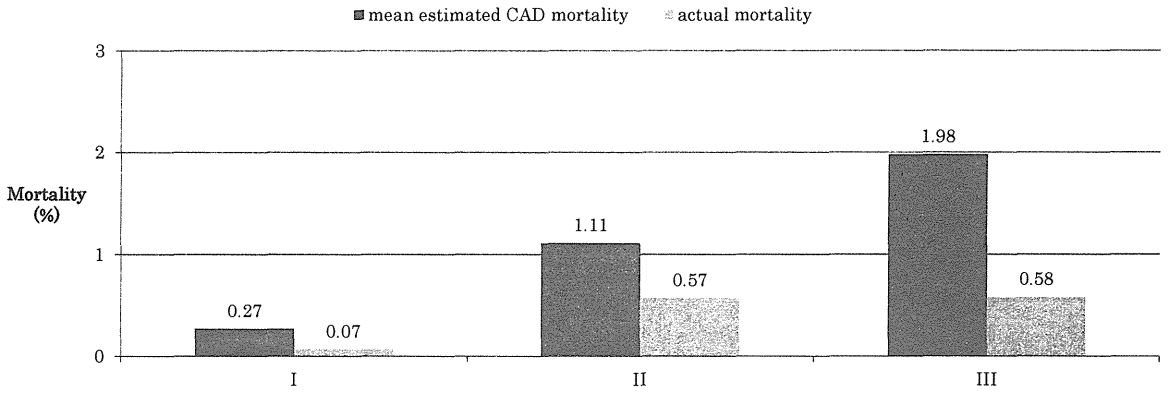
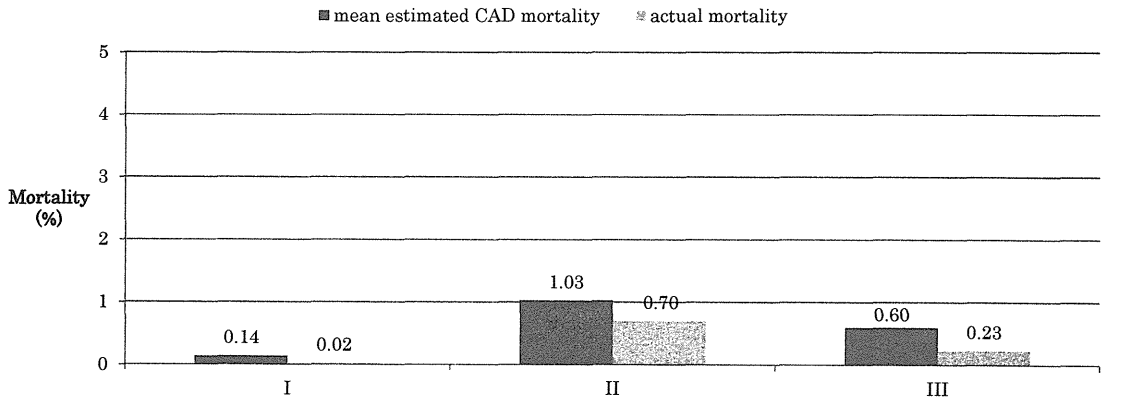


	I	II	III
Difference between two mortality [2]	0.20	0.54	1.40
Number of participants	1387	1765	11939
Number of death	1	10	69

1) Category I:<0.5%, Category II: ≥0.5% <2.0%, Category III: ≥2.0% or DM

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each category

Fig. 3A. Mean estimated CAD mortality of men in NIPPON DATA80 and actual mortality of men in EPOCH-JAPAN according to the JAS Guidelines 2012.[1] Hosmer–Lemeshow test (χ^2 statistic=127.69, d.f.=1, $P<0.001$)

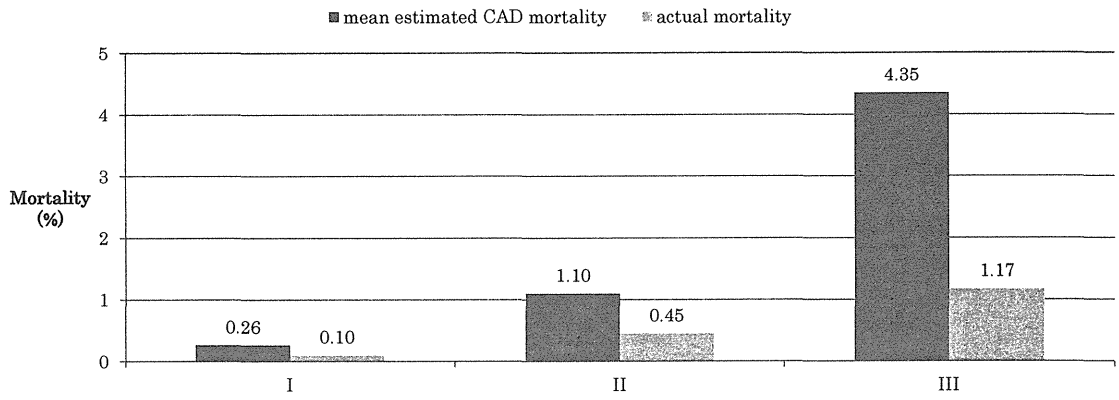


	I	II	III
Difference between two mortality [2]	0.12	0.33	0.37
Number of participant	4236	1142	13211
Number of death	1	8	31

1) Category I:<0.5%, Category II: ≥0.5% <2.0%, Category III: ≥2.0% or DM

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each category

Fig. 3B. Mean estimated CAD mortality of women in NIPPON DATA80 and actual mortality of women in EPOCH-JAPAN according to the JAS Guidelines 2012.[1] Hosmer–Lemeshow test (χ^2 statistic=34.89, d.f.=1, $P<0.001$)

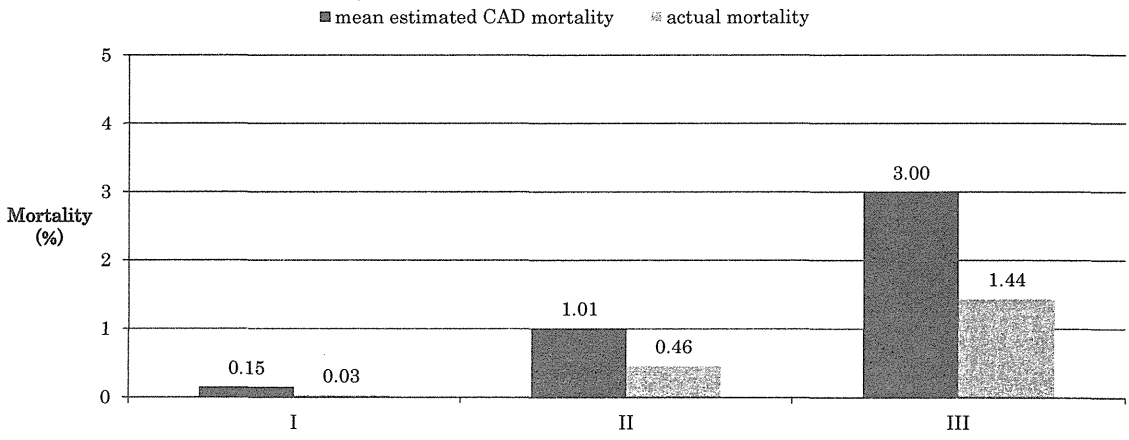


	I	II	III
Difference between two mortality [2]	0.16	0.65	3.18
Number of participants	5168	5719	4204
Number of death	5	26	49

1) Category I:<0.5%, Category II: ≥0.5% <2.0%, Category III: ≥2.0%

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each category

Fig. 4A. Mean estimated CAD mortality of men in NIPPON DATA80 and actual mortality of men in EPOCH-JAPAN according to the JAS Guidelines 2012 excluding the inclusion criteria of DM in Category III.[1] Hosmer–Lemeshow test (χ^2 statistic=129.67, d.f.=1, $P<0.001$)



	I	II	III
Difference between two mortality [2]	0.12	0.55	1.56
Number of participants	12847	4772	970
Number of death	4	22	14

1) Category I:<0.5%, Category II: ≥0.5% <2.0%, Category III: ≥2.0%

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each category

Fig. 4B. Mean estimated CAD mortality of women in NIPPON DATA80 and actual mortality of women in EPOCH-JAPAN according to the JAS Guidelines 2012 excluding the inclusion criteria of DM in Category III.[1] Hosmer–Lemeshow test (χ^2 statistic=34.58, d.f.=1, $P<0.001$)

lute difference between the mean estimated and actual mortality was relatively small. However, in higher CAD mortality groups, the mean estimated mortality was much higher than the actual mortality. For stroke, in both sexes, the mean estimated mortality was almost concordant with the actual mortality in low/moderate mortality groups, and the mean estimated mortality was higher than the actual mortality in higher estimated mortality groups. For three categories of JAS guidelines 2012, the mean estimated CAD mortality was higher than its actual mortality in all categories. However, the actual mortality in Category III did not increase significantly from Category II in men while that in Category III was lower than Category II in women. When we did not consider the presence of DM in risk classification, the actual mortality increased in ascending order of category in both sexes.

Comparison studies have been performed in the well-established risk charts such as the FRS and SCORE (Systematic Coronary Risk Evaluation) in the previous study[7-11, 28-30]. However, while there exist several risk assessment charts in Japan such as the NIPPON DATA80[15], Hisayama study[26], JALS-ECC[31], JMS cohort study[32, 33], and Suita study[3], few studies have evaluated the calibration with external Japanese cohort studies. Hisayama's study had attempted to develop and validate a new cardiovascular risk prediction model. Two-thirds of their participants were randomly assigned to a risk prediction model derivation cohort ($n=1,756$) and the remaining one-third of the participants were reserved as an independent validation cohort ($n=878$). Among subjects allocated to the derivation cohort, a new risk prediction model was developed using Cox's proportional hazards model, in which age, sex, SBP, diabetes, LDL cholesterol, high-density lipoprotein cholesterol, and smoking status were included as risk factors. The performance of the risk prediction model was tested among subjects allocated to the validation cohorts. However, the limitation of Hisayama's study was the lack of external validation[26], that is, the split sample as an internal validation was performed to justify the risk prediction model. To the best of our knowledge, this is the first study to review and evaluate ND80RAC with the large-scale nationwide Japanese cohort study.

The baseline period was different between NIPPON DATA80 and most cohort studies in EPOCH-JAPAN except NIPPON DATA80. The baseline survey of NIPPON DATA80 started from 1980, while the majority of the cohort studies in EPOCH-JAPAN started after 1990. In Japan, age-adjusted mortality from stroke increased after World War II until 1965 and significantly declined until 1990[13]. According to the vital statistics in Japan, the mortality from myocardial infarction in men was also decreased from 1980 to 1990[34]. The above-mentioned trend in mortality may be one reason for lower actual mortality in EPOCH-JAPAN than the mean estimated CAD/stroke mortality calculated by ND80RAC. Furthermore, mortality in the elderly is significantly decreasing during the last decade in Japan[2], which could also contribute to reduce the actual mortality in the elderly who were classified into the high-risk category.

Furthermore, due to the remarkable medical progress, the percutaneous coronary intervention had become one of the standard therapies for CAD since 1980s and the prevalence of stroke care units was associated with reduced in-hospital mortality. In addition, the advanced therapeutic agents such as statin have contributed to the cause of decreasing CAD mortality, particularly in high-risk individuals[35]. These factors could also explain the difference between the mean estimated mortality calculated by ND80RAC and the actual cumulative mortality in EPOCH-JAPAN, particularly in higher mortality groups.

The participants of most cohort studies in EPOCH-JAPAN were community dwellers or workers who participated in annual checkups performed under the health service law[36]. Accordingly, the participants of EPOCH-JAPAN could be considered to have high motivation for being healthy. In addition, participants of annual checkups usually could get health education or advice at their health checkups continuously after the baseline survey. In addition, workers have to get annual checkups under the law every year, and their health conditions are strictly managed after every checkup. Thus, the participants of EPOCH-JAPAN may be healthier than the general Japanese population. A previous study has reported that incidence and mortality due to CVD in the participants of annual checkups were much lower than those in non-participants[37]. On the other hand, the baseline survey of NIPPON DATA80 was performed in 1980, and such health checkup system by law has not been established yet. Furthermore, in NIPPON DATA80, all household members aged ≥30 years in 300 randomly selected census tracts across Japan were invited to participate with a high participation rate (76.6%). Thus, the participants of NIPPON DATA80 could have different characteristics from those of EPOCH-JAPAN.

We also calibrated the mean estimated CAD mortality and its actual cumulative mortality according to the three categories of JAS guidelines 2012. When we did not consider the presence of DM in risk

classification, the actual mortality increased in the ascending order of category in both sexes (**Fig. 4**). DM is one of the important risk factors for CAD/stroke. However, the results of the present study may indicate the need for reconsideration of the definition of diabetes when we estimate an individual's absolute risk for CAD/stroke for apparently healthy community dwellers. DM is associated with several complications[38, 39], and previous studies in Japan have already shown that DM complications were one of the major risk factors in CVD[40-42]. Furthermore, the prediction tools in the Japanese general population have demonstrated that the predicted risk for CVD due to DM, which was usually defined by glucose level or self-reported diabetes history, was nearly equivalent or even smaller than that of smoking[3, 31-33]. Therefore, when we estimate the individual's absolute risk for CVD, it may be important not only to diagnose DM by self-reported medical checkup but also to consider the disease duration of DM or the presence of DM complications such as nephropathy, neuropathy, visual acuity, and retinopathy.

The number of participants in the present study decreased from 101,977 in 12 cohorts due to death to 33,680. As we showed the characteristics of EPOCH-JAPAN including 12 cohorts with information about the cause of death in **Supplemental Table 1** and those of the participants of the present study in **Supplemental Table 2**, the percentage of participants in men and current smokers and total cholesterol were higher, and the glucose level was lower in the participants of the present study than those in EPOCH-JAPAN including 12 cohorts. Thus, these changes of the baseline characteristics may affect the results of the present study. However, because the values of all risk factors used in ND80RAC were required to estimate the probability of CAD/stroke mortality in the present study, we excluded 38,079 missing values/outliers of risk factors, most of which were blood glucose from Oyabe study and JACC. In addition, we excluded 7,029 participants with a history of cardiovascular disease and 13,747 participants who were <40 years or >75 years. These exclusion criteria may also affect the results of the present study. However, we believe that EPOCH-JAPAN in the present study was the best available candidate for an external cohort study to calibrate ND80RAC in the present situation.

The present study has several limitations. At first, the mortality from CAD was small, particularly in women. Another large-scale cohort study may be necessary to certify the risk chart for CAD in women. Second, the dataset did not include the information of the cholesterol-lowering therapy such as statins. While the baseline surveys in 6 cohorts in EPOCH-JAPAN were conducted before the first statin usage was started in 1989[34], other 3 cohorts were conducted around 1990. Therefore, the effect of statins had little impact on most of the participants at baseline. Finally, we could not consider the risk factors suggested by JAS guidelines 2012 such as HDL-C, family history of premature CAD, past history of peripheral artery disease, chronic kidney disease, and impaired glucose tolerance in these categorization.

In conclusion, the estimated CAD mortality by ND80RAC tended to be higher than the actual mortality in the population of which baseline survey was more recently performed. ND80RAC was established in approximately 10,000 nationwide general populations for the first time as a health-education tool in Japan for primary prevention to estimate the risk of CVD mortality. The tool was expected to assess the compatibility to other nationwide Japanese populations, and we could finally perform the calibration study after integrating the dataset of EPOCH-JAPAN, which includes $>30,000$ nationwide individuals. In the present study, we showed the need for calibration of the health-education tools by constructing a nationwide larger-scaled cohort study. We also showed the need for revision or re-establishment of the tools due to the change in backgrounds of Japanese population, including the remarkable development of medicine and medical technology, or for a better definition of risk factors and other endpoints.

Acknowledgements

We are grateful to all of the participants in each cohort study. We thank Mrs. Toshimi Yoshida (Shiga University of Medical Science) and Mrs. Satoko Narikawa (Keio University) for expert clerical assistance.

Appendix

The Evidence for Cardiovascular Prevention from Observational Cohorts in Japan (EPOCH−JAPAN) Research Group consists of the following investigators. Chairperson: Hirotsugu Ueshima (Shiga University of Medical Science); Co−Chairperson: Tomonori Okamura (Keio University School of Medicine);

Executive committee: Hirotsugu Ueshima (Shiga University of Medical Science), Yutaka Imai (Tohoku University Graduate School of Pharmaceutical Sciences), Takayoshi Ohkubo (Teikyo University School of Medicine), Fujiko Irie (Ibaraki Prefecture), Hiroyasu Iso, Akihiko Kitamura (Osaka University Graduate School of Medicine), Yutaka Kiyohara (Kyushu

University Graduate School of Medicine), Katsuyuki Miura (Shiga University of Medical Science), Yoshitaka Murakami (Toho University), Hideaki Nakagawa (Kanazawa Medical University), Takeo Nakayama (Kyoto University School of Public Health), Akira Okayama (Research Institute of Strategy for Prevention), Toshimi Sairenchi (Dokkyo Medical University), Shigeyuki Saitoh (Sapporo Medical University), Kiyomi Sakata (Iwate Medical University), Akiko Tamakoshi (Hokkaido University Graduate School of Medicine), Ichiro Tsuji (Tohoku University Graduate School of Medicine), Michiko Yamada (Radiation Effects Research Foundation), Masahiko Kiyama (Osaka Center for Cancer and Cardiovascular Disease Prevention), Yoshihiro Miyamoto (National Cerebral and Cardiovascular Center), Shizukiyo Ishikawa (Jichi Medical University), Hiroshi Yatsuya (Fujita Health University) and Tomonori Okamura (Keio University School of Medicine)

Funding Sources

This research was supported by a grant-in-aid from the Ministry of Health, Labour and Welfare, Health and Labor Sciences research grants, Japan (Research on Health Services: H17-Kenkou-007; Comprehensive Research on Cardiovascular Disease and Life-Related Disease: H18-Junkankitou [Seishuu]-Ippan-012; Comprehensive Research on Cardiovascular Disease and Life-Related Disease: H19-Junkankitou [Seishuu]-Ippan-012; Comprehensive Research on Cardiovascular and Life-Style Related Diseases: H20-Junkankitou [Seishuu]-Ippan-013; Comprehensive Research on Cardiovascular and Life-Style Related Diseases: H23-Junkankitou [Seishuu]-Ippan-005), and an Intramural Research Fund (22-4-5) for Cardiovascular Diseases of National Cerebral and Cardiovascular Center; and Comprehensive Research on Cardiovascular and Life-Style Related Diseases: H26-Junkankitou [Seisaku]-Ippan-001.

Conflict of Interest Disclosures

None.

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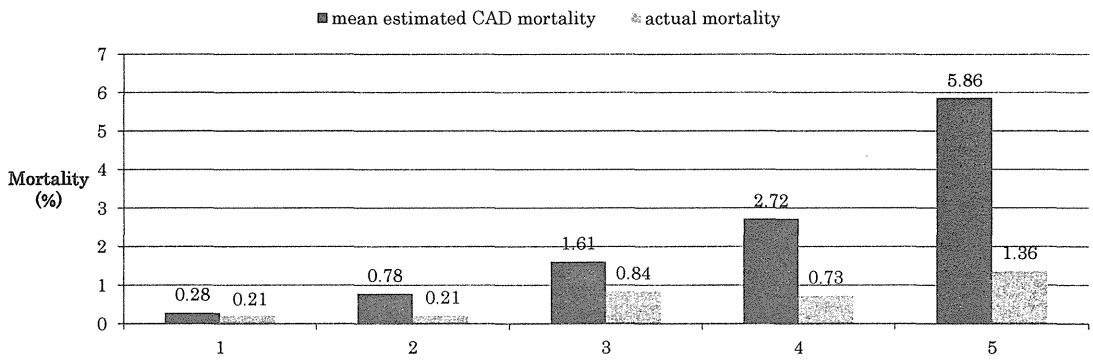
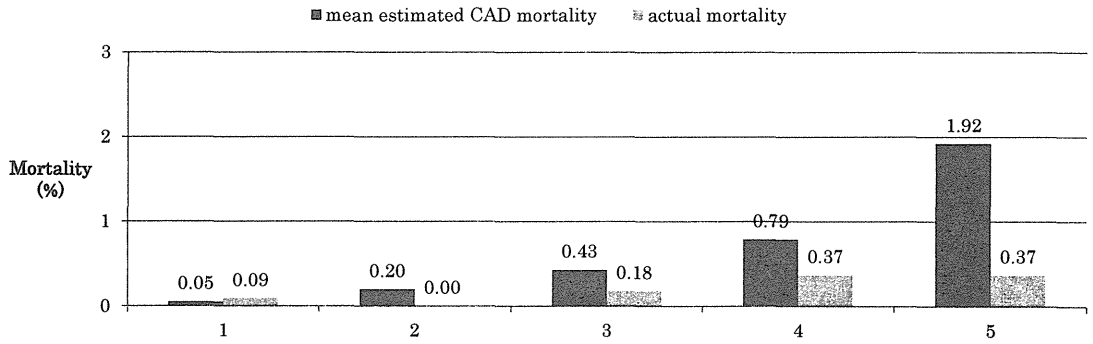


Difference between two mortality [2]	0.07	0.57	0.77	1.99	4.50
Number of participants	954	955	954	955	955
Number of deaths	2	2	8	7	13

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 1A
Analysis between quintile of mean estimated CAD mortality of men in NIPPON DATA80 and actual mortality of men in Osaki Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=57.06, d.f.=3, $P<0.001$)

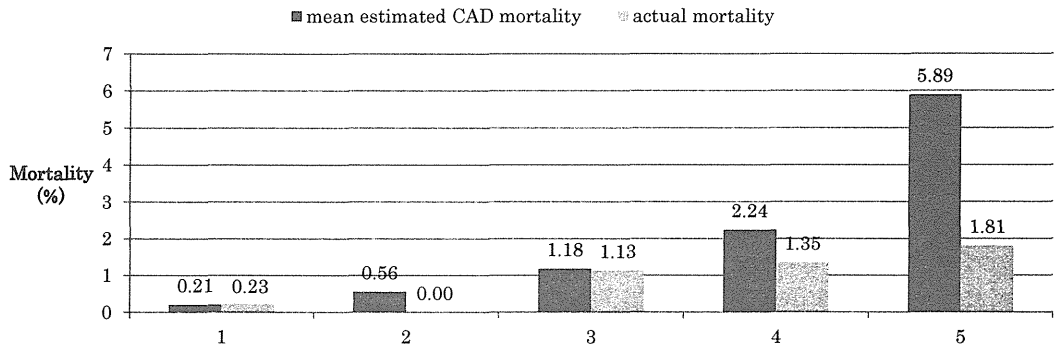


Difference between two mortality [2]	0.04	0.20	0.25	0.42	1.55
Number of participants	1081	1081	1083	1082	1082
Number of deaths	1	0	2	4	4

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 1B
Analysis between quintile of mean estimated CAD mortality of women in NIPPON DATA80 and actual mortality of women in Osaki Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=20.33, d.f.=3, $P<0.001$)

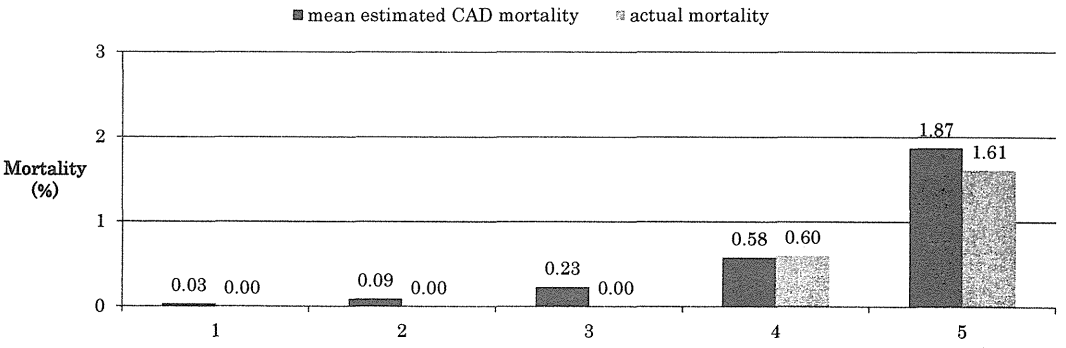


Difference between two mortality [2]	0.02	0.56	0.05	0.89	4.08
Number of participants	442	443	443	443	443
Number of deaths	1	0	5	6	8

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 2A
Analysis between quintile of mean estimated CAD mortality of men in NIPPON DATA80 and actual mortality of men in Suita Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=17.43, d.f.=3, $P<0.001$)

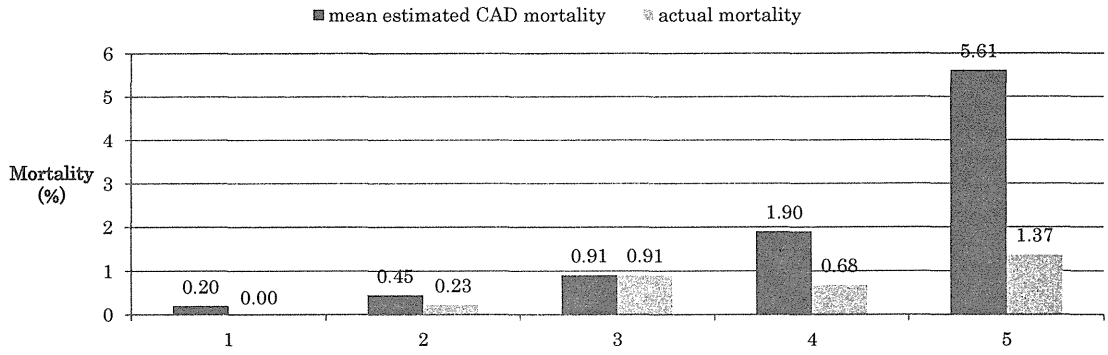


Difference between two mortality [2]	0.03	0.09	0.23	0.02	0.26
Number of participants	495	495	497	496	496
Number of deaths	0	0	0	3	8

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 2B
Analysis between quintile of mean estimated CAD mortality of women in NIPPON DATA80 and actual mortality of women in Suita Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=1.92, d.f.=3, $P=0.59$)

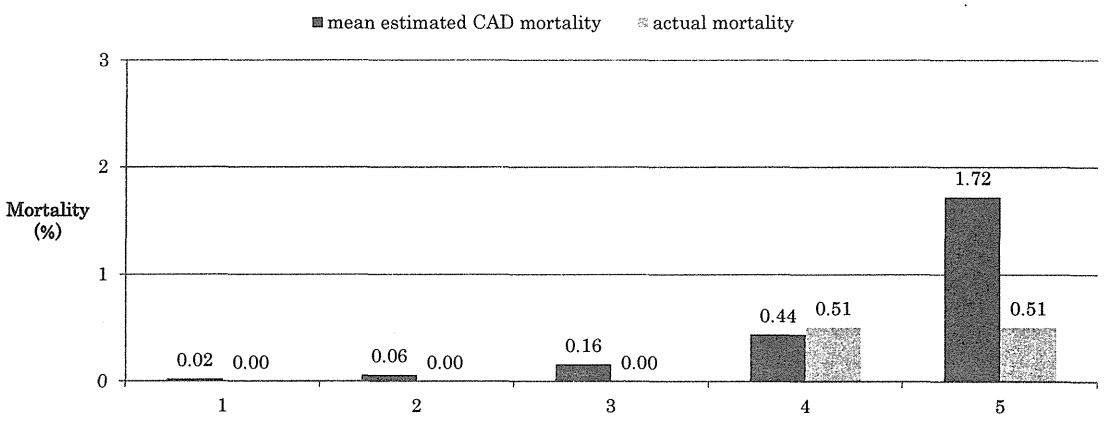


Difference between two mortality [2]	0.20	0.22	0.00	1.22	4.24
Number of participants	437	438	438	438	438
Number of deaths	0	1	4	3	6

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 3A
Analysis between quintile of mean estimated CAD mortality of men in NIPPON DATA80 and actual mortality of men in NIPPON DATA90.[1] Hosmer–Lemeshow test (χ^2 statistic=19.7, d.f.=3, $P<0.001$)

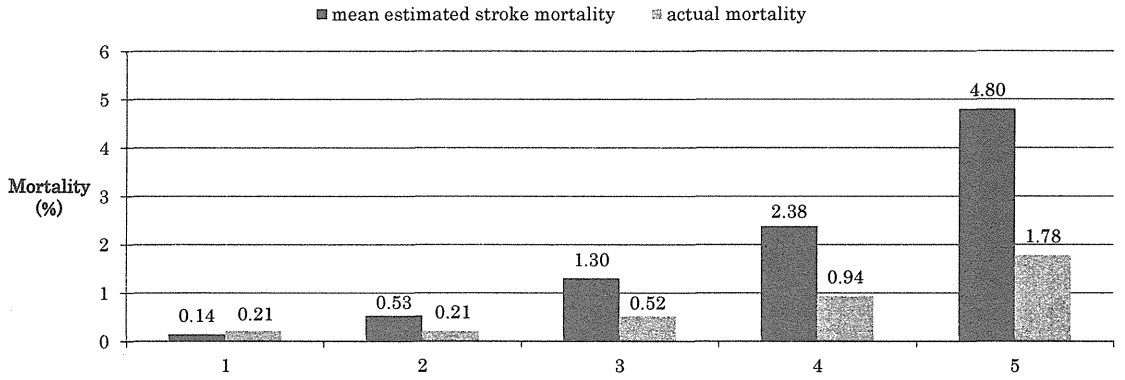


Difference between two mortality [2]	0.02	0.06	0.16	0.07	1.21
Number of participants	587	588	588	588	588
Number of deaths	0	0	0	3	3

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated CAD mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 3B
Analysis between quintile of mean estimated CAD mortality of women in NIPPON DATA80 and actual mortality of women in NIPPON DATA90.[1] Hosmer–Lemeshow test (χ^2 statistic=6.57, d.f.=3, $P=0.09$)

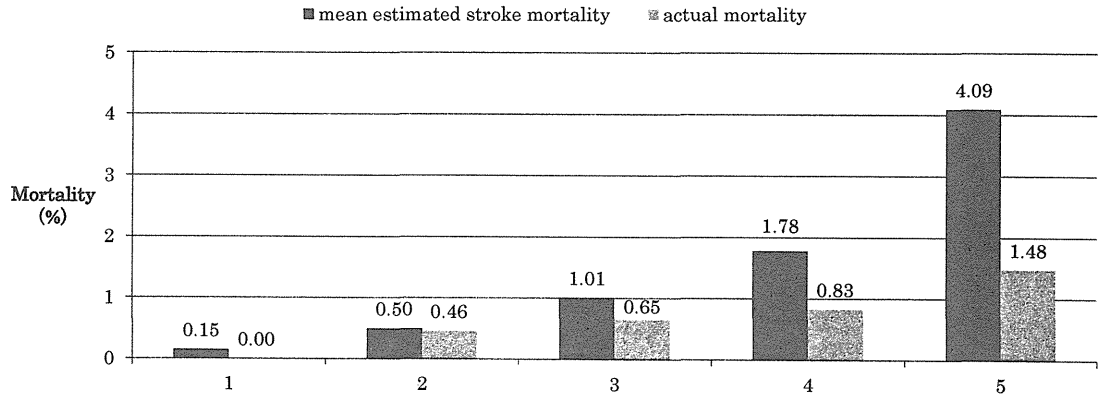


Difference between two mortality [2]	0.07	0.32	0.78	1.44	3.02
Number of participants	954	955	954	955	955
Number of deaths	2	2	5	9	17

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated stroke mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 4A
Analysis between quintile of mean estimated stroke mortality of men in NIPPON DATA80 and actual mortality of men in Osaki Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=34.2, d.f.=3, $P<0.001$)

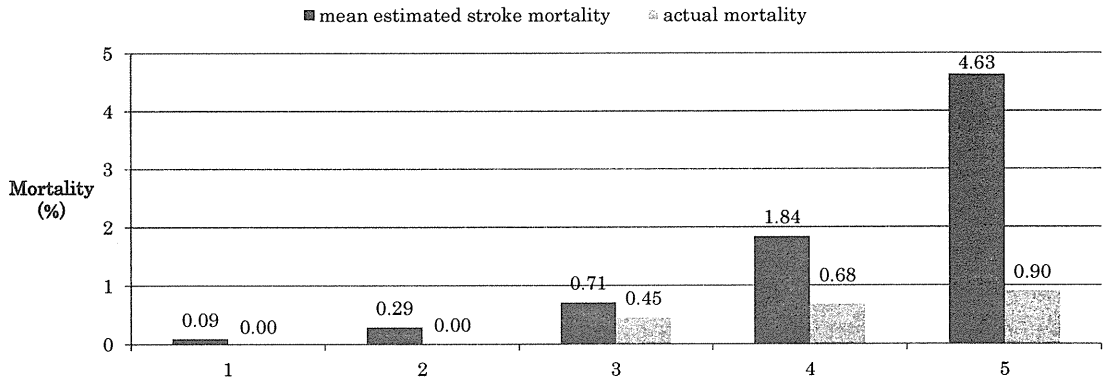


Difference between two mortality [2]	0.15	0.04	0.36	0.95	2.61
Number of participants	1081	1082	1082	1082	1082
Number of deaths	0	5	7	9	16

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated stroke mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 4B
Analysis between quintile of mean estimated stroke mortality of women in NIPPON DATA80 and actual mortality of women in Osaki Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=27.5, d.f.=3, $P<0.001$)

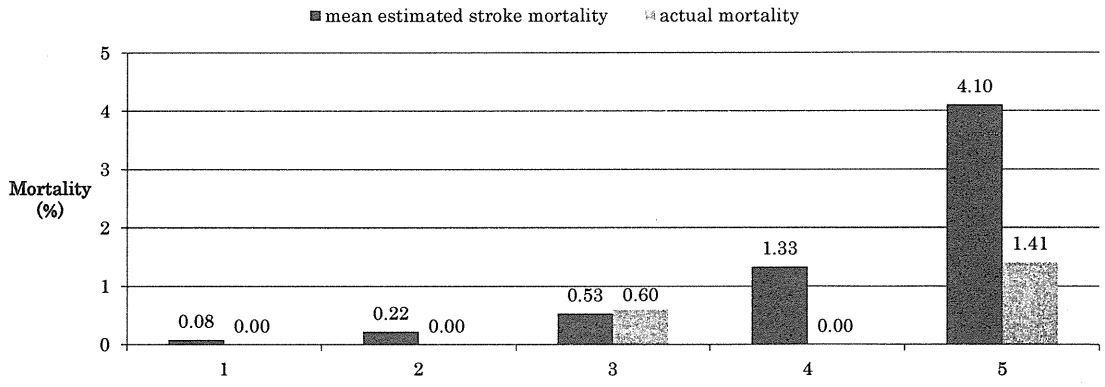


Difference between two mortality [2]	0.09	0.29	0.26	1.16	3.73
Number of participants	442	443	443	443	443
Number of deaths	0	0	2	3	4

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated stroke mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 5A
Analysis between quintile of mean estimated stroke mortality of men in NIPPON DATA80 and actual mortality of men in Suita Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=19.4, d.f.=3, $P<0.001$)

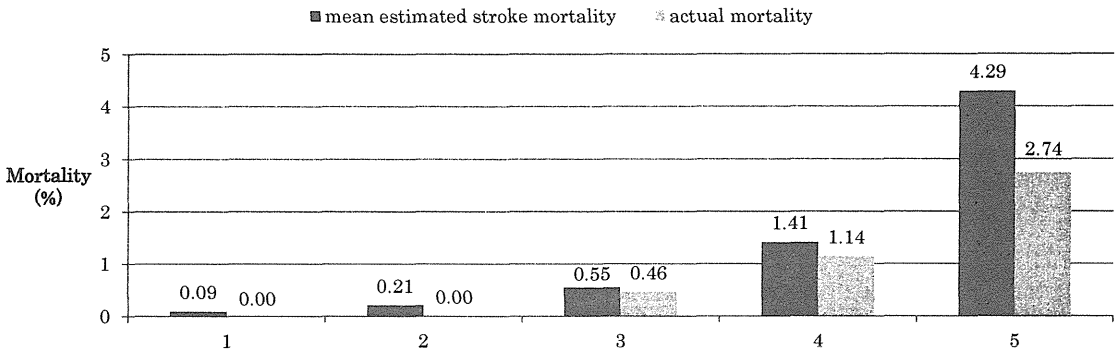


Difference between two mortality [2]	0.08	0.22	0.07	1.33	2.69
Number of participants	495	496	496	496	496
Number of deaths	0	0	3	0	7

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated stroke mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 5B
Analysis between quintile of mean estimated mortality of women in NIPPON DATA80 and actual mortality of women in Suita Cohort.[1] Hosmer–Lemeshow test (χ^2 statistic=17.4 d.f.=3, $P<0.001$)

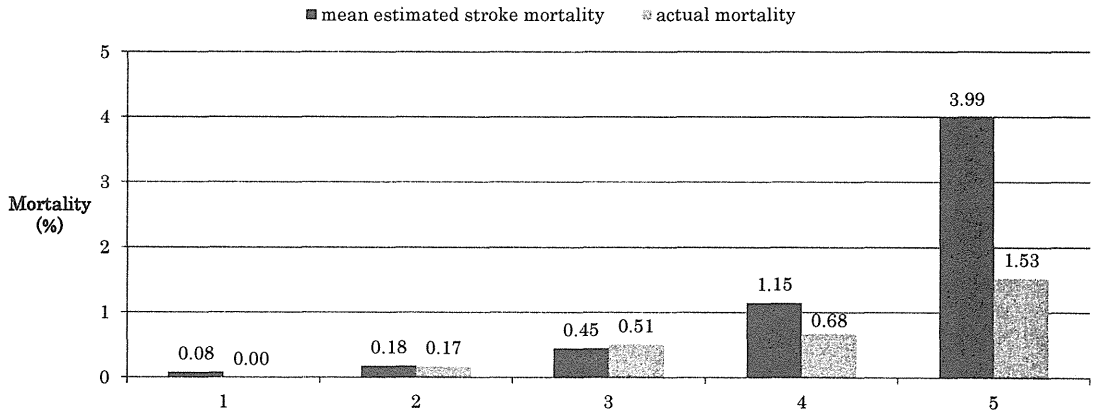


Difference between two mortality [2]	0.09	0.21	0.09	0.27	1.55
Number of participants	437	438	438	438	438
Number of deaths	0	0	2	5	12

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated stroke mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 6A
Analysis between quintile of estimated mean stroke mortality of men in NIPPON DATA80 and actual mortality of men in NIPPON DATA90.[1] Hosmer–Lemeshow test (χ^2 statistic=4.18, d.f.=3, P=0.24)



Difference between two mortality [2]	0.08	0.01	0.06	0.47	2.46
Number of participants	587	588	588	588	588
Number of deaths	0	1	3	4	9

1) Estimated from NIPPON DATA80 risk chart

2) Calculated the difference between the mean estimated stroke mortality and the actual cumulative mortality in each quintile

Supplemental Fig. 6B
Analysis between quintile of mean estimated stroke mortality of women in NIPPON DATA80 and actual mortality of women in NIPPON DATA90.[1] Hosmer–Lemeshow test (χ^2 statistic=11.0, d.f.=3, P=0.01)

Supplemental Table 1. Baseline characteristics of EPOCH-JAPAN by cohorts including the 12 cohorts with the cause of death

Cohort Name	N	Men, N (%)	Age, years (SD)	Systolic blood pressure, mmHg (SD)	Total cholesterol, mg/dL (SD)	Blood glucose, mg/dL (SD)	Current Smoker (%)
Tanno-Sobetsu	2489	1097 (44.1)	47 (11)	132 (20)	189 (37)	93 (17)	36.3
Ohsaki	16238	6907 (42.5)	62 (9)	131 (18)	204 (35)	107 (30)	25.7
Ohasama	3174	1269 (40.0)	58 (13)	131 (17)	196 (37)	117 (46)	21.0
Oyabe	5197	1624 (31.3)	57 (11)	127 (20)	194 (36)	.	19.3
YKK workers[1]	7039	4380 (62.2)	38 (10)	119 (15)	190 (35)	93 (13)	38.9
Suita	6448	3092 (48.0)	55 (13)	128 (22)	207 (37)	99 (19)	31.2
RERF cohort[2]	4670	1521 (32.6)	62 (12)	135 (23)	210 (40)	105 (33)	23.5
Hisayama	2736	1162 (42.5)	60 (12)	134 (22)	206 (42)	106 (24)	25.0
JACC[3]	30265	11044 (36.5)	57 (10)	133 (19)	198 (37)	.	21.9
NIPPON DATA80[4]	9442	4157 (44.0)	51 (13)	136 (21)	189 (34)	130 (36)	32.6
NIPPON DATA90	8099	3405 (42.0)	53 (14)	135 (21)	203 (38)	103 (32)	28.5
Osaka	6180	2228 (36.1)	55 (13)	134 (21)	210 (37)	100 (27)	24.2
Total	101977	41886 (41.1)	56 (13)	132 (20)	200 (37)	107 (31)	26.3

1) YKK: Yoshida Kogyo Kabushikigaisya
2) RERF: Radiation Effects Research Foundation
3) JACC: Japan Collaborative Cohort
4) NIPPON DATA: National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged

Supplemental Table 2. Baseline characteristics of EPOCH-JAPAN by cohorts in the present study

Cohort Name	N	Men, N (%)	Age, years (SD)	Systolic blood pressure, mmHg (SD)	Total cholesterol, mg/dL (SD)	Blood glucose, mg/dL (SD)	Current Smoker (%)
Tanno-Sobetsu	1606	743 (46.3)	50 (7)	132 (20)	190 (38)	93 (18)	36.2
Ohsaki	10182	4773 (46.9)	60 (9)	131 (17)	204 (35)	106 (29)	26.1
Ohasama	729	245 (33.6)	58 (8)	132 (16)	202 (36)	117 (40)	16.7
YKK workers[1]	2798	1884 (67.3)	47 (5)	119 (16)	203 (35)	94 (14)	39.2
Suita	4693	2214 (47.2)	57 (10)	129 (22)	211 (37)	100 (20)	29.9
RERF cohort[2]	3402	1082 (31.8)	58 (9)	132 (21)	212 (39)	101 (27)	25.3
Hisayama	2305	990 (43.0)	56 (9)	131 (20)	207 (42)	105 (24)	25.5
NIPPON DATA90[3]	5128	2189 (42.7)	55 (10)	138 (20)	207 (38)	104 (34)	28.3
Osaka	2837	971 (34.2)	55 (8.9)	127 (16)	212 (37)	99 (26)	25.0
Total	33680	15091 (44.8)	56 (10)	131 (19)	206 (37)	102 (27)	28.1

1) YKK: Yoshida Kogyo Kabushikigaisya
2) RERF: Radiation Effects Research Foundation
3) NIPPON DATA: National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged

Advance Publication
Journal of Atherosclerosis and Thrombosis, Vol. 23, No. 8 Journal of Atherosclerosis and Thrombosis 1
Original Article
Accepted for publication: December 23, 2015
Published online: February 12, 2016

Serum γ-glutamyltransferase and Mortality due to Cardiovascular Disease in Japanese Men and Women

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Aim: Whether the association between serum γ-glutamyltransferase (γ-GTP) levels and total cardiovascular disease (CVD) mortality is independent of alcohol drinking in East Asian populations is not well known. We conducted a pooled analysis of Japanese men and women that enabled an analysis restricted to never-drinkers.

Methods: A total of 15,987 men and 25,053 women aged 40–79 years, pooled from seven cohort studies throughout Japan, were followed-up to examine sex-specific relationship between serum γ-GTP levels and total CVD mortality. Cox regression model was used that was adjusted for age, smoking status, body mass index, and systolic blood pressure and serum triglyceride, total cholesterol, aspartate aminotransferase, and alanine aminotransferase levels.

Results: During an average follow-up of 8.7 years, we documented 361 and 340 deaths from total CVD, 146 and 168 from stroke, and 101 and 53 from coronary heart disease (CHD) for men and women, respectively. Among the never-drinkers, hazard ratios (HRs) for mortality for one standard deviation of log-γ-GTP for men were 1.89 (1.00–3.58) for stroke, 1.04 (0.57–1.90) for CHD, and 1.43 (1.04–1.96) for total CVD. For women, HRs were 1.28 (1.06–1.54), 1.81 (1.34–2.44), and 1.30 (1.14–1.49), respectively.

Conclusion: γ-GTP may be a risk factor for total CVD mortality independent of alcohol drinking status in Japanese men and women.

J Atheroscler Thromb, 2016; 23: 000-000.

Key words: γ-glutamyltransferase, Cardiovascular disease, Stroke, Coronary heart disease, Epidemiology

Introduction

Serum γ-glutamyltransferase (γ-GTP) is commonly used as a diagnostic indicator for liver dysfunction, which is often a consequence of long-term alcohol drinking[1]. It has also been considered as a potential marker for oxidative stress and inflammation[2,3]. A previous meta-analysis showed that serum γ-GTP levels were positively associated with cardiovascular disease (CVD) risks in both men and women even within the normal range[4]. However, the meta-analysis reported the existence of significant heterogeneity because of Asian studies. So far, only three Asian studies have examined the associations between γ-GTP levels and CVD, including two Japanese studies that

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Received: August 21, 2015
Accepted for publication: December 23, 2015

Advance Publication
Journal of Atherosclerosis and Thrombosis
Accepted for publication: December 23, 2015
Published online: February 12, 2016

found no association in men[5, 6] and one Korean study that found an inverse association in the sample combining men and women[7]. Asians differ from Caucasians in terms of alcohol drinking habits and innate alcohol metabolism, i.e., aldehyde dehydrogenase polymorphism. Furthermore, stroke incidence is higher than coronary heart disease (CHD) in Asians. In addition, the meta-analysis found statistically significant association in nondrinkers only for CHD but not for stroke. Therefore, further studies were warranted in Asian population and for stroke in relation to γ-GTP levels. In the present study, we utilized the merit of large-scale pooled database of major cohort studies in Japan to analyze the association of serum γ-GTP levels with mortalities from CHD and stroke as well as the association in never-drinking men and women.

Methods

Study Participants

The Evidence for Cardiovascular Prevention from Observational Cohorts in Japan is a pooled project incorporating a meta-analysis of individual participant data from 13 well-qualified Japanese cohorts. The project was designed to examine the relationship between health examination measures (laboratory measures and lifestyle factors) and cause-specific mortality in Japanese populations. Each cohort was followed-up for approximately 10 years and included 1,000 or more participants. The details of this project have been described previously[8-14]. Serum γ-GTP levels at baseline were available in seven cohorts (N=54,467): the Ohsaki cohort, the Ohasama study, the YKK factory workers cohort, the Radiation Effects Research Foundation cohort, the Hisayama study, the JACC study, and NIPPON DATA 90. We excluded those with histories of CVD at baseline (N=5,160), those who were <40 years or >80 years of age (N=7,322), and individuals who had high (>50 IU/L) aspartate aminotransferase (AST) levels (N=1,349) or alanine aminotransferase (ALT) levels (N=2,358) in an attempt to exclude possible confounding of apparent liver disease. These exclusions left 41,040 participants comprising 15,987 men and 25,053 women for the present analyses.

Exposure and CVD Outcomes

Serum γ-GTP concentration was determined using a colorimetric assay; ALT and AST levels were measured using the ultraviolet method. In each cohort, mortality ascertainment was systematically conducted by reviewing death certificates. The underlying cause of death was based on either ICD-9 or ICD-10. Classification codes used in the study were as follows: death from stroke (430–438; I60–I69), CHD (410–414; I20–I25), and CVD (390–459; I00–I99). The present study was approved by the Ethical Review Committee of Keio University and Shiga University of Medical Science.

Statistical Analysis

Men and women were separately analyzed in this study. Cox proportional hazards models stratified by cohort[15] were performed to estimate hazard ratios (HRs) and their corresponding 95% confidence intervals (95% CI) for CVD outcomes according to baseline serum γ-GTP levels. In the stratified Cox model, individual participant data from all studies are pooled, whereas the model accounts for the clustering of participants within studies. Because cohorts are almost identical to the areas, except two nation-wide cohorts (JACC and NIPPON DATA 90) for which area information had not been incorporated into the present pooled dataset, we did not additionally adjust for area. There were up to 10% of missing values in all the continuous variables of total individuals except for age. Instead of excluding those with missing values from the multivariable analysis, we had included them by using dummy variables that had a value missing. We first adjusted for continuous age (age-adjusted model) and subsequently adjusted for smoking status (never, past, 1–20/day, and ≥ 21/day) and sex-specific quartiles of body mass index (BMI) (kg/m^2), systolic blood pressure (mmHg), serum triglyceride levels (mg/dL), serum total cholesterol levels (mg/dL), and AST (IU/L) and ALT (IU/L) levels (multivariable-adjusted model). The analyses were performed among never-drinkers first, then among the whole subjects adjusting for drinking status (never, quit, and regular) in the multivariable model. Statistical analyses were performed using SAS 9.2 for Windows (SAS Inc, Cary, NC, USA), and two sided p<0.05 was considered to be statistically significant.

Results

Table 1 shows the baseline characteristics according to γ-GTP quartiles. Age, proportion of regular drinker and current smoker, BMI, systolic blood pressure, serum triglycerides levels, serum total cholesterol levels, AST, and ALT were positively associated with γ-GTP quartiles, whereas age was inversely associated with γ-GTP levels in men. Regular drinkers comprised 72% of men and 29% of women.

During an average follow-up of 8.7 years, we

Accepted for publication: December 23, 2015
Published online: February 12, 2016

Table 1. Sex-specific means and proportions of cardiovascular risk factors according to quartiles of γ-GTP at baseline

	Risk factors	Quartiles of γ-GTP (IU/L)			
		Q1 (low)	Q2	Q3	Q4 (high)
Men	Quartile range (IU/L)	1-16	17-24	25-40	41-837
	No. of participants	4123	4074	3841	3949
	Age (year)	59.6 (10.8)	58.6 (10.6)	57.7 (10.3)	55.7 (9.7)
	Never drinker, *n* (%)	1512 (36.7)	992 (24.4)	641 (16.7)	285 (7.2)
	Quit drinker, *n* (%)	323 (7.8)	256 (6.3)	179 (4.7)	114 (2.9)
	Regular drinker, *n* (%)	2196 (53.3)	2729 (67.0)	2955 (76.9)	3483 (88.2)
	Never smoker, *n* (%)	1035 (25.1)	918 (22.5)	772 (20.1)	644 (16.3)
	Former smoker, *n* (%)	1038 (25.2)	1029 (25.3)	985 (25.6)	892 (22.6)
	1-20 cigarettes a day, *n* (%)	1378 (33.4)	1324 (32.5)	1183 (30.8)	1333 (33.8)
	≥ 21 cigarettes a day, *n* (%)	438 (10.6)	488 (12.0)	552 (14.4)	736 (18.6)
	Body mass index (kg/m^2)	22.0 (2.6)	22.8 (2.8)	23.4 (2.9)	23.9 (2.8)
	Systolic blood pressure (mmHg)	129.0 (18.8)	130.2 (18.5)	133.2 (18.7)	136.1 (18.5)
	Serum triglycerides (mg/dL)	102.2 (55.0)	120.2 (73.1)	137.8 (87.3)	172.6 (122.0)
	Serum total cholesterol (mg/dL)	187.5 (32.6)	194.8 (32.7)	199.0 (34.3)	201.5 (37.7)
	Aspartate aminotransferase (IU/L)	21.5 (5.9)	22.6 (6.0)	24.0 (6.3)	26.9 (7.2)
	Alanine aminotransferase (IU/L)	17.2 (7.2)	19.6 (7.8)	22.5 (9.0)	26.3 (9.5)
Women	Quartile range (IU/L)	1-9	10-13	14-18	19-435
	No. of participants	5466	7785	5447	6355
	Age (year)	55.4 (10.4)	57.3 (10.0)	58.8 (9.6)	58.9 (9.1)
	Never drinker, *n* (%)	4462 (81.6)	5799 (74.5)	3904 (71.7)	4262 (67.1)
	Quit drinker, *n* (%)	42 (0.8)	107 (0.4)	74 (0.3)	107 (0.4)
	Regular drinker, *n* (%)	1004 (18.4)	1986 (25.5)	1543 (23.3)	2093 (32.9)
	Never smoker, *n* (%)	4861 (88.9)	6196 (79.6)	4198 (77.1)	4714 (74.2)
	Former smoker, *n* (%)	53 (1.0)	104 (1.3)	76 (1.4)	94 (1.5)
	1-20 cigarettes a day, *n* (%)	139 (2.5)	236 (3.0)	187 (3.4)	327 (5.2)
	≥ 21 cigarettes a day, *n* (%)	9 (0.2)	15 (0.2)	17 (0.3)	42 (0.7)
	Body mass index (kg/m^2)	22.4 (2.8)	23.0 (3.0)	23.7 (3.3)	24.4 (3.4)
	Systolic blood pressure (mmHg)	127.6 (19.1)	128.1 (18.8)	131.2 (19.7)	133.0 (19.4)
	Serum triglycerides (mg/dL)	95.6 (51.8)	106.7 (60.5)	123.9 (72.6)	141.2 (87.2)
	Serum total cholesterol (mg/dL)	199.1 (34.9)	207.2 (35.2)	214.4 (35.4)	218.5 (37.3)
	Aspartate aminotransferase (IU/L)	18.9 (5.1)	20.2 (5.2)	21.4 (5.5)	23.7 (6.8)
	Alanine aminotransferase (IU/L)	13.9 (5.7)	15.1 (6.0)	17.3 (6.9)	21.8 (8.9)

documented 361 and 340 deaths from total CVD, 146 and 168 from stroke, and 101 and 53 from CHD for men and women respectively. Among never-drinkers, the respective numbers were 82 and 252 from total CVD, 25 and 126 from stroke, and 31 and 38 from CHD, respectively. The one SD of log-γ-GTP was 0.70 for men and 0.56 for women. Serum γ-GTP level was seemingly positively associated with stroke mortality in never-drinking men. The multivariable HR (95% CI) for one SD increase of log-γ-GTP was 1.89 (1.00–3.58) (**Table 2**). Although the significant HRs were observed in Q2 and Q3 but not in Q4 compared with Q1 of γ-GTP quartile for total CVD mortality, the HR for one SD increase of log-γ-GTP was statistically significant (multivariable HR: 1.17, 95% CI: 1.03–1.33). However, it was not related to CHD mortality (multivariable HR: 1.04, 95% CI: 0.57–1.90) in never-drinking men. In never-drinking women, one SD increase of log-γ-GTP was significantly positively associated with mortalities from stroke, CHD, and total CVD. Furthermore, never-drinking women in the quartile Q4 had more than four-time higher CHD mortality risk compared with Q1 (multivariable HR: 4.49, 95% CI: 1.41–14.32).

The associations were essentially similar in total participants in both men and women (**Table 3**).

Advance Publication
Journal of Atherosclerosis and Thrombosis
Accepted for publication: December 23, 2015
Published online: February 12, 2016

Table 2. Sex-specific, age- and multivariable-adjusted hazard ratios and 95% confidence intervals for mortality from cardiovascular disease according to quartiles of γ-GTP and one SD increment of log γ-GTP in never-drinkers

	Quartiles of γ-GTP				HR1[†]
	Q1 (low)	Q2	Q3	Q4 (high)	
Men					
Quartile range (IU/L)	1-16	17-24	25-40	41-837	
No. at risk	1,512	992	641	285	
Person-years	13,371	8,599	5,575	2,543	
Stroke					
No. of mortality	11	8	4	2	
Mortality rate	0.82	0.93	0.72	0.79	
Age adjusted HR	1.00	1.50 (0.59-3.83)	1.35 (0.41-4.52)	2.14 (0.44-10.28)	1.46 (0.86-2.46)
Multivariable HR[§]	1.00	1.59 (0.56-4.50)	1.56 (0.41-5.98)	4.14 (0.72-23.91)	1.89 (1.00-3.58)
Coronary heart disease					
No. of mortality	9	12	8	2	
Mortality rate	0.67	1.40	1.44	0.79	
Age adjusted HR	1.00	2.19 (0.91-5.29)	2.49 (0.92-6.72)	1.67 (0.35-8.00)	1.69 (1.31-2.19)
Multivariable HR[§]	1.00	2.02 (0.79-5.13)	2.10 (0.70-6.27)	1.69 (0.32-9.02)	1.04 (0.57-1.90)
Total cardiovascular diseases					
No. of mortality	30	30	21	5	
Mortality rate	2.24	3.49	3.77	1.97	
Age adjusted HR	1.00	1.74 (1.04-2.93)	2.20 (1.22-3.95)	1.44 (0.55-3.80)	1.33 (1.00-1.77)
Multivariable HR[§]	1.00	1.90 (1.09-3.30)	2.41 (1.27-4.57)	1.78 (0.64-4.96)	1.43 (1.04-1.96)
Women					
Quartile range (IU/L)	1-9	10-13	14-18	19-435	
No. at risk	4,462	5,799	3,904	4,262	
Person-years	40,944	52,008	34,286	37,580	
Stroke					
No. of mortality	23	35	25	43	
Mortality rate	0.56	0.67	0.73	1.14	
Age adjusted HR	1.00	1.06 (0.61-1.81)	0.99 (0.55-1.78)	1.64 (0.96-2.79)	1.32 (1.11-1.55)
Multivariable HR[§]	1.00	1.17 (0.67-2.06)	1.09 (0.58-2.02)	1.60 (0.87-2.92)	1.28 (1.06-1.54)
Coronary heart disease					
No. of mortality	5	8	9	16	
Mortality rate	0.12	0.15	0.26	0.43	
Age adjusted HR	1.00	1.52 (0.49-4.71)	2.46 (0.80-7.56)	4.26 (1.50-12.07)	1.43 (0.89-2.29)
Multivariable HR[§]	1.00	1.53 (0.47-4.98)	2.51 (0.76-8.25)	4.49 (1.41-14.32)	1.81 (1.34-2.44)
Total cardiovascular diseases					
No. of mortality	47	68	53	84	
Mortality rate	1.15	1.31	1.55	2.24	
Age adjusted HR	1.00	1.04 (0.71-1.52)	1.07 (0.71-1.62)	1.66 (1.13-2.42)	1.28 (1.14-1.45)
Multivariable HR[§]	1.00	1.11 (0.75-1.66)	1.16 (0.75-1.79)	1.77 (1.15-2.71)	1.30 (1.14-1.49)

Mortality rate is expressed as /1000 person-years.

[†]HR1: HR for 1 SD of log γ-GTP.

[§]Multivariable HR: adjusted for age (continuous), smoking status (never, former, 1-20/day and ≥ 21/day), body mass index (sex-specific quartile), systolic blood pressure (sex-specific quartiles), serum triglycerides levels (sex-specific quartiles), serum total cholesterol levels (sex-specific quartiles), aspartate aminotransferase (sex-specific quartiles) and alanine aminotransferase (sex-specific quartiles).

Advance Publication
Journal of Atherosclerosis and Thrombosis
Accepted for publication: December 23, 2015
Published online: February 12, 2016

Table 3. Sex-specific, age- and multivariable-adjusted hazard ratios and 95% confidence intervals for mortality from cardiovascular disease according to quartiles of γ-GTP and one SD increment of log γ-GTP in total participants

	Quartiles of γ-GTP				HR1[†]
	Q1 (low)	Q2	Q3	Q4 (high)	
Men					
Quartile range (IU/L)	1-16	17-24	25-40	41-837	
No. at risk	4,123	4,074	3,841	3,949	
Person-years	35,697	34,752	32,487	33,606	
Stroke					
No. of mortality	39	40	31	36	
Mortality rate	1.09	1.15	0.95	1.07	
Age adjusted HR	1.00	1.25 (0.80-1.96)	1.24 (0.76-2.00)	1.69 (1.06-2.71)	1.31 (1.11-1.54)
Multivariable HR[§]	1.00	1.23 (0.77-1.94)	1.23 (0.74-2.06)	1.76 (1.02-3.03)	1.39 (1.14-1.68)
Coronary heart disease					
No. of mortality	28	29	24	20	
Mortality rate	0.78	0.83	0.74	0.60	
Age adjusted HR	1.00	1.19 (0.70-2.02)	1.15 (0.66-2.00)	1.08 (0.60-1.96)	1.01 (0.82-1.25)
Multivariable HR[§]	1.00	1.25 (0.72-2.15)	1.15 (0.63-2.11)	1.03 (0.52-2.06)	0.98 (0.76-1.26)
Total cardiovascular diseases					
No. of mortality	97	105	85	74	
Mortality rate	2.72	3.02	2.62	2.20	
Age adjusted HR	1.00	1.27 (0.96-1.67)	1.27 (0.95-1.71)	1.31 (0.96-1.79)	1.14 (1.02-1.27)
Multivariable HR[§]	1.00	1.32 (0.99-1.76)	1.33 (0.96-1.82)	1.39 (0.97-1.99)	1.17 (1.03-1.33)
Women					
Quartile range (IU/L)	1-9	10-13	14-18	19-435	
No. at risk	5,466	7,785	5,447	6,355	
Person-years	49,579	68,477	47,223	55,107	
Stroke					
No. of mortality	30	45	35	58	
Mortality rate	0.61	0.66	0.74	1.05	
Age adjusted HR	1.00	0.95 (0.59-1.52)	0.97 (0.58-1.60)	1.45 (0.91-2.32)	1.30 (1.13-1.50)
Multivariable HR[§]	1.00	1.02 (0.62-1.67)	1.03 (0.60-1.76)	1.44 (0.85-2.44)	1.28 (1.09-1.50)
Coronary heart disease					
No. of mortality	7	9	14	23	
Mortality rate	0.14	0.13	0.30	0.42	
Age adjusted HR	1.00	1.04 (0.38-2.83)	2.28 (0.90-5.82)	3.50 (1.44-8.49)	1.72 (1.39-2.12)
Multivariable HR[§]	1.00	0.97 (0.35-2.75)	2.11 (0.79-5.68)	3.33 (1.24-8.93)	1.82 (1.41-2.34)
Total cardiovascular diseases					
No. of mortality	59	93	69	119	
Mortality rate	1.19	1.36	1.46	2.16	
Age adjusted HR	1.00	0.99 (0.71-1.39)	0.97 (0.68-1.40)	1.52 (1.09-2.12)	1.29 (1.17-1.43)
Multivariable HR[§]	1.00	1.04 (0.73-1.47)	1.01 (0.69-1.48)	1.58 (1.08-2.29)	1.32 (1.17-1.47)

Mortality rate is expressed as /1000 person-years.
[†]HR1: HR for 1 SD of log γ-GTP.
[§]Multivariable HR: adjusted for age (continuous), drinking status (never, quit, and regular), smoking status (never, former, 1-20/day and ≥ 21/day), body mass index (sex-specific quartile), systolic blood pressure (sex-specific quartiles), serum triglycerides levels (sex-specific quartiles), serum total cholesterol levels (sex-specific quartiles), aspartate aminotransferase (sex-specific quartiles) and alanine aminotransferase (sex-specific quartiles).

Advance Publication
Journal of Atherosclerosis and Thrombosis
Accepted for publication: December 23, 2015
Published online: February 12, 2016

Discussion

In this study, we performed meta-analysis of individual participants data consisting of 41,040 Japanese with no history of CVD or overt liver dysfunction. We demonstrated that serum γ-GTP level was positively associated with stroke and total CVD mortality in men and women and CHD mortality in women. These associations did not alter when the analysis was restricted to never-drinker. Our finding extended previously reported positive association mainly in Caucasians[4, 16], to East Asian, and to stroke mortality in both men and women. We have confirmed that the association was independent of alcohol drinking habit as well as systolic blood pressure and other potential confounding variables. We found stronger association of γ-GTP with CHD than with stroke in women but not in men where only the association with stroke was observed. The finding for CHD is not consistent with those of several previous studies that found associations in both sexes[17] or two other studies including men independent of alcohol consumption[18, 19]. Although there is no clear explanation for this finding, one possibility could be inaccuracy of participants' self-report of drinking status, particularly in those with high γ-GTP. The fact that alcohol drinking has protective association with CHD might have distorted the current finding[20]. However, there would not have been significant stigma of reporting of alcohol consumption as almost four-fifths of the men were regular drinkers at baseline; we did not consider the chance of intentional misreporting being high.

We have examined the association separately for men and women in the present study. The present finding on CVD mortality would be consistent with 24-year follow-up British Regional Heart Study including men that revealed a positive dose–response association for CVD mortality independent of alcohol consumption[19]. We have extended this finding to stroke mortality in Japanese men and women and also to CHD mortality in Japanese women. Nevertheless, studies exist that reported inconsistent findings for stroke. A Finnish study was consistent with ours in both men and women[21]. An Austrian study and British Women's Heart and Health Study did not find association in women[4, 22]. On the contrary, one Japanese study, which was not included in the present individual participant data meta-analysis, observed positive association only in women[6]. The reason for the discrepancies is not clear, but the present finding would be more reliable as it is an individual participant data meta-analysis intended to overcome inter-study variations in the findings.

There could be a few explanations for the present findings. Serum γ-GTP level is a maker of oxidative stress and inflammation, both of which can play a role in the pathophysiology of CVD[2, 3]. Moreover, γ-GTP may trigger oxidation of low-density lipoproteins and contribute to plaque formation, maturation, and rupture[23, 24]. Another possible explanation would be the increased γ-GTP as an indicator of nonalcoholic fatty liver disease (NAFLD)[25]. It was reported that elevation of big fraction of γ-GTP was specific to NAFLD[26]. Although we have excluded individuals with high ALT (>50 IU/L) blood level at baseline[27] and further adjusted for continuous ALT in the statistical model, it remained to be elucidated whether and how much the present finding was attributed to NAFLD. Further studies are needed to explore pathophysiological mechanisms under the present findings and to evaluate the usefulness of serum γ-GTP levels for risk prediction.

A strength of the present study is that we pooled data from several Japanese cohort studies with long follow-up period (over 10 years). This enabled us to utilize a large number of never-drinking participants to eliminate the confounding of alcohol drinking status on the association between serum γ-GTP levels and CVD mortality, particularly pertinent for East-Asian populations where never-drinking men were scarce. Additionally, the large sample size of current study allows us to exclude individuals with elevated serum AST or ALT in an attempt to eliminate possible confounding of γ-GTP elevation from apparent liver diseases such as long-term medication use and hepatitis C virus infection[28], of which the prevalence is high in Japan.

Some limitations of our study merit consideration. First, the participants in most of the cohort studies volunteered to undertake the community health examination, and for that reason, their characteristics might be different to those of unwilling non-participants or the general population. This would influence the absolute effect measure (mortality rate) and might underestimate the risk. However, these differences would have little effect on relative effect measures, such as HR. Second, several potential confounding factors were unavailable in the current study such as treatment of hypertension and diabetes mellitus, high-density lipoprotein cholesterol, and physical activity; however, in the previous studies done by others, adjustments for these factors did not significantly alter the results[5, 17, 22]. Third, the HRs for the higher quartiles of γ-GTP in the total participant might be overestimated, because we did not collect the information on the alcohol consumption, which are assumed