due to cancer. The three major causes of cancer death were stomach cancer ($n=131$), lung cancer ($n=107$) and liver cancer ($n=50$), a total that represented 52% of deaths due to cancer. Of all the deaths, 34% ($n=617$) were due to non-cardiovascular or non-cancer diseases. There were 35 deaths due to non-cancer liver disease (liver cirrhosis, $n=26$), which represented approximately 5% of all-cause deaths when deaths due to liver cancer were included ($n=85$).

Table 2 shows the number of deaths and multivariable-adjusted HR for the major causes of death except for cardiovascular disease according to TC stratification. Mortality from cancer was not associated with TC levels in either gender although it was highest in the lowest TC group. The mortality from non-cancer or non-cardiovascular disease was also not associated with the TC level. We found there was a positive association between the lowest TC group and increased risk for all-cause mortality in men (HR = 1.21 (95% CI, 1.01–1.45), women (HR = 1.26; 95% CI, 0.99–1.60) and in the combined data from both genders (HR = 1.19; 95% CI, 1.03–1.37). The highest TC group also had an increased risk for all-cause mortality in men (HR = 1.44; 95% CI, 0.90–2.31), women (HR = 1.24; 95% CI, 0.90–1.71) and in the combined data from both genders (HR = 1.36; 95% CI, 1.05–1.77).

Table 3 shows the number of deaths and multivariable-adjusted HRs for cardiovascular and liver disease. Mortality from cardiovascular disease was the highest in the highest TC group in both genders, with significantly higher HR in

Table 2
The number of deaths and multivariable-adjusted HRs (95% CIs) for cancer, non-cardiovascular, non-cancer and all-cause mortality; according to serum total cholesterol level in a 17.3-year follow-up study, NIPPON DATA80

Baseline serum total cholesterol level (stratum mean), mmol/L	No. of persons	Person-years	Cancer		Non-cardiovascular, non-cancer		All-cause	
			No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)
Men								
<4.14 (3.74)	851	13768	92	1.22 (0.90, 1.64)	90	1.28 (0.94, 1.74)	259	1.21 (1.01, 1.45)
4.14–4.65 (4.39)	1000	17000	84	1.00	78	1.00	241	1.00
4.66–5.17 (4.91)	937	16057	74	1.08 (0.79, 1.47)	75	1.26 (0.92, 1.74)	223	1.18 (0.98, 1.42)
5.18–5.68 (5.41)	648	11113	44	1.01 (0.67, 1.46)	44	1.32 (0.90, 1.93)	140	1.25 (1.01, 1.55)
5.69–6.20 (5.90)	354	6192	22	0.88 (0.54, 1.41)	26	1.21 (0.77, 1.89)	76	1.08 (0.83, 1.41)
6.21–6.70 (6.41)	167	2872	13	1.12 (0.62, 2.03)	5	0.49 (0.20, 1.21)	34	1.02 (0.71, 1.47)
6.71– (7.30)	78	1365	6	1.20 (0.52, 2.76)	6	1.57 (0.67, 3.64)	19	1.44 (0.90, 2.31)
Women								
<4.14 (3.78)	952	16784	34	1.19 (0.76, 1.86)	42	1.34 (0.89, 2.00)	118	1.26 (0.99, 1.60)
4.14–4.65 (4.40)	1183	21011	47	1.00	56	1.00	165	1.00
4.66–5.17 (4.91)	1142	20011	53	0.96 (0.64, 1.43)	62	1.02 (0.71, 1.47)	185	0.98 (0.79, 1.21)
5.18–5.68 (5.40)	925	16155	46	0.88 (0.58, 1.33)	61	1.00 (0.69, 1.45)	171	0.92 (0.74, 1.21)
5.69–6.20 (5.91)	528	9252	23	0.68 (0.41, 1.14)	36	0.92 (0.60, 1.41)	106	0.92 (0.74, 1.14)
6.21–6.70 (6.40)	275	4751	10	0.58 (0.29, 1.16)	23	1.19 (0.73, 1.95)	54	0.88 (0.68, 1.12)
6.71– (7.20)	176	2960	10	0.88 (0.44, 1.77)	13	1.01 (0.55, 1.88)	50	1.24 (0.90, 1.71)
Men and women combined								
<4.14 (3.76)	1803	30552	126	1.21 (0.95, 1.55)	132	1.26 (0.99, 1.61)	377	1.19 (1.03, 1.37)
4.14–4.65 (4.39)	2183	38011	131	1.00	134	1.00	406	1.00
4.66–5.17 (4.91)	2079	36068	127	1.03 (0.80, 1.31)	137	1.16 (0.91, 1.48)	408	1.09 (0.95, 1.26)
5.18–5.68 (5.40)	1573	27268	90	0.96 (0.73, 1.26)	105	1.15 (0.89, 1.50)	311	1.07 (0.92, 1.25)
5.69–6.20 (5.91)	882	15444	45	0.78 (0.55, 1.10)	62	1.05 (0.77, 1.43)	182	0.98 (0.82, 1.17)
6.21–6.70 (6.40)	442	7623	23	0.89 (0.52, 1.27)	28	0.97 (0.64, 1.47)	88	0.96 (0.76, 1.22)
6.71– (7.23)	254	4325	16	1.01 (0.60, 1.72)	19	1.19 (0.73, 1.95)	69	1.36 (1.05, 1.77)

HR, hazard ratio; 95% CI, 95% confidence interval. HR was adjusted for age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking category and alcohol intake category. Gender was also adjusted while a sex-combined analysis was performed.

Table 3
The number of deaths and multivariable-adjusted hazard ratios for cardiovascular and liver disease mortality according to serum total cholesterol level, NIPPON DATA80

Baseline serum total cholesterol level (stratum mean), mmol/L	No. of persons	Person-years	Cardiovascular						Liver disease	
			All		Coronary heart disease		Stroke		No. of deaths	HR (95% CI)
			No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)		
Men										
<4.14 (3.74)	851	13768	77	1.15 (0.84, 1.58)	10	1.07 (0.46, 2.51)	43	1.21 (0.78, 1.89)	32	2.74 (1.36, 5.52)
4.14–4.65 (4.39)	1000	17000	79	1.00	12	1.00	40	1.00	11	1.00
4.66–5.17 (4.91)	937	16057	74	1.24 (0.90, 1.71)	12	1.21 (0.54, 2.71)	32	1.08 (0.68, 1.73)	10	1.07 (0.45, 2.52)
5.18–5.68 (5.41)	648	11113	52	1.51 (1.05, 2.17)	12	2.11 (0.92, 4.84)	27	1.53 (0.92, 2.54)	3	0.59 (0.16, 2.15)
5.69–6.20 (5.90)	354	6192	28	1.19 (0.77, 1.85)	9	2.17 (0.89, 5.25)	11	0.97 (0.49, 1.90)	2	0.59 (0.13, 2.68)
6.21–6.70 (6.41)	167	2872	16	1.44 (0.83, 2.48)	7	3.74 (1.44, 9.76)	3	0.53 (0.16, 1.73)	0	–
6.71– (7.30)	78	1365	7	1.68 (0.77, 3.69)	3	3.77 (1.02, 13.9)	2	0.97 (0.23, 4.07)	0	–
Women										
<4.14 (3.78)	952	16784	42	1.21 (0.81, 1.79)	7	0.94 (0.36, 2.45)	17	1.05 (0.58, 1.92)	9	3.13 (1.04, 9.42)
4.14–4.65 (4.40)	1183	21011	62	1.00	12	1.00	30	1.00	5	1.00
4.66–5.17 (4.91)	1142	20011	70	1.00 (0.71, 1.42)	10	0.73 (0.31, 1.71)	28	0.77 (0.46, 1.30)	5	0.90 (0.26, 3.12)
5.18–5.68 (5.40)	925	16155	64	0.91 (0.64, 1.30)	12	0.92 (0.41, 2.08)	31	0.80 (0.48, 1.33)	2	0.35 (0.07, 1.83)
5.69–6.20 (5.91)	528	9252	47	1.03 (0.70, 1.52)	10	1.14 (0.48, 2.70)	22	0.85 (0.58, 1.49)	4	1.07 (0.28, 4.10)
6.21–6.70 (6.40)	275	4751	21	0.95 (0.58, 1.57)	3	0.74 (0.20, 2.68)	9	0.70 (0.33, 1.50)	2	1.25 (0.24, 6.61)
6.71– (7.20)	176	2960	27	1.84 (1.15, 2.93)	9	3.33 (1.35, 8.18)	11	1.31 (0.65, 2.67)	0	–
Men and women combined										
<4.14 (3.76)	1803	30552	119	1.11 (0.86, 1.42)	17	0.91 (0.49, 1.71)	60	1.14 (0.80, 1.62)	41	3.03 (1.70, 5.43)
4.14–4.65 (4.39)	2183	38011	141	1.00	24	1.00	70	1.00	16	1.00
4.66–5.17 (4.91)	2079	36068	144	1.12 (0.89, 1.42)	22	1.01 (0.56, 1.81)	60	0.92 (0.65, 1.30)	15	1.02 (0.50, 2.06)
5.18–5.68 (5.40)	1573	27268	116	1.13 (0.88, 1.46)	24	1.42 (0.79, 2.56)	58	1.06 (0.74, 1.52)	5	0.53 (0.19, 1.45)
5.69–6.20 (5.91)	882	15444	75	1.12 (0.84, 1.49)	19	1.67 (0.90, 3.11)	33	0.94 (0.61, 1.43)	6	0.97 (0.38, 2.51)
6.21–6.70 (6.40)	442	7623	37	1.14 (0.79, 1.65)	10	1.84 (0.86, 3.91)	12	0.69 (0.37, 1.29)	2	0.62 (0.14, 2.71)
6.71– (7.23)	254	4325	34	1.90 (1.29, 2.79)	12	3.81 (1.84, 7.91)	13	1.38 (0.75, 2.54)	0	–

HR, hazard ratio; 95% CI, 95% confidence interval. HR was adjusted for age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking category and alcohol intake category. Gender was also adjusted while a sex-combined analysis was performed.

women and when the gender data were combined. The mortality for coronary heart disease suggested a positive graded relationship with TC when the gender data were combined. For men, the HR in the second highest TC group was 3.74 (95% CI, 1.44–9.76), while the HR in the highest TC group was 3.77 (95% CI, 1.02–13.9). For women, although a graded relationship was not observed, the highest TC group had a significantly increased risk of death from coronary heart disease. Mortality from stroke was not associated with TC levels in either gender. The mortality from cerebral hemorrhage was the highest in the lowest TC group in men (HR = 3.77; 95% CI, 1.35–10.5), while the mortality from cerebral infarction was not associated with TC levels in either gender (data not shown). The lowest TC group was positively associated with an increased risk for death from liver disease in men, women and the combined gender data.

The association between TC group and cause-specific mortality after excluding deaths within the first 5 years of follow-up was essentially similar to those shown in Table 2 and 3 (data not shown).

When all-cause mortality was calculated after exclusion of liver disease (Fig. 1), the increased HR in the lowest TC group disappeared (HR = 1.10, 95% CI, 0.95–1.28, for combined data of men and women). In contrast, in the combined data, the positive relationship between the highest TC group and all-cause mortality remained significant with an increase in HR (HR = 1.41, 95% CI, 1.12–1.38). After further excluding deaths within the first 5 years of follow-up, the magnitude of the HR in the lowest TC group decreased (HR = 1.05; 95% CI, 0.89–1.24), whereas the HR in the highest TC group increased even further (HR = 1.48; 95% CI, 1.12–1.96). Repeating these analyses on data grouped according to gender showed nearly identical results (data not shown).

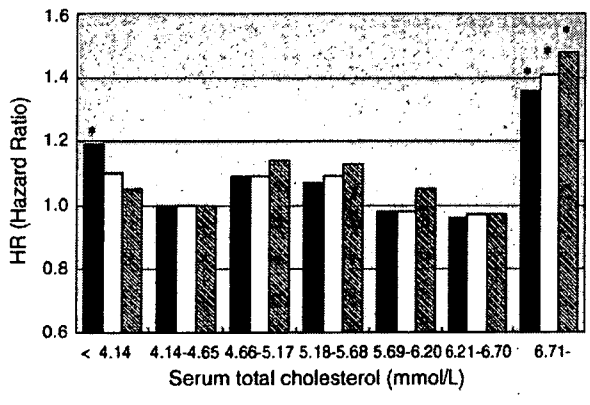

Fig. 1. Multivariable-adjusted hazard ratios (HR) for all-cause mortality grouped according to serum total cholesterol after adjustment for gender, age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking and alcohol intake. Black bars show HR for all-cause mortality among all participants. White bars show HR for all-cause mortality after exclusion of deaths due to liver disease during the entire follow-up period. Hatched bars show HR for all-cause mortality after further exclusion of all-cause deaths within the first 5 years of follow-up. ($^{*}P < 0.05$).

In addition to stratifying participants based on clinical TC cut-off values, we also grouped all participants according to the quintile of serum TC. When we used the second quintile (4.16–4.59 mmol/L) as a reference group, we observed a significant increase in all-cause (HR = 1.21, 95% CI, 1.05–1.40) and liver disease mortality (HR = 2.91, 95% CI, 1.58–5.35) in the lowest TC quintile (<4.16 mmol/L, 161 mg/dl). This was similar to the results obtained in the lowest TC group (<4.14 mmol/L, 160 mg/dl) when clinical TC cut-off values were used to group the participants. However, the highest TC quintile (≥5.61 mmol/L, 217 mg/dl) was not associated with an increase in all-cause or any cause-specific mortality except for coronary heart disease (HR = 2.01; 95% CI, 1.16–3.51). When HR for all-cause mortality was calculated after exclusion of liver disease, the increased HR in the lowest TC quintile disappeared. Gender-specific analysis also showed similar results (data not shown).

4. Discussion

This 17.3-year cohort study of the Japanese population showed a positive association between the lowest (<4.14 mmol/L) or highest (≥6.71 mmol/L) TC levels and an increased risk of all-cause mortality. However, the relationship between low TC and all-cause mortality disappeared when deaths due to liver disease were excluded, with only the highest TC group showing a significant increase in all-cause mortality. The strengths of the present study were a high response rate in the baseline survey at which time several biological markers were measured and a long duration of follow-up of randomly selected subjects. The large number of person-years in the study also allowed us to use multivariable analysis to examine the relationship between high serum TC and all-cause mortality using cut-off points set higher than previous studies in Asian populations [5–8].

The prevalence of hepatitis C virus (HCV) infection in Japanese residents born before World War II has been estimated to be approximately 5–7%, [21,22] a level considerably higher than in Western countries [23–25]. Because the majority of our study participants belonged to the pre-World War II generation, the prevalence of HCV infection in our study cohort would be expected to be relatively high. It has recently been revealed that a low serum cholesterol level in individuals with chronic HCV infection is a predictor of both liver fibrosis [26] and liver cancer [9] Another study indicated that subjects with genotype 1b hepatitis C viral infection (the most common genotype of the HCV in Japan) had significantly lower serum cholesterol levels than those infected with hepatitis B virus or genotype 2a HCV, even in the pre-cirrhosis period [27].

These results suggest that hypocholesterolemia in Japan is associated with the prevalence of persistent infection with HCV. Low serum TC may be a response to liver dysfunction caused by progressive fibrotic changes rather than a primary cause of liver fibrosis. We believe these findings may partly

explain the relationship we observed between low TC and all-cause death in Japan. An epidemic of HCV infection occurred mainly in the pre-war generation of the Japanese population as a result of commercial blood transfusions carried out during the two decades after World War II [28]. This interpretation is also supported by our previous finding that a history of earlier blood transfusion was associated with hypocholesterolemia in a rural Japanese community [29].

Most cohort studies in non-Western populations have failed to demonstrate a positive relationship between high serum TC and all-cause mortality [5–8]. We found participants with a TC level ≥6.71 mmol/L, a higher level than US criteria (≥6.21 mmol/L), had an increased risk of all-cause mortality, mainly as a consequence of coronary heart disease. It was reported in the 1960 and 1970s that a cohort of Japanese people born before World War II had markedly lower serum TC levels [30]. Although subjects may have had elevated TC at the baseline survey, it was not possible to determine the duration of elevated TC levels prior to the baseline measurement. A "lag time" between exposure to high serum TC levels and the occurrence of coronary heart disease may provide an explanation of the higher cut-off value for TC in the Japanese population. Accordingly, the effect of high serum TC on both coronary heart disease and all-cause mortality may be attenuated.

Similar to previous studies in Japan, we found no positive relationship between cholesterol levels and stroke [31,32]. In fact, we observed the highest mortality for cerebral hemorrhage in the lowest TC group in men. Some studies, [8,31] but not all, [33] reported that hypocholesterolemia was associated with a higher risk of cerebral hemorrhage. However, we were unable to determine if this was a causal relationship.

The present study had some limitations. First, a single cholesterol measurement at the baseline survey may have underestimated the relationship between TC and mortality due to regression dilution effects [34]. Second, we divided the population into seven TC groups with an unbalanced number of participants based on the combination of clinical criteria because the prevalence of hypercholesterolemia (6.71 mmol/L or greater) was very small in this population. Third, we did not measure antibodies against the hepatitis C virus, and non-fasting blood collection might have affected serum glucose levels. Fourth, the change in the ICD coding from version 9 to version 10 during follow-up period may have been a confounding factor in the diagnosis of the cause of death. However, ICD coding was done by specialists in the Ministry of Health and Welfare, not by researchers. Furthermore, mortality from stroke and cancer is known to be accurately reported on death certificates in Japan [35,36]. Although underestimation of coronary heart disease deaths during the use of ICD 9 is possible, [37] this should make it more difficult to show a positive association between high TC and death due to coronary heart disease. Thus, the positive association that we observed may be conservative.

In conclusion, as in the Western populations, we showed that high serum levels of TC in the Japanese general population were positively associated with all-cause mortality, although the cut-off point appeared to be higher in Japanese residents than Westerners. Furthermore, the relationship between hypocholesterolemia and liver diseases, such as liver cancer, liver cirrhosis and hepatitis, may increase all-cause mortality in hypocholesterolemic Japanese residents.

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SHORT COMMUNICATION

Japanese men have larger areas of visceral adipose tissue than Caucasian men in the same levels of waist circumference in a population-based study

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Visceral adipose tissue (VAT) is an independent risk factor for metabolic and cardiovascular disorders. There has been no study that demonstrated different abdominal fat distribution between Asian and Caucasian men. As the Japanese are less obese but more susceptible to metabolic disorders than Caucasians, they may have larger VAT than Caucasians at similar levels of obesity. We compared the abdominal fat distribution of the Japanese ($n=239$) and Caucasian-American ($n=177$) men aged 40–49 years in groups stratified by waist circumference in a population-based sample. We obtained computed tomography images and determined areas of VAT and subcutaneous adipose tissue (SAT). We calculated VAT to SAT ratio (VSR). The Japanese men had a larger VAT and VSR in each stratum, despite substantially less obesity overall. In multiethnic studies, difference in abdominal fat distribution should be considered in exploring factors related to obesity.
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Keywords: abdominal adipose tissue; visceral adipose tissue; Japanese; Caucasian

Visceral adipose tissue (VAT) is an independent risk factor for coronary heart disease (CHD),[1] hypertension,[2] type-2 diabetes,[3] and impaired glucose tolerance (IGT).[4] Accumulation of VAT is a form of obesity related to environmental factors such as diet and physical inactivity.[5] As the Japanese are less obese but more susceptible to metabolic disorders than Caucasians,[5] they may have larger VAT than Caucasians at similar levels of obesity. There have been reports of racial differences in abdominal fat distribution between Caucasians and Africans,[6] Hispanics,[7] and Asians.[8,9] Previous studies have shown that Asian-American and Caucasian men had no significant difference in VAT among volunteer participants.[8] Likewise, a meta-analysis showed no significant difference in VAT between Japanese and Caucasian men.[9] In these studies, VAT was compared after adjusting for other factors such as age and obesity. There has been no report directly comparing VAT between the Japanese and Caucasian men in a population-based sample. Therefore, we compared the abdominal fat distribution of the Japanese and Caucasian-American men aged 40–49 years in groups stratified by waist circumference in a population-based sample.

We recruited 240 American men aged 40–49 years (Caucasians: 82%), randomly selected from Allegheny County, Pennsylvania, US, and 240 Japanese men aged 40–49 years, randomly selected from Kusatsu, Shiga, Japan. We confined our analyses to Caucasian and Japanese men, because African men have been compared with Caucasians elsewhere,[6] and we had only one man who categorized himself as neither Caucasian nor African-American. In order to compare the abdominal adipose tissue (AAT) distribution at similar levels of waist circumference, participants with extremely large or small waist circumference (beyond the levels of mean ± 2 (s.d.)) were excluded from the present analysis. After excluding 44 non-Caucasian-Americans and 20 outliers in waist circumference, 416 (239 Japanese and 177 Caucasian) men were examined. Waist circumference

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was measured twice at the umbilical level at the end of the exhalation phase while the participant was standing upright, and the mean of the two measurements was calculated. Areas of the whole AAT and VAT were determined at the level between the fourth and fifth lumbar vertebrae, using computed tomography images obtained with the same apparatus at each site (GE-Imatron C150). Areas of subcutaneous adipose tissue (SAT) were calculated as AAT minus VAT, and VAT to SAT ratio (VSR) was calculated as VAT/SAT. The technician was blinded to the race/ethnicity and other identifiable information about the participants The intraclass correlation coefficients at our reading center are 0.99 for SAT and 0.99 for VAT. Written informed consent was obtained from each participant. The study protocol was reviewed and approved by the Institutional Review Boards of University of Pittsburgh, US, and Shiga University of Medical Science, Japan.

We stratified the participants into quartile strata (from the lowest Q1 to the highest Q4) by waist circumference. We compared the means of continuous variables using *t*-tests, and considered the difference significant at *P*-value <0.05 in two-tailed tests. We performed statistical analyses using a Statistical Package for the Social Sciences (SPSS Japan Inc. version 11.0 J, Tokyo, Japan).

Table 1 shows AAT, SAT, VAT, and VSR in each waist stratum and overall. Pearson's correlation coefficients between waist circumference and AAT were 0.90 for the Caucasian men and 0.92 for the Japanese men. The Japanese men had a significantly larger VSR than the Caucasian men in all waist circumference strata. The Japanese men had a significantly larger VAT than the Caucasian men in Q2 and Q3. In Q1 and Q4, the Japanese men had a larger VAT, although this difference was not statistically significant. The Japanese men had a larger VAT in each stratum, despite

Table 1 Waist circumference, areas of whole abdominal adipose tissue, subcutaneous adipose tissue, visceral adipose tissue, and ratio of visceral to subcutaneous adipose tissue measured at between 4th and 5th vertebrae, according to quartile groups of waist circumference and total in Caucasian-American and Japanese men aged 40–49 years

	Caucasian	*Japanese*	P
Q1 (waist circumference: 67.95–82.80 cm)			
n (% of the total)	11 (6.2%)	93 (38.9%)	
Age (years)	45.7±2.8	45.1±2.8	0.507
Abdominal adipose tissue (cm^2)	111.3±23.1	105.5±36.0	0.476
Subcutaneous adipose tissue (cm^2)	63.7±14.7	52.6±19.8	0.040
Visceral adipose tissue (cm^2)	47.6±10.1	52.9±19.1	0.164
Ratio of visceral to subcutaneous adipose tissue	0.76±0.12	1.06±0.32	<0.001
Q2 (waist circumference: 82.85–88.85 cm)			
n (% of the total)	29 (16.4%)	74 (31.0%)	
Age (years)	44.9±2.7	45.0±3.0	0.771
Abdominal adipose tissue (cm^2)	159.0±33.2	166.7±27.0	0.270
Subcutaneous adipose tissue (cm^2)	88.7±23.0	81.8±14.6	0.142
Visceral adipose tissue (cm^2)	70.2±17.5	84.8±19.7	0.001
Ratio of visceral to subcutaneous adipose tissue	0.84±0.28	1.06±0.29	0.001
Q3 (waist circumference: 88.90–96.75 cm)			
n (% of the total)	57 (32.2%)	49 (20.5%)	
Age (years)	45.0±2.9	44.9±2.7	0.769
Abdominal adipose tissue (cm^2)	201.5±37.6	210.7±23.1	0.125
Subcutaneous adipose tissue (cm^2)	116.0±27.0	109.0±17.1	0.108
Visceral adipose tissue (cm^2)	85.5±24.5	101.7±20.1	<0.001
Ratio of visceral to subcutaneous adipose tissue	0.77±0.26	0.96±0.25	<0.001
Q4 (waist circumference: 97.00–114.25 cm)			
n (% of the total)	80 (45.2%)	23 (9.6%)	
Age (years)	45.1±3.0	45.3±2.7	0.757
Abdominal adipose tissue (cm^2)	292.8±50.4	267.6±35.4	0.009
Subcutaneous adipose tissue (cm^2)	175.0±38.6	147.3±32.1	0.001
Visceral adipose tissue (cm^2)	117.8±34.0	120.3±26.1	0.702
Ratio of visceral to subcutaneous adipose tissue	0.71±0.26	0.86±0.29	0.026
Total			
n (% of the total)	177 (100%)	239 (100%)	
Age (years)	45.1±2.9	45.1±2.8	0.954
Waist circumference (cm)	95.4±8.2	85.3±8.2	<0.001
Abdominal adipose tissue (cm^2)	230.2±74.6	161.6±61.4	<0.001
Subcutaneous adipose tissue (cm^2)	134.9±50.1	82.3±35.6	<0.001
Visceral adipose tissue (cm^2)	95.2±35.6	79.3±30.9	<0.001
Ratio of visceral to subcutaneous adipose tissue	0.75±0.26	1.02±0.30	<0.001

significantly smaller waist circumference, AAT, SAT, and VAT than the Caucasian men overall. In the waist circumference stratification, a higher proportion of the Japanese men was in the lower quartiles (Q1 and Q2), whereas a higher proportion of the Caucasian men was in higher quartiles (Q3 and Q4).

In this study, we demonstrated that the Japanese men had larger VAT and VSR than the Caucasian men in every waist circumference stratum. To our knowledge, this is the first report to compare the distribution of abdominal adipose tissue between Japanese and Caucasian men directly in a standardized manner.

These findings of greater VAT in Japanese men may help explain the greater incidence of type 2 diabetes in this population compared to Caucasian men.[5,10] As the Japanese have a larger VAT even at lower levels of obesity, it leads to a higher risk than Caucasians, because VAT is an independent predictor of type 2 diabetes[3] and IGT.[4] In fact, in the Diabetes Prevention Program, the obesity inclusion criterion was lower for Asians (BMI $\geqslant 22\,kg/m^2$) than other races (BMI $\geqslant 24\,kg/m^2$),[11] based on the notion that the Japanese have higher risk of type 2 diabetes at lower levels of obesity.[10]

The reason we failed to demonstrate significant VAT differences in Q1 and Q4 may be due to insufficient sample sizes caused by the fact that waist circumference distribution was skewed to opposite directions in the two groups, resulting in a smaller proportion of either population in these strata. In fact, AAT in Q4 was smaller in the Japanese men, regardless of waist circumference stratification and the exclusion of outliers in waist circumference. This difference may be caused by the Caucasian men's negatively skewed waist circumference distribution. However, we were able to demonstrate that the mean values of VAT were larger in the Japanese than Caucasian men, and that VSR was significantly larger in the Japanese than Caucasian men in all waist circumference strata.

The strengths of our study are; (1) direct comparison in a standardized manner; (2) randomly sampled participants from general populations; and (3) narrow age-range of 40–49 years exempting us from age adjustment. There was actually no significant age difference between the Japanese and Caucasian men in any strata or overall.

The weakness of our study is the lack of women. Park *et al*.[8] reported larger proportion of VAT in AAT in women, but no significant difference in VAT itself. They also reported a positive interaction between age and race in women. Although they reported no interaction in men, a similar interaction may have been observed in men if we had a broader age-range in our study.

In conclusion, Japanese men have larger VAT and VSR than Caucasian men across the same level of waist circumference. In multiethnic studies, difference in abdominal fat distribution should be considered in exploring factors related to obesity.

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ORIGINAL ARTICLE

What factors are associated with high plasma B-type natriuretic peptide levels in a general Japanese population?

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There are few community-based epidemiologic studies that have dealt with risk factors for heart failure in non-Western populations. It has been reported that the measurement of plasma B-type natriuretic peptide (BNP) is useful for detecting patients with asymptomatic heart failure. To clarify the determinants of high plasma BNP level, the association of BNP with cardiovascular risk factors in community dwelling residents was examined. The plasma BNP levels were measured in 686 residents aged 35–69 years who received annual health check-up. The relationship of BNP to blood pressure, blood haemoglobin, serum cholesterol (total and high-density lipoprotein cholesterol), plasma glucose, electrocardiographic (ECG) findings, urinary salt excretion, and lifestyle factors (smoking and alcohol consumption) were cross-sectionally analysed. The plasma BNP geometric mean was 13.7 pg/ml. Both linear and logistic regression analyses indicated that the plasma BNP levels were positively associated with age, urinary salt excretion, higher blood pressure, high R-wave voltage in the 12-lead ECG (Minnesota Code 3-1 or 3-3), and female gender. Plasma BNP levels were inversely associated with blood haemoglobin levels. Gender-specific analysis showed similar results. However, plasma BNP did not correlate with other cardiovascular risk factors such as serum lipids.
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Introduction

Approximately 15% of deaths in Japan are due to heart diseases, of which about one-third are due to heart failure.[1] In 2001, mortality due to heart failure was 36.9 per 100 000 person-years, which is approximately two-thirds of that due to coronary heart disease (56.4 per 100 000 person-years).[1] The risk factors for coronary heart disease have been well described in several epidemiologic studies in Japan.[2–6] However, there are few available epidemiologic studies that deal with the risk factors for heart failure, even though it is a major problem in the Japanese population.[7,8] Accordingly, it is very important to clarify the risk factors for heart failure in Japan.

Congestive heart failure is usually regarded as the end-stage of the progressive deterioration of left ventricular function, which cannot be compensated for by cardiovascular homeostatic mechanisms.[9,10] Although heart failure is usually progressive, it can remain asymptomatic for many years. Thus, it would be of benefit to identify latent patients who have asymptomatic left ventricular dysfunction. However, in the general population, it is difficult and expensive in the primary care setting to screen the general population using Doppler echocardiography or exercise tolerance tests to diagnose left ventricular dysfunction.

B-type natriuretic peptide (BNP) is synthesized and released from the myocardium in response to an increase in ventricular filling pressure.[11] Recently, it was reported that the measurement of plasma BNP has a high sensitivity and a high specificity for detecting patients with asymptomatic heart failure or left ventricular dysfunction.[12–15] However, there are only a few studies that have examined the factors that are associated with high plasma BNP levels in the non-Western population.[16,17]

The purpose of this study is to clarify the risk factors for high plasma BNP levels, which is an important marker of asymptomatic heart failure, in a Japanese general population.

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Population and methods

Study population

The participants were 957 residents aged 35–69 years, who received regular annual health check-ups for the residents except for employees under the Health and Medical Service Law for the Aged, in SA town, Shiga Prefecture, a rural community in Western Japan. Well-trained nurses interviewed each participant to obtain a medical history and lifestyle information such as smoking and alcohol consumption. Of the 827 participants who gave informed consent, 13 participants did not have the complete data needed for the analysis. Of a total of 814 eligible participants, 128 were excluded for the following reasons: past or present history of coronary heart disease ($n=18$), diabetes mellitus ($n=48$), atrial fibrillation ($n=2$), and having symptoms suspected of heart failure, such as some clinical conditions that preclude physical exercise ($n=60$). No participants had a past or present history of renal disease. Thus, 686 residents aged 35–69 years participated in the study (209 men and 477 women; mean age $\pm$ s.d., 56.1 ± 9.7 years).

All the procedures of this study were reviewed and approved by the Institutional Review Board of Shiga University of Medical Science (No.14-10, 2002).

Clinical examination

The body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). The blood pressure was measured twice after 5 min of rest using an automatic sphygmomanometer (COLIN CORPORATION, BP-103i II, Aichi, Japan) placed on the right arm of participants in the sitting position. The mean of the two measurements was used for this analysis. The blood pressure was classified into the following four categories using WHO criteria of 1999:[18] optimal and normal—systolic blood pressure (SBP) under 130 mmHg and diastolic blood pressure (DBP) under 85 mmHg; high normal—SBP 130–139 mmHg and/or DBP 85–89 mmHg; grade 1—SBP 140–159 mmHg and/or DBP 90–99 mmHg; and grades 2 and 3—SBP 160 mmHg or greater and/or DBP 100 mmHg or greater.

Blood samples were drawn from an antecubital vein of nonfasting participants, and then analysed in one laboratory (KINKIYOKEN, Shiga). Plasma samples for the BNP measurements were transferred immediately to tubes with 1.0 mg/ml of EDTA-2Na and 500 kallikrein inhibitory units (KIU)/ml of aprotinin. Plasma was obtained by centrifugation at 3000 rpm for 10 min at 4°C and stored at −80°C until analysis. Plasma BNP concentration was measured with specific immunoradiometric assays for human BNP (ShionoRIA BNP kit, Shionogi & Co., Ltd, Osaka, Japan).[12–14,16,19,20] For BNP, the intra- and inter-assay coefficients of variation for this assay were 1.3 and 3.2%, respectively. Plasma BNP level of 18 pg/ml or greater were considered indicative of potential left ventricular dysfunction. This was based on a previous study conducted in the UK that showed this BNP cutoff value had a sensitivity of 77% and a specificity of 87% in 1252 participants aged 25–74 years for diagnosing left ventricular systolic dysfunction (left ventricular ejection fraction 30% or less).[15]

Total cholesterol and high-density lipoprotein (HDL) cholesterol in serum were measured enzymatically. Lipid measurement at the reporting laboratory has been standardized at the Osaka Medical Center for Health Science and Promotion, by a member of the Cholesterol Reference Method Laboratory Network (CRMLN).[21,22] Plasma glucose was measured by the hexokinase method. Blood haemoglobin was determined by the latex coagulation method.

Electrocardiography (ECG) was performed by standard 12-lead ECG after the patient had rested sufficiently. Findings of high R-wave voltage, ST-T depression, and an inverse- or flat-T-wave in the ECG were defined according to the Minnesota Code (MC).[23] High R-wave voltage in the 12-lead ECG was defined by the following: an R-wave in V5 or V6 of 2.6 mV or greater (MC 3-1) and/or the height of the R-wave in V1 plus V5 or V6 of 3.5 mV or greater (MC 3-3). Other findings that were documented if present included ST-T depression (MC 4-1 or 4-3), and inverse or flat T-waves (MC 5-1 or 5-3).

Daily salt excretion was estimated by Tanaka's formulas,[24] which estimate populational daily urinary salt excretion from the sodium and creatinine levels in casual urine samples. Using a self-reported questionnaire administered by well-trained nurses, the participants were asked about daily alcohol intake and smoking habits.

Statistical analyses

The possible determinants of BNP were divided into quartiles or categories. Geometric means of BNP were used for the analysis of each determinant because the distribution of BNP was positively skewed. To compare these with the crude geometric means of BNP in each quartile or category, analysis of variance was used. Comparisons with age- and gender-adjusted geometric means of BNP were performed using analysis of covariance. Gender-specific analysis was also performed.

Linear regression analysis was used to clarify the contribution of each independent variable to BNP. Multiple logistic regression analysis was used to assess the contribution of each independent variable to a high plasma BNP level (18 pg/ml or greater). The significance of the interaction of sex with risk factors related to BNP was tested using an inter-

action term in multivariate models in the gender-combined analysis.

The Statistical Package for the Social Sciences (SPSS Japan Inc., version 11.0J, Tokyo, Japan) was used for the analyses. All probability values were two-tailed and all confidence intervals were estimated at the 95% level.

Results

Table 1 shows the means and the prevalence of risk factors. The mean plasma BNP was 13.7 pg/ml in the entire population, 10.7 pg/ml in men and 15.3 pg/ml in women.

There was no relationship between BNP level and each quartile for BMI, DBP, total cholesterol, HDL cholesterol, plasma glucose, and current smoking. Table 2 shows the geometric means of BNP according to the quartiles or categories (blood pressure category, high R-wave voltage, and current alcohol consumption) for each risk factor that was statistically significant in the analysis of variance or covariance. SBP, Grade 2 or severe hypertension category (SBP $\geqslant$ 160 mmHg and/or DBP $\geqslant$ 100 mmHg), high R-wave voltage in the ECG, and daily salt excretion were positively associated with BNP, and their values were higher in the higher BNP quartiles. There was a statistically significant relationship between the BNP levels and haemoglobin quartiles, with higher BNP levels in patients with haemoglobin values in the lower quartiles.

Since the interaction term between sex and risk factors related to BNP was not statistically significant in the multivariate regression analyses,

Table 1 Levels and prevalence of risk characteristics for males, females, combined among 686 subjects aged 35–69 years old in SA-Town, Shiga, Japan, 2002

Variables	*Males* (n = *209*)	*Females* (n = *477*)	*Combined* (n = *686*)
	mean±*s.d.*	*mean*±*s.d.*	*mean*±*s.d.*
Age (years)	57.1±9.1	55.6±9.9	56.1±9.7
Body mass index (kg/m)	23.8±2.9	23.0±3.0	23.3±3.0
Systolic blood pressure (mmHg)	130.1±18.0	124.0±18.3	125.8±18.4
Diastolic blood pressure (mmHg)	82.4±11.1	75.5±11.1	77.6±11.5
Total cholesterol (mmol/l)	5.29±0.79	5.59±0.91	5.50±0.89
High density lipoprotein (HDL) cholesterol (mmol/l)	1.39±0.38	1.63±0.39	1.56±0.40
Plasma glucose (mmol/l)	5.31±0.77	5.07±0.50	5.15±0.60
Haemoglobin (g/dl)	14.7±1.0	12.9±1.1	13.5±1.4
Salt excretion (g/day, estimated)	12.6±3.4	12.1±3.3	12.3±3.3
B type natriuretic peptide (BNP) (pg/ml, geometric mean)	10.7	15.3	13.7
	Prevalence (%)	*Prevalence (%)*	*Prevalence (%)*
ECG findings			
High R-wave voltage[a]	13.4	5.9	8.0
ST-depression[b]	0.0	1.9	1.3
Inverse or flat T-wave[c]	1.4	2.1	1.9
Blood pressure category[d]			
Optimal+normal	47.4	62.5	57.9
High–normal	16.3	14.5	15.0
Grade 1	27.8	17.6	20.7
Grade 2+3	8.6	5.5	6.4
Subject using antihypertensive agents	14.4	14.7	14.6
Smoking habit			
Nonsmoker	24.4	91.8	71.3
Ex smoker	23.4	1.9	8.5
Current smoker	52.2	6.3	20.3
Alcohol consumption			
Nondrinker	22.5	76.7	60.2
Ex drinker	1.0	0.4	0.6
Current drinker	76.6	22.9	39.2
Menopause	—	69.0	—
High plasma BNP (18 pg/ml or greater)	24.4	43.2	37.5

[a]High R-wave voltage:high R criteria:V5 or V6 > 2.6 mV, and/or V1 and V5 or V6 > 3.5 mV.
[b]ST-depression: the criteria for ST depression was the Minnesota Code 4-1 or 4-3.
[c]Inverse- or flat-T-wave: the criteria for inverse- or flat-T was the Minnesota Code 5-1 or 5-3.
[d]Blood pressure category: Optimal+normal: SBP < 130 mmHg and DBP < 85 mmHg, high–normal: SBP 130–139 mmHg and/or DBP 85–89 mmHg, grade 1:SBP 140–159 mmHg and/or DBP 90–99 mmHg, grade 2+3: SBP $\geqslant$ 160 mmHg and/or DBP $\geqslant$ 100 mmHg.

Table 2 Plasma BNP levels and quintiles for proportional variables among 686 males and females aged 35–69 years old in SA-Town, Shiga, Japan, 2002

Variables	*Number of subjects*	*Crude geometric mean (pg/ml)*	P*	*Age- and gender-adjusted geometric mean (pg/ml)*	P**
Systolic blood pressure (mmHg)					
Quartile 1 −111.3	171	12.7	0.004	13.6	0.041
Quartile 2 111.4–123.4	170	13.0		13.3	
Quartile 3 123.5–138.9	169	12.9		12.5	
Quartile 4 139.0–	176	16.5		15.5	
Blood pressure category[a]					
Optimal+normal	397	12.7	0.001	13.2	0.047
High–normal	103	14.1		13.2	
Grade 1	142	15.0		14.6	
Grade 2+3	44	19.5		17.8	
Haemoglobin (g/dl)					
Quartile 1 −12.5	159	17.5	0.000	17.0	0.000
Quartile 2 12.6–13.3	168	15.6		15.2	
Quartile 3 13.4–14.2	175	13.6		13.2	
Quartile 4 14.3–	184	10.0		10.8	
High R-wave voltage in the ECG[b]					
−	631	13.3	0.000	13.3	0.000
+	55	20.3		19.7	
Salt excretion (g/day, estimated)					
Quartile 1 −9.9	166	11.0	0.000	11.7	0.000
Quartile 2 10.0–11.8	171	12.3		12.3	
Quartile 3 11.9–13.9	171	15.7		15.1	
Quartile 4 14.0–	178	16.5		16.2	
Alcohol consumption					
Non-/ex drinker	417	14.4	0.037	13.1	0.053
Current drinker	269	12.7		14.8	

[a]Blood pressure category: Optimal+normal: SBP < 130 mmHg and DBP < 85 mmHg, high–normal: SBP 130–139 mmHg and/or DBP 85–89 mmHg, grade 1: SBP 140–159 mmHg and/or DBP 90–99 mmHg, grade 2+3: SBP ⩾ 160 mmHg and/or DBP ⩾ 100 mmHg.
[b]High R-wave voltage+high R criteria: V5 or V6 > 2.6 mV, and/or V1 and V5 or V6 > 3.5 mV.
*P: analysis of variance, **P: analysis of covariance.

the following analyses were carried out to combined men and women with adjustment for gender.

Table 3 shows the partial regression coefficients from the linear regression analysis. In this model, age, daily salt excretion, high R-wave voltage, female gender, and SBP were positively associated with plasma BNP levels. Blood haemoglobin was negatively associated with BNP levels. The multiple correlation coefficient (R) of this model was 0.49 and the degrees of freedom (df)-adjusted coefficient of determination (R^2) was 0.23 ($F = 30.0$, $P < 0.001$). Alcohol consumption was positively associated with BNP levels, although it did not reach statistical significance ($P = 0.051$). BMI showed no association with BNP levels.

Table 4 shows the odds ratios of each risk factor with a high plasma BNP level (18 pg/ml or greater) determined using multiple logistic regression analysis. Age, daily salt excretion, high R-wave voltage, female gender, and grade 2 or greater hypertension were positively associated with high plasma BNP levels, and blood haemoglobin concentration was negatively associated. The significant relationship between BNP and salt excretion was also observed even after we excluded participants with high R-wave voltage or participants taking antihypertensive agents, although the relationship between BNP and SBP or hypertension disappeared when these patients were excluded.

Gender-specific analysis showed that plasma BNP levels were significantly correlated with age, urinary salt excretion, and low haemoglobin for each gender. We also observed positive relations of plasma BNP levels with SBP and high R-wave voltage for each gender, which indicated similar magnitude of regression coefficients or odds ratio, although the relation with SBP for women and high R-wave voltage for men did not reach statistical significance.

Further analysis adjusting for administration of antihypertensive agents, smoking, serum lipids, and plasma glucose did not substantially affect the results shown in Tables 3 (data not shown in the table).

Table 3 Determinants of plasma BNP levels: linear regression analysis, 686 males and females aged 35–69 years old in SA-Town, Shiga, Japan, 2002

Variables	*Partial regression coefficients*	*s.e.*[a]	t	P
Age (10 years)	0.210	0.029	7.296	0.000
Haemoglobin (1 s.d., 1.36 g/dl)	−0.196	0.034	−5.681	0.000
Salt excretion (1 s.d., 3.4 g/day)	0.133	0.027	4.892	0.000
High R-wave voltage in the ECG (0 = no, 1 = yes)[b]	0.325	0.098	3.326	0.001
Gender (0 = male, 1 = female)	0.251	0.080	3.140	0.000
Systolic blood pressure (1 s.d., 18.4 mmHg)	0.070	0.029	2.454	0.014
Alcohol consumption (0 = non- or ex drinker, 1 = current drinker)	0.123	0.062	1.987	0.051
Body mass index (1 s.d., 3.0 kg/m^2)	−0.021	0.028	−0.759	0.448

[a]Standard error.
[b]High R-wave voltage was defined by height of R-wave in the ECG ;V5 or V6 is 2.6 mV or greater, and/or height of R-wave for V1 plus V5 or V6 is 3.5 mV or greater.

Table 4 Multivariate odds ratio and 95% confidence intervals for having high plasma BNP (⩾18.0 pg/ml), 686 males and females aged 35–69 years old in SA-Town, Shiga, Japan, 2002

Variables	*Odds ratio*	*95% confidence interval*		
Age (years)	1.05	1.03	—	1.07
Haemoglobin (g/dl)	0.69	0.58	—	0.81
Salt excretion (g/day)	1.09	1.03	—	1.15
High R-wave voltage in the ECG (0 = no, 1 = yes)[a]	2.05	1.10	—	3.81
Gender (0 = male, 1 = female)	2.01	1.18	—	3.41
Blood pressure category[b]				
Optimal+normal	1.00		—	
High–normal	0.92	0.56	—	1.51
Grade 1	1.28	0.82	—	2.01
Grade 2+3	2.09	1.04	—	4.22
Alcohol consumption (0 = non- or ex drinker, 1 = current drinker)	1.45	0.97	—	2.17
Body mass index (kg/m^2)	0.99	0.93	—	1.06

[a]High R-wave voltage was defined by height of R-wave in the ECG; V5 or V6 is 2.6 mV or greater, and/or height of R-wave for V1 plus V5 or V6 is 3.5 mV or greater.
[b]Blood pressure category: Optimal+normal: SBP < 130 mmHg and DBP < 85 mmHg, high–normal: SBP 130–139 mmHg and/or DBP 85–89 mmHg, grade 1: SBP 140–159 mmHg and/or DBP 90–99 mmHg, grade 2+3: SBP ⩾ 160 mmHg and/or DBP ⩾ 100 mmHg.

Discussion

The present study suggests that higher blood pressure, urinary salt excretion (a surrogate measure of dietary salt intake), high R-wave voltage in the ECG, and low blood haemoglobin as well as age and female gender are important determinants of plasma BNP levels in a general Japanese population.

Previous studies have reported a positive relationship between heart failure and hypertension.[19,25] The present study has also shown a positive relationship between grade 2 or greater hypertension and high levels of plasma BNP. Similar to previous reports dealing with Western populations, hypertension of moderate or greater degree may be one of the risk factors for asymptomatic heart failure or left ventricular dysfunction in the Japanese population. Hypertension, which is derived mainly from increased systemic vascular resistance and/or expanded intravascular volume, causes a sustained increase in left ventricular afterload that decreases cardiac output or ejection fraction, ultimately resulting in congestive heart failure.[19]

In general, urinary salt excretion is nearly equal to the dietary salt intake. High salt intake is an important factor that can expand intravascular volume, and it is a major causal risk factor for hypertension.[26–29] A recent study suggested that high salt intake *per se*, independent of hypertension, can have a harmful effect on the general population owing to the high risk of total mortality and mortality due to coronary heart disease and stroke.[30,31] A previous study reported that chronic high dietary salt intake increases the plasma concentration of BNP, even in the absence of hypertension.[32] It has also been reported that high dietary salt intake is related to the incidence of congestive heart failure in overweight men and women in the United States.[20] Furthermore, it has been emphasized that high salt intake is a risk factor for left ventricular hypertrophy.[33–35] High salt intake may be directly correlated to plasma BNP concen-

tration due to an increase in the circulating blood volume, which may indirectly lead to an increase in myocardial mass due to hypertension. The relationship between BNP and daily salt excretion was present after exclusion of subjects taking antihypertensive agents or those who had high R-wave voltage.

BNP, as a cardioprotective factor, has a diuretic effect that promotes the excretion of water and sodium by the kidneys.[36,37] As a result of the high sodium excretion promoted by BNP, the amount of urinary salt excretion may overestimate the actual dietary salt intake in participants with high plasma BNP levels. However, participants with high urinary salt excretion are continually exposed to high salt intake, which results in a situation that high BNP secretion is needed in order to protect their circulatory system. Consequently, we believe that a high salt intake may be a causal risk factor for heart failure or left ventricular dysfunction.

Left ventricular hypertrophy, which is usually accompanied by hypertension, is an example of target organ damage caused by an increase in circulating blood volume and/or vascular resistance occurring over many years. In the Framingham study, cardiac mass was assessed using echocardiography.[12] In the present study, we used the presence of high R-wave voltage in the 12-lead ECG as an index of left ventricular hypertrophy. An ECG is a more convenient and inexpensive method than an echocardiogram, and it is suitable for mass screening in the community. Moreover, people aged 40 years or greater in Japan are able to have an annual ECG under the Health and Medical Service Law for the Aged or the Industrial Safety and Health Law. In a previous study, significantly higher BNP levels were noted in patients with heart disease or hypertension who had abnormal electrocardiographic findings, such as high R-wave voltage.[38] Our finding seems to be consistent with this previous study, although our participants were healthy community dwelling residents.

Hypertension, high salt excretion, and high R-wave voltage in the ECG, which are associated with high BNP levels, are also the classical risk factors for stroke reported in previous Japanese cohort studies.[39–42] However, serum total cholesterol, which is a risk factor for ischaemic heart disease and not for stroke in Japan,[2–5] was not associated with plasma BNP levels. Since mortality due to ischaemic heart disease in Japan is lower than that in Western populations,[1,6,43] it may be reasonable to assume that the risk factors for latent heart failure are similar to those for stroke in the Japanese population.

Another interesting finding in the present study was the negative correlation between blood haemoglobin and BNP. It has been reported that anaemia is an independent prognostic factor for mortality in congestive heart failure patients living in the community.[44] Our result suggests that a low blood haemoglobin concentration, even within the clinically normal range, is associated with high plasma BNP. A reduced haemoglobin concentration might be a maker for advanced heart failure that may occur as a result of haemodilution due to volume overload and renal insufficiency.[44] Other factors in heart failure that are associated with anaemia include iron deficiency, chronic inflammation, and impaired erythropoietin production.[45]

Several limitations of this study should be acknowledged. First, we dealt with high R-wave voltage in the ECG as a marker for left ventricle hypertrophy, which may not always reflect true cardiac mass. Although body mass may affect R-wave amplitude, we statistically adjusted for the effect of body mass index. Unfortunately, due to the low prevalence of other abnormal findings in the ECG such as ST-T depression (1.3%) and inverse or flat T-waves (1.9%), we were not able to use these findings in our analysis. Second, our study used a cross-sectional design, which does not prove causal relations between plasma BNP levels and the above-mentioned risk factors.

In conclusion, we clarified the relationship between the elevated plasma BNP and hypertension, urinary salt excretion, high R-wave voltage in the ECG, age, and low haemoglobin concentration in a Japanese general population. We found some possible determinants for the elevation of plasma BNP in the Japanese general population. These factors — age, urinary salt excretion, hypertension — are similar to the classical risk factors for stroke in Japan.

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CARDIOVASCULAR DISEASE

Much lower prevalence of coronary calcium detected by electron-beam computed tomography among men aged 40–49 in Japan than in the US, despite a less favorable profile of major risk factors

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Background Since World War II (WWII), exposures to westernized lifestyle have occurred in many non-Western countries, including Japan. National surveys showed that risk factor profiles for atherosclerosis around 1990 were similar in men in the post WWII birth cohorts in the US and Japan. We compared the degree of coronary calcium and other factors in men in the post WWII birth cohort: men aged 40–49 in the US and Japan.

Methods We conducted a cross-sectional study examining randomly selected 100 men from Kusatsu, Japan, and 100 men from Allegheny County, US. Coronary calcium was assessed using electron-beam computed tomography.

Results Systolic blood pressure, total cholesterol, low density lipoprotein (LDL)-cholesterol, and smoking rates were higher among the Japanese (122.6 ± 14.1 versus 113.7 ± 9.6 mmHg, $P < 0.01$; 5.72 ± 0.90 versus 4.99 ± 0.81 mmol/l (220.9 ± 34.6 versus 192.8 ± 31.3 mg/dl), $P < 0.01$; 3.52 ± 1.01 versus 3.10 ± 0.78 mmol/l (136.0 ± 39.0 versus 119.7 ± 30.0 mg/dl), $P < 0.01$; and 48 versus 15%, $P < 0.01$, respectively). Triglycerides and fibrinogen were similar. High density lipoprotein (HDL)-cholesterol was higher among the Japanese. Body mass index, fasting insulin, and C-reactive protein were higher among the Americans. Prevalence of coronary artery calcium score >0 was strikingly lower among the Japanese than the Americans (13% versus 47%, $P < 0.01$).

Conclusions Much lower prevalence of coronary calcium despite a less favourable profile of many major independent risk factors in the Japanese might imply that there are strong protective factors against atherosclerosis in the Japanese. Further investigation is of critical importance.

Keywords Subclinical atherosclerosis, electron-beam computed tomography, US, Japan, epidemiology, risk factors, post World War II birth cohort, coronary calcium

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Since World War II (WWII), exposures to westernized lifestyle have occurred in many non-Western countries, including Japan. Men born after WWII in Japan have undergone major changes in diet and other lifestyle factors, including heavy cigarette smoking, increase in the levels of total and low density lipoprotein cholesterol (LDL-C), increase in cholesterol intake in diet (which is higher than in the US), increase in body weight and obesity, substantial increase in alcohol consumption, and increase in western foods.[1] Comparing the survey data from national representative samples around 1990, risk factor profiles for coronary heart disease (CHD): total cholesterol, and systolic and diastolic blood pressure, were very similar in men in the post WWII birth cohorts in the US and Japan, except for much higher prevalence of cigarette smoking in Japan and much higher prevalence of obesity in the US.[2] We compared CHD mortality in men aged 35–44 between the US and Japan and found that CHD mortality in Japan is still considerably lower than in the US: 23.7/100 000 for the US and 8.7/100 000 for Japan.[3] After careful review, the differences do not appear to be explained by differences in potential misclassification of causes of death.[3] These observations imply that there may be important protective factors that reduce risk of CHD in Japan.

Differences in genetic factors are unlikely to fully explain the difference in CHD mortality because studies of Japanese migrants to the US clearly illustrated the increase in CHD morbidity and mortality in Japanese men in the US.[4] Epidemiological investigations monitoring and comparing CHD morbidity and mortality in the post WWII birth cohorts, i.e. age <50, between the countries would be very important. Such studies are, however, practically impossible to conduct because incidence of CHD is too low in the post WWII birth cohort.

Electron-beam computed tomography (EBT) is a non-invasive method for defining subclinical atherosclerosis of coronary arteries and accurately quantifying coronary artery calcium.[5] Calcification within the coronary arteries is highly correlated with the extent of atherosclerosis. Coronary artery calcification occurs in small amounts in the early lesions of atherosclerosis in the second and third decades of life.[5] It is found frequently in advanced lesions and in older age.

The key question is 'Do Japanese men in the post WWII birth cohort with similar risk factor profiles as men in the US have as much or less coronary atherosclerosis?' In this study, we tested the null hypothesis that there is no difference in the prevalence of coronary artery calcium detected by EBT between men aged 40–49 in the US and Japan. We also compared the traditional risk factors and other factors between the two populations.

Methods

Subjects

Participants were 40–49 years of age and residents in Allegheny County, Pennsylvania (PA) or Kusatsu City, Shiga, Japan. Exclusion criteria included: (1) clinical cardiovascular disease, (2) type 1 diabetes, (3) cancer except skin cancer in the past 2 years, (4) renal failure, and (5) genetic familial hyperlipidaemias.

In Kusatsu City, subjects were randomly selected using the Basic Residents' Register which has each resident's information on name, birth date, address, household, and other details. From the Register, 300 men aged 40–49 were randomly selected and were contacted consecutively via phone. Among 203 men contacted from May 2001 to December 2002, 100 men agreed to participate in the study and were examined. The rate of participation was 49%. Of these 100, two subjects were excluded in the current analyses because EBT images were not obtained appropriately.

In Allegheny County, PA, subjects were volunteers. In May 2002, the study was announced through the University of Pittsburgh Medical Center (UPMC) Health Plan with the eligibility criteria. The UMPC Health Plan is a major health care insurer in the area of Pittsburgh, covering the majority of University workers and students. From June to October 2002, 100 subjects participated in the study. Among them, 99 were Caucasians.

Informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of Shiga University Medical Science, Otsu, Japan, and University of Pittsburgh, Pittsburgh, US.

Study protocol

Participants were instructed to abstain from food or drink beginning at 8 p.m. on the previous day. On the examination day, their body weight and height were measured while they were wearing light clothing without shoes. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). Waist circumference was measured at the level of the umbilicus while participant was standing erect. Blood pressure was measured in the right arm of seated participants after the participant emptied his bladder and sat quietly for 5 minutes, using an appropriate-sized cuff, with a standard mercury sphygmomanometer. The average of two measurements of the first (systolic) and fifth (diastolic) Korotkoff sounds was used for the analysis.

Venipuncture was performed early in the clinic visit after a 12-hour fast. The serum was kept at room temperature for 45 minutes. The plasma was then stored on ice and centrifuged within 60 minutes. Multiple aliquots of plasma or serum were prepared and frozen at −70°C. Serum samples were shipped on dry ice to the Heinz Laboratory, Department of Epidemiology, University of Pittsburgh, to measure lipids, glucose, and insulin. Serum lipids were measured with the standardized methods according to the Centers for Disease Control and Prevention, including total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides. LDL-C was estimated by the Friedewald equation.[6] When the value of triglycerides exceeded 4.52 mmol/l (400 mg/dl), LDL-C was measured directly using an automated spectrophotometric assay, LDL Direct Liquid Select (Equal Diagnostics, Exton US). There were two such subjects at each site. Serum glucose was determined using the hexokinase–glucose 6-phosphate dehydrogenase enzymatic assay. Serum insulin was determined using radio immunoassay (Linco Research Inc., St Charles, US).

Samples were shipped on dry ice to the Laboratory for Clinical Biochemistry Research, University of Vermont, to measure C-reactive protein (CRP) and fibrinogen. CRP was measured by a calorimetric competitive enzyme-linked immunosorbent assay. Fibrinogen was measured in an automated clot-rate assay using the ST4 instrument (Diagnostica Stago, Parsippany, US).

A self-administered questionnaire was used to obtain information on demography, smoking habits, alcohol drinking, fish intake, and other factors. Alcohol drinking was assessed as whether a participant drinks beer, wine, liquor, or sake with the

frequency of drinking. Frequency of fish intake was assessed as <1/week, 1/week, 2–4/week, or >4 times/week.

Electron-beam computed tomography (EBT)

The scanning was done using a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, US) at both sites. Scanners were calibrated regularly by technologists following a standardized protocol. Measures of water density [0 Hounsfield Unit (HU)], air (-1000 HU), and calcification (threshold 130 HU) were uniform in the two scanners.

Scanning was performed in a standardized protocol to obtain 30–40 contiguous 3 mm thick transverse images from the level of the aortic root to the apex of the heart. Images were obtained during maximal breath holding using ECG triggering so that each 100 m second exposure was obtained during the same phase of the cardiac cycle. The amount of radiation exposure received from the EBT procedure is approximately 0.7 rem to the chest. All scan data were saved to optical disc.

Readings of the scanning were done centrally at the Cardiovascular Institute, Pittsburgh, using a DICOM (Digital Imaging and Communications in Medicine) workstation and software by AccuImage (AccuImage Diagnostic Corporation, San Francisco, US). The software program implements the widely accepted Agatston scoring method.[7] Coronary artery calcium was considered to be present when three contiguous pixels (area = 1 mm^2) >130 HU were detected overlying the vessels of interest. A calcium score was then calculated for each region of interest by multiplying the area of all significant pixels by a grade number (1, 2, 3, 4) indicative of the peak computed tomography number (HU). The individual region of interest scores were then summed for a total coronary calcium score. The reading was evaluated by one trained radiology technician. The reproducibility of the EBT scans from this laboratory had an intraclass correlation of 0.99.[8]

Since the distribution of coronary calcium score was very skewed, the data were analysed in a categorical form. To calculate the prevalence of coronary calcium, two cut points were used: >0 as well as <10, and ≥10.

Statistical analysis

Values are expressed as means ± standard deviation (SD) and were compared with the use of a two-sample t-test. Continuous variables that showed highly skewed distribution (CRP and triglycerides) were compared with the use of the Mann-Whitney U test, and values were expressed as median and inter-quartile range. Dichotomous data (prevalence of coronary artery calcium, cigarette smoking, alcohol drinking, and fish eating) were compared with the use of the χ^2 statistics. All *P*-values were two-tailed. *P*-value <0.05 was considered as significant. SPSS software (release 11.5.0, SPSS Inc., Chicago, US) was used for all statistical analyses.

Results

Comparison of the major independent risk factors between men in the US and Japan

Levels of many major independent risk factors were less favourable among the Japanese men than among the American men (Table 1). Levels of systolic blood pressure were significantly higher among the Japanese men by about 10 mmHg. Prevalence of hypertension defined as levels of systolic blood pressure ≥140 mmHg, or levels of diastolic blood pressure ≥90 mmHg, or on anti-hypertensive medication was 19.4% for the Japanese and 11.0% for the Americans. Levels of total cholesterol as well as LDL-C were significantly higher among the Japanese men by about 0.73 mmol/l (28 mg/dl) and 0.41 mmol/l (16 mg/dl), respectively. Prevalence of current cigarette smoking was substantially higher among the Japanese men. Levels of HDL-C, in contrast, were significantly higher among the Japanese men by about 0.23 mmol/l (9 mg/dl).

Table 1 Comparison of major independent risk factors and other factors between the American and Japanese men

	US (n = 100)	Japan (n = 98)	*P*
Age (year)	44.6 ± 2.9	44.7 ± 2.8	0.74
Systolic blood pressure (mmHg)	113.7 ± 9.6	122.6 ± 14.1	<0.01
Diastolic blood pressure (mmHg)	78.4 ± 5.8	78.6 ± 10.4	0.99
Total cholesterol (mmol/l)	4.99 ± 0.81	5.72 ± 0.90	<0.01
Triglycerides (mmol/l)	1.31 (0.89, 1.89)	1.48 (1.02, 2.04)	0.17[b]
High density lipoprotein cholesterol (mmol/l)	1.19 ± 0.30	1.42 ± 0.39	<0.01
Low density lipoprotein cholesterol (mmol/l)	3.10 ± 0.78	3.52 ± 1.01	0.01
Smoker (%)	15.0	48.0	<0.01[c]
Alcohol[a] (%)	16.0	45.9	<0.01[c]
Fasting blood glucose (mmol/l)	5.29 ± 0.51	5.74 ± 0.49	<0.01
Insulin (pmol/l)	86.8 ± 45.8	56.9 ± 26.4	<0.01
Body mass index (kg/m^2)	27.0 ± 3.3	23.3 ± 3.1	<0.01
Waist circumference (cm)	96.4 ± 9.8	84.7 ± 8.5	<0.01
Height (cm)	180.6 ± 6.6	170.2 ± 5.1	<0.01
C reactive protein (mg/l)	0.9 (0.45, 2.20)	0.43 (0.21, 0.88)	<0.01[b]
Fibrinogen (μmol/l)	7.09 ± 1.95	6.95 ± 1.85	0.81

[a] Drink alcohol every day.
[b] Mann–Whitney test.
[c] Chi-square test.

Comparison of other factors between men in the US and Japan

Differences were observed in obesity, levels of insulin, CRP and glucose, and some lifestyle factors, which may relate to major independent risk factors or atherosclerosis (Table 1). Anthropometry data showed that the American men were much more obese and had greater waist circumferences. Levels of insulin as well as CRP were much higher among the American men. Levels of triglycerides as well as fibrinogen were similar between the two populations. Levels of glucose were significantly higher among the Japanese men by about 0.44 mmol/l (8 mg/dl). Prevalence of diabetes defined as levels of fasting blood sugar ≥6.0 mmol/l (126 mg/dl) or being on anti-diabetic medication was 2.0% for both the Japanese and the Americans.