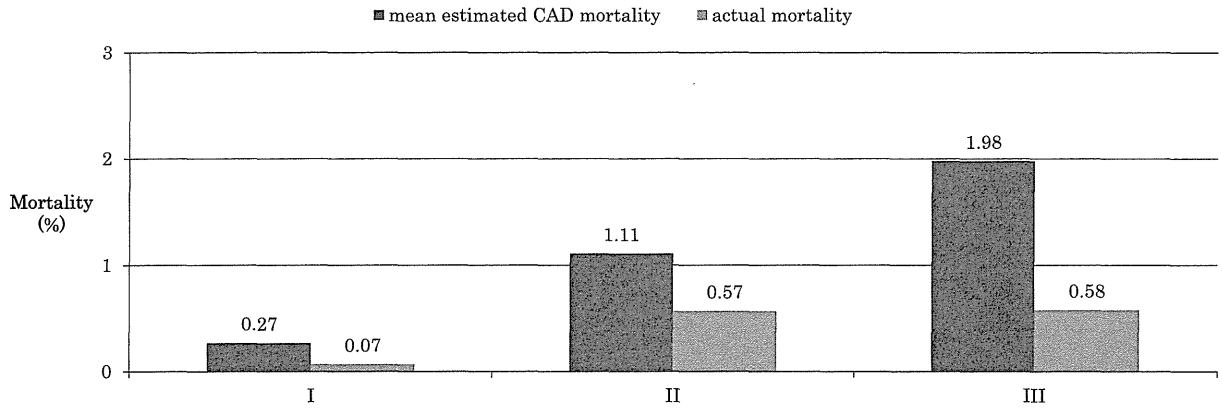
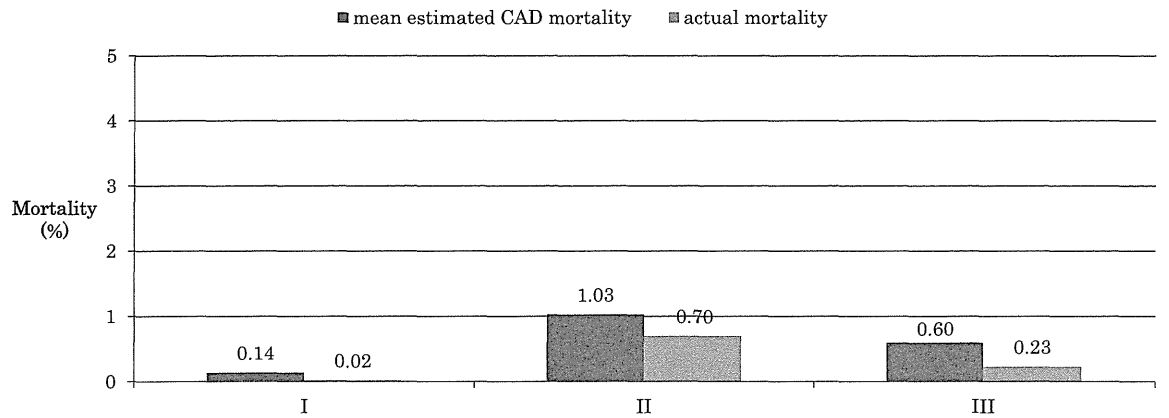


	I	II	III
Difference between two mortality [2]	0.20	0.54	1.40
Number of participants	1387	1765	11939
Number of death	1	10	69

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, $P<0.001$)

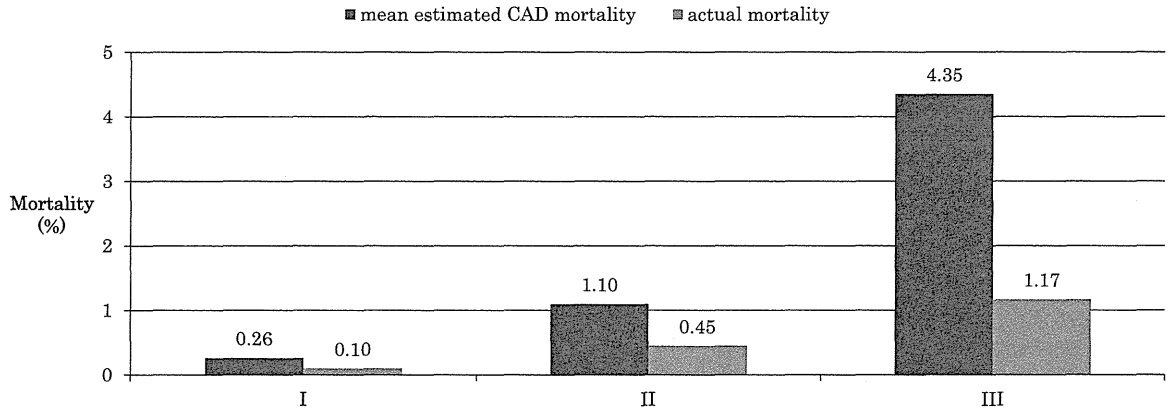


	I	II	III
Difference between two mortality [2]	0.12	0.33	0.37
Number of participant	4236	1142	13211
Number of death	1	8	31

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, $P<0.001$)

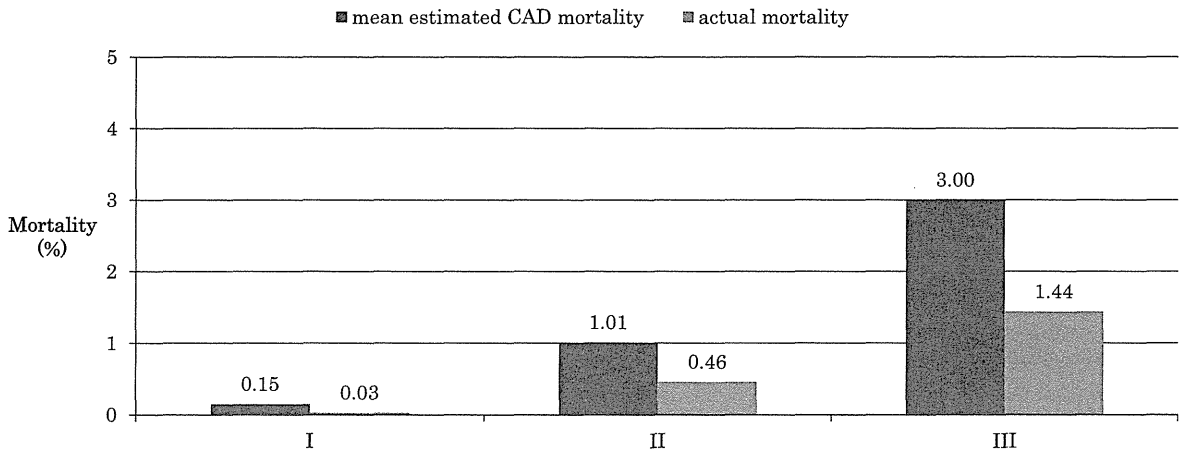


	I	II	III
Difference between two mortality [2]	0.16	0.65	3.18
Number of participants	5168	5719	4204
Number of death	5	26	49

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.

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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)

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Conflict of Interest Disclosures

None.

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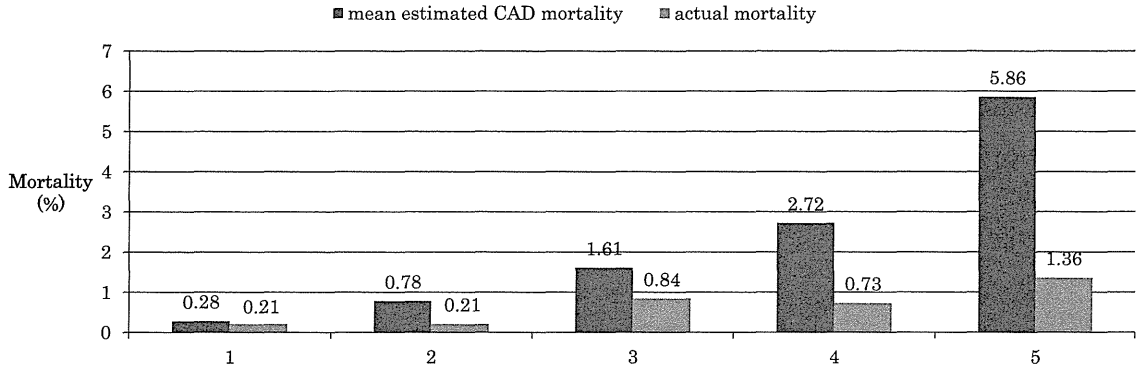
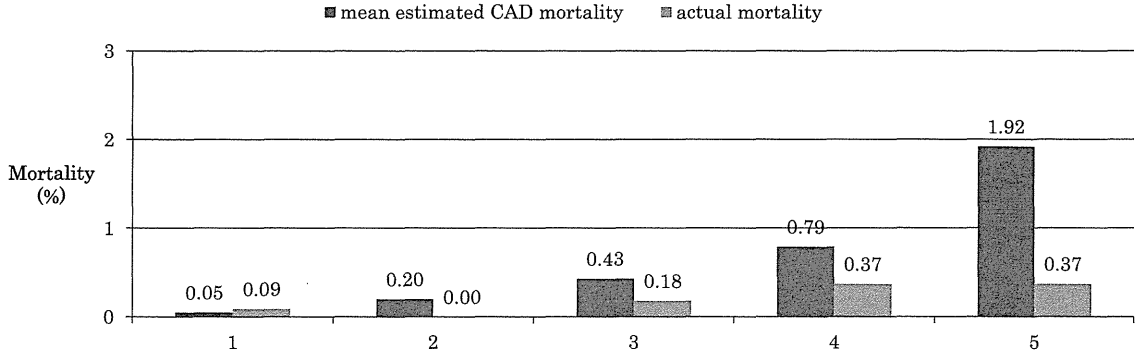


Difference between two mortality [2]	0.07	0.57	0.77	1.99	4.50
Number of participants	954	955	954	955	955
Number of deaths	2	2	8	7	13

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, $P<0.001$)

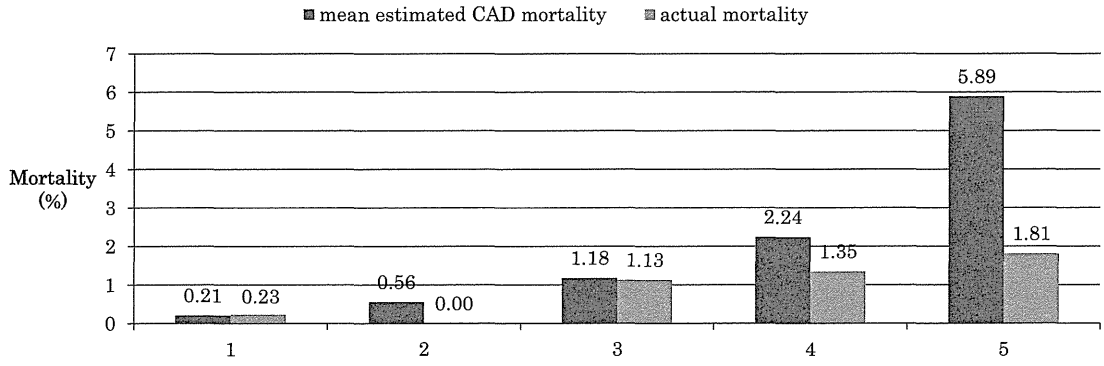


Difference between two mortality [2]	0.04	0.20	0.25	0.42	1.55
Number of participants	1081	1081	1083	1082	1082
Number of deaths	1	0	2	4	4

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, $P<0.001$)

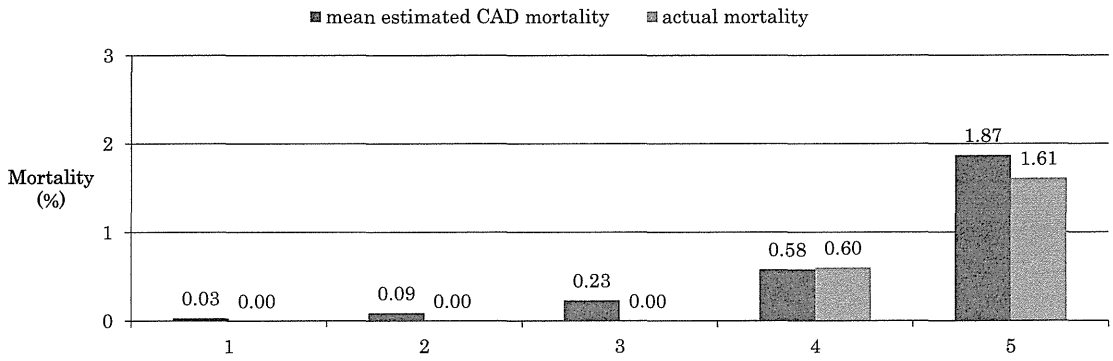


Difference between two mortality [2]	0.02	0.56	0.05	0.89	4.08
Number of participants	442	443	443	443	443
Number of deaths	1	0	5	6	8

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, $P<0.001$)

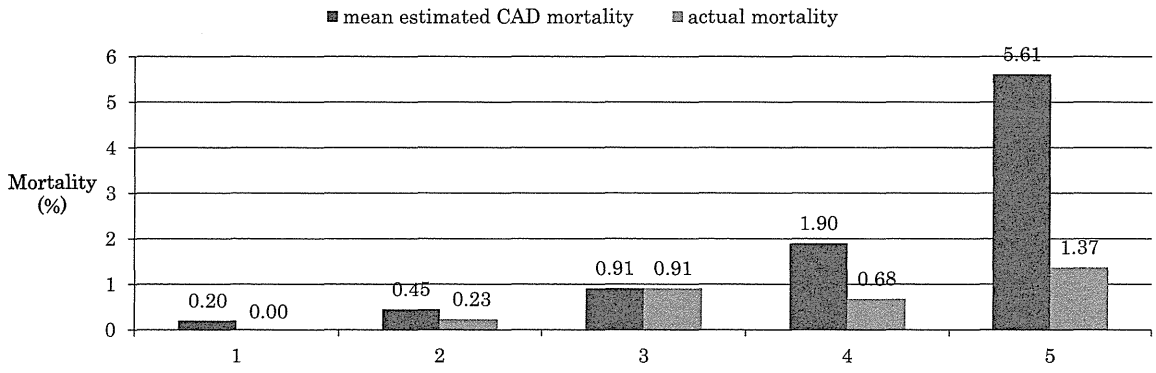


Difference between two mortality [2]	0.03	0.09	0.23	0.02	0.26
Number of participants	495	495	497	496	496
Number of deaths	0	0	0	3	8

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, $P=0.59$)

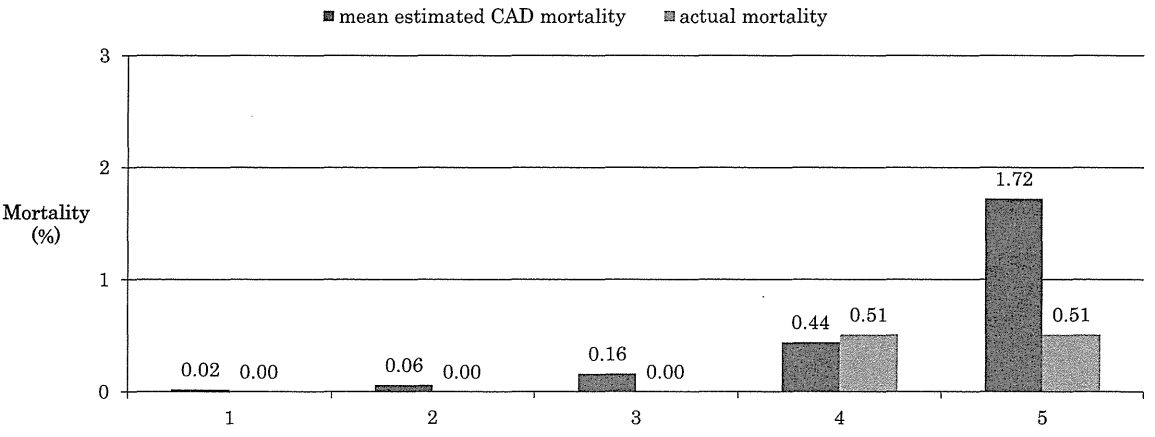


Difference between two mortality [2]	0.20	0.22	0.00	1.22	4.24
Number of participants	437	438	438	438	438
Number of deaths	0	1	4	3	6

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, $P<0.001$)

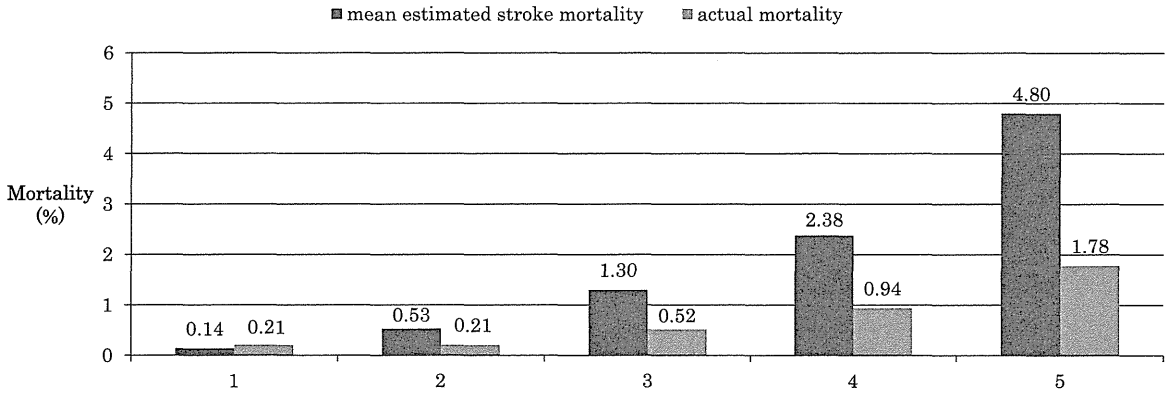


Difference between two mortality [2]	0.02	0.06	0.16	0.07	1.21
Number of participants	587	588	588	588	588
Number of deaths	0	0	0	3	3

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, $P=0.09$)

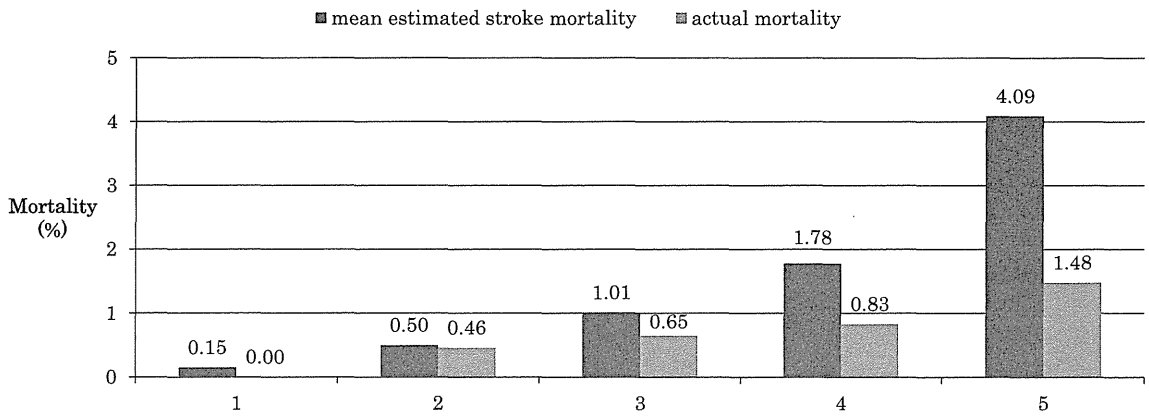


Difference between two mortality [2]	0.07	0.32	0.78	1.44	3.02
Number of participants	954	955	954	955	955
Number of deaths	2	2	5	9	17

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, $P<0.001$)

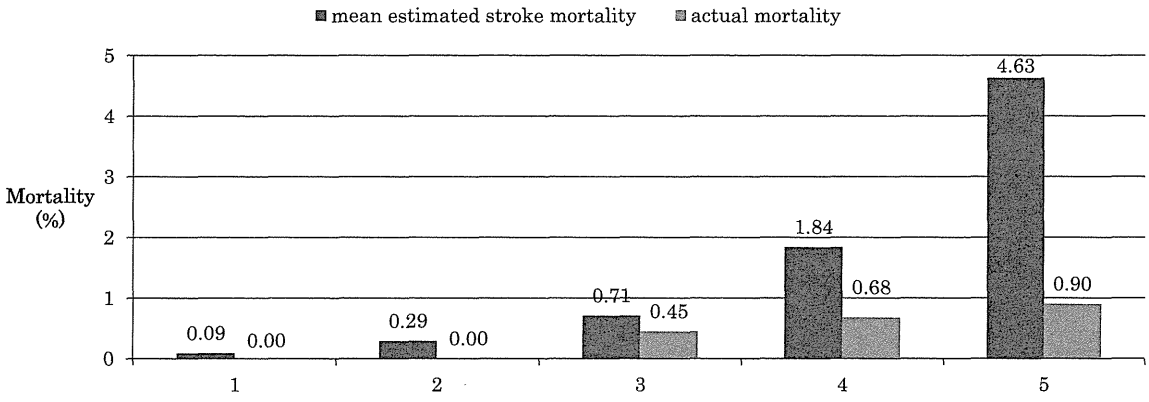


Difference between two mortality [2]	0.15	0.04	0.36	0.95	2.61
Number of participants	1081	1082	1082	1082	1082
Number of deaths	0	5	7	9	16

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, $P<0.001$)

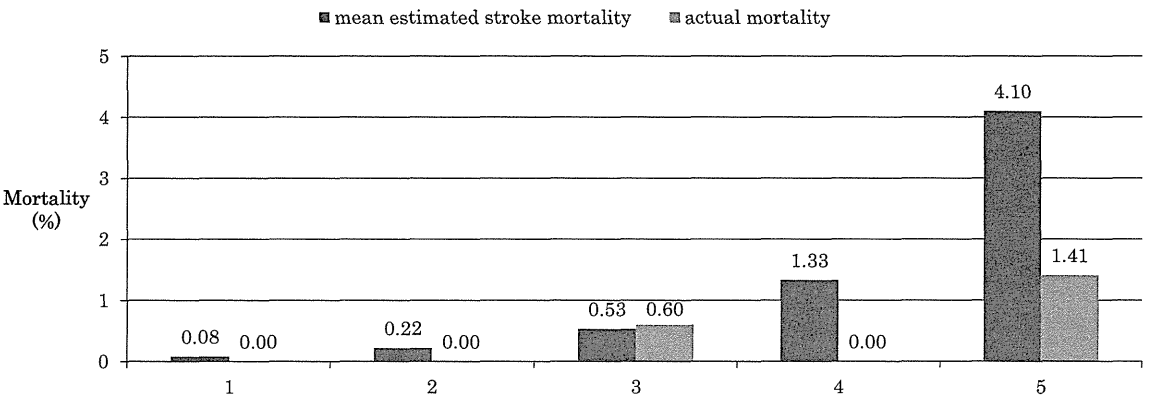


Difference between two mortality [2]	0.09	0.29	0.26	1.16	3.73
Number of participants	442	443	443	443	443
Number of deaths	0	0	2	3	4

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, $P<0.001$)

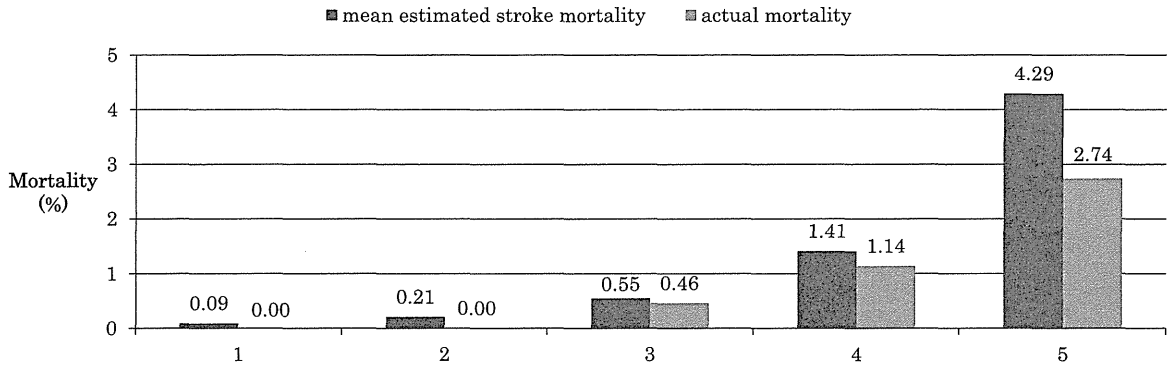


Difference between two mortality [2]	0.08	0.22	0.07	1.33	2.69
Number of participants	495	496	496	496	496
Number of deaths	0	0	3	0	7

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, $P<0.001$)

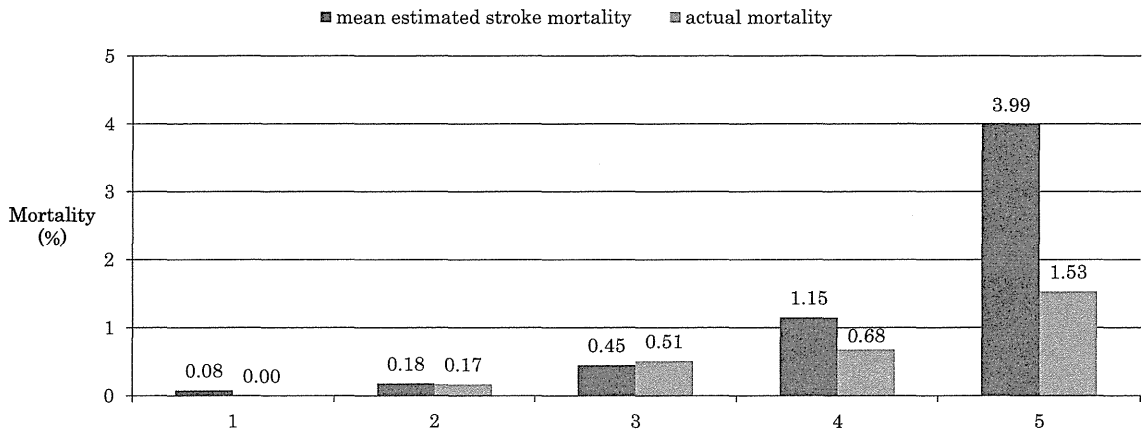


Difference between two mortality [2]	0.09	0.21	0.09	0.27	1.55
Number of participants	437	438	438	438	438
Number of deaths	0	0	2	5	12

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

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Original Article

Association Between Serum Long-Chain n-3 and n-6 Polyunsaturated Fatty Acid Profiles and Glomerular Filtration Rate Assessed by Serum Creatinine and Cystatin C Levels in Japanese Community-Dwellers

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ABSTRACT

Background: Plasma concentration of n-3 polyunsaturated fatty acids (PUFAs) has been reported to be associated with renal function in Western populations. However, few studies have investigated the association between serum long-chain n-3 and n-6 PUFA profiles and renal function in a Japanese population with high marine-derived long-chain n-3 PUFA intake.

Methods: A cross-sectional study was performed in 549 Japanese rural community-dwellers aged 40 to 64 years. In adjusted analysis of covariance, we assessed the relationship between estimated glomerular filtration rate (eGFR) and tertiles of serum long-chain n-3 and n-6 PUFA profiles ([eicosapentaenoic acid {EPA} + docosahexaenoic acid {DHA}]:arachidonic acid [AA]). GFR was estimated by Japanese specific equations using serum creatinine and cystatin C ($eGFR_{cre}$ and $eGFR_{cys}$). Using multivariate-adjusted linear regression models, we also assessed the relationships between eGFRs and several n-3 and n-6 PUFAs, which have been suggested to be associated with renal function.

Results: In all participants, higher dietary fish intake as assessed by a semi-quantitative questionnaire was associated with higher serum value of (EPA+DHA):AA. Participants in the higher (EPA+DHA):AA tertiles had non-significantly higher $eGFR_{cre}$ and significantly higher $eGFR_{cys}$ ($P = 0.016$). In addition, $eGFR_{cys}$ in T_2+T_3 of (EPA+DHA):AA was significantly higher than that in T_1 (adjusted mean $eGFR_{cys}$, T_1: 87 ml/min/1.73 m^2, T_2+T_3: 91 ml/min/1.73 m^2; $P < 0.01$). Among the PUFAs, only (EPA+DHA) was significantly associated with $eGFR_{cys}$.

Conclusions: Serum (EPA+DHA):AA, which reflects an individual's fish intake, might be associated with $eGFR_{cys}$ in Japanese community-dwellers.

Key words: epidemiology; (EPA+DHA):AA; population-based study

INTRODUCTION

N-3 polyunsaturated fatty acids (PUFAs) have been suggested to be protective against the development of renal dysfunction. According to a previous community-based study in Italy, plasma concentration of n-3 PUFAs was inversely associated with age-associated decline in estimated glomerular filtration rate (eGFR).[1] The Japanese population is unique because it has particularly high fish intake; consequently, Japanese people tend to have high serum long-chain n-3 PUFA levels,[2] which may be associated with low risk of coronary artery disease.[3,4] However, the relationship between serum long-chain n-3 PUFA levels and renal function has not been investigated in Japanese community-dwellers.

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Arachidonic acid (AA), which is classified as an n-6 PUFA, has been considered to have inflammatory and thrombotic effects because many (though not all) eicosanoids derived from AA are considered to be inflammatory, whereas EPA- and DHA-derived eicosanoids are considered to be protective against inflammation induced by AA.[5] Accordingly, previous studies in Japanese patients have investigated the relationship between cardiac events and serum n-3 PUFA:AA ratios, which are markers for balance of n-3 PUFAs and AA.[5] These studies have shown that higher EPA:AA and (EPA+DHA):AA ratios were associated with lower risk of cardiac events.[5–7] However, few community-based epidemiological studies have investigated the relationship between kidney function and long-chain n-3 PUFA:AA ratios.

The Japanese Society of Nephrology has developed 2 equations to estimate GFR, using serum creatinine (Cre) and cystatin C (Cys C) levels.[8] Serum Cys C is currently being considered as a potential replacement for Cre as a filtration marker because it is not affected by dietary intake and muscle mass.[9,10]

To investigate the relationships between eGFR and serum long-chain n-3 and n-6 PUFA profiles in community-dwellers, we performed a cross-sectional study in 549 Japanese men and women aged 40–64 years. GFR was estimated by 2 equations for the Japanese population, using serum Cre and Cys C.

METHODS

Study participants

The data from the baseline survey of the Sasayama study were analyzed. The Sasayama study is a population-based cohort study in which the endpoints are increased medical expenditures, worsening of quality of life, or cerebral and cardiovascular disease (CVD) risk factors, such as hypertension, diabetes mellitus, and dyslipidemia.

The study participants consisted of Japanese national health insurance (NHI) beneficiaries living in Sasayama City in Western Japan's Hyogo Prefecture who had undergone a medical examination between May 2012 and February 2013. The NHI system is one of the insurance systems in Japan, which is for non-employees, such as self-employed individuals, farmers, fishermen, and their dependents. During this time period, a total of 1131 NHI beneficiaries aged 40–64 years underwent a medical examination, and 675 individuals agreed to participate in the study. Written informed consent was obtained from each participant. Of these 675 participants, 126 were excluded due to 1 or more of the following reasons: non-fasting visit ($n = 82$), missing data ($n = 37$), or triglyceride level ≥400 mg/dL ($n = 7$). The remaining 549 individuals (237 men and 312 women, mean [standard deviation {SD}] age: 57 [7] years) were included in the present study. The present study was approved by the Hyogo College of Medicine Ethics Committee.

Data collection and standardization

Height and weight while wearing socks and light clothing were measured, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Blood pressure was measured using an automatic sphygmomanometer after a 5-minute rest. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or use of medication for hypertension.

The participants were asked to respond to questionnaires about lifestyle-related factors, such as medication, smoking (current smoker or not), alcohol consumption (current drinker or not), and fish intake. The questionnaires included question about the frequency of fish intake per week, and the portion size of fish consumed in his or her typical meal using full-scale photos of 80 g of cooked fish. Then, each participant's total fish intake per week was calculated by summing the values that were calculated by multiplying the frequency and portion size.

Blood samples after an overnight fast were obtained from all participants. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides, and glucose levels were measured by enzymatic methods. Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula. Diabetes was defined as fasting blood glucose ≥126 mg/dL and/or HbA1c ≥ 6.5% (measured according to National Glycohemoglobin Standardization Program [NGSP] standards) and/or current use of insulin or oral medication for diabetes.

Fatty acid concentrations were measured using gas chromatography (GC-17A; Shimazu Corp, Kyoto, Japan) in the same commissioned clinical laboratory center (SRL Inc., Tokyo, Japan).[11] Serum total PUFA concentration was calculated as the sum of n-6 PUFA concentration (linoleic acid [LA, 18:2n-6], γ-linolenic acid [18:3n6], dihomo-γ-linolenic acid [20:3n6], and arachidonic acid [AA, 20:4n6]) and n-3 PUFA concentration ([α-linolenic acid [18:3n3], eicosapentaenoic acid [EPA, 20:5n3], docosapentaenoic acid [22:5n3], and docosahexaenoic acid [DHA, 22:6n3]).[2] Long-chain n-3 PUFAs were calculated as the sum of EPA, docosapentaenoic acid, and DHA.

Serum Cre was measured using the enzymatic method, and serum Cys C was measured using the colloidal gold technique.[12] GFR (mL/min/1.73 m^2) was estimated using the following 2 equations, which were developed by the Japanese Society of Nephrology: equation 1: $eGFR_{cre} = 194 \times Cre^{-1.094} \times age^{-0.287}$ (× 0.739 if female),[8] and equation 2: $eGFR_{cys} = 104 \times Cys\ C^{-1.019} \times 0.996^{age}$ (× 0.929 if female) − 8.[8,13]

Statistical analysis

Sex-specific and sex-combined analyses were performed. To show the characteristics of the study participants classified according to tertiles of the (EPA+DHA):AA ratio, mean (SD) or median were calculated for continuous variables, and the

percentage was calculated for dichotomous variables. The crude and age- and sex-adjusted geometric means of fish intake per week were compared among the tertiles of the (EPA+DHA):AA ratio with Bonferroni's correction for multiple post-hoc comparisons.

To investigate which variables among the long-chain n-3 and n-6 PUFA profiles show large standardized coefficients in relation to $eGFR_{cre}$ and $eGFR_{cys}$, linear regression models were used after adjusting for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, and current smoking and drinking. In these models, the long-chain n-3 and n-6 PUFA profiles included the serum concentrations of EPA, DHA, EPA+DHA, and long-chain n-3 PUFA, as well as EPA:AA, DHA:AA, (EPA+DHA):AA, and long-chain n-3 PUFA:AA ratios.

Among the tertiles of the (EPA+DHA):AA ratio, $eGFR_{cre}$ and $eGFR_{cys}$ were compared by analysis of covariance (ANCOVA) with Bonferroni's correction for multiple post-hoc comparisons after adjusting for the following confounders: Model 1 included age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, and current smoking and drinking; Model 2 included variables in Model 1 plus log-transformed C-reactive protein (CRP) measured using a high-sensitivity CRP assay. Because the fish intake of Japanese population was generally higher than that providing the maximal preventive effect for CVD in the previous studies,[14] $eGFR_{cre}$ and $eGFR_{cys}$ were also compared among the participants in the lowest tertile (T_1) and those in the other tertiles (T_2+T_3) of the (EPA+DHA):AA ratio after adjusting for the same confounders mentioned above.

Because several n-3 and n-6 PUFAs have been suggested to be associated with renal function in previous studies,[1,15] multiple linear regression models were used to confirm the contribution of serum PUFA concentration to $eGFR_{cre}$ and $eGFR_{cys}$ after adjusting for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, and current smoking and drinking. Serum concentrations of EPA+DHA, AA, linoleic acid, and α-linolenic acid were included in Model 1, and serum concentrations of EPA+DHA and n-6 PUFA were included in Model 2.

All P values were two-tailed, and the significance level was set at $P < 0.05$. The statistical package SPSS 20.0J for Windows (SPSS, Tokyo, Japan) was used to perform the analyses.

RESULTS

In all participants, the mean (SD) concentration of serum PUFA was 1457.3 (239.0) μg/mL total PUFA, 286.7 (95.8) μg/mL n-3 PUFA, 260.6 (92.5) μg/mL long-chain n-3 PUFA, and 1170.6 (198.6) μg/mL n-6 PUFA. The mean $eGFR_{cre}$ was 73 (13) mL/min/1.73 m^2, and the mean $eGFR_{cys}$ was 89 (16) mL/min/1.73 m^2. Eighty-one individuals had chronic kidney disease (CKD) defined by $eGFR_{cre}$ <60 mL/min/1.73 m^2, and 12 individuals had CKD defined by $eGFR_{cys}$ <60 mL/min/1.73 m^2.

Table 1 shows the characteristics of the participants according to serum (EPA+DHA):AA tertile in all participants. Age, BMI, and prevalence of hypertension and diabetes were higher in the higher (EPA+DHA):AA tertile. The percentage of medication for dyslipidemia was lower in the higher (EPA+DHA):AA tertile. eTables 1 and 2 show the sex-specific characteristics of the participants. In men, prevalence of smoking was higher in the lowest tertile compared to other groups (43.6% in T_1, 25.0% in T_2, and 29.1% in T_3). Figure shows the relationships between serum (EPA+DHA):AA tertile and geometric mean of fish intake (g/week). The higher (EPA+DHA):AA tertile was significantly associated with higher fish intake (trend $P < 0.001$, $P < 0.001$ between T_1 and T_2, and $P < 0.05$ between T_2 and T_3). In sex-specific analysis, the results were similar.

eTable 3 shows the relationships between eGFRs and n-3 and n-6 PUFA profiles in multivariate-adjusted linear regression models. All long-chain n-3 PUFA concentrations and ratios of long-chain n-3 PUFA to AA showed significant relationships with $eGFR_{cys}$. These concentrations and ratios did not show significant relationship with $eGFR_{cre}$; however, n-3 PUFA:AA ratios showed higher coefficients for $eGFR_{cre}$ than n-3 PUFA concentrations.

Table 2 shows the association between serum (EPA+DHA):AA tertiles and $eGFR_{cre}$ in ANCOVA after adjusting for the confounders. In men, higher (EPA+DHA):AA tertiles were associated with higher $eGFR_{cre}$ without statistical significance. In women, adjusted means of $eGFR_{cre}$ were the same among (EPA+DHA):AA tertiles. In all participants, higher (EPA+DHA):AA tertiles were associated with higher $eGFR_{cre}$ without statistical significance. Table 3 shows the association between (EPA+DHA):AA tertiles and $eGFR_{cys}$ in ANCOVA after adjusting for the confounders. In men, higher (EPA+DHA):AA tertiles were significantly associated with higher $eGFR_{cys}$. In women, higher (EPA+DHA):AA tertiles were associated with higher $eGFR_{cys}$ without statistical significance. In all participants, higher (EPA+DHA):AA tertiles were significantly associated with higher $eGFR_{cys}$, and Bonferroni's correction for multiple post-hoc comparisons showed significant differences between T_1 and T_2 ($P < 0.05$) and between T_1 and T_3 ($P < 0.05$). In addition, as shown in Table 4, $eGFR_{cre}$ was higher in T_2+T_3 than in T_1 without statistical significance, and $eGFR_{cys}$ was significantly higher in T_2+T_3 than in T_1 in all participants.

In addition, eGFRs were also compared among tertiles of fish intake (T_1, T_2, and T_3) in all participants by ANCOVA after adjusting for the confounders described as Model 1. Mean $eGFR_{cre}$ was 72 mL/min/1.73 m^2 in T_1, 75 mL/min/1.73 m^2 in T_2, and 73 mL/min/1.73 m^2 in T_3 (P for ANCOVA = 0.204), and mean $eGFR_{cys}$ was 88 mL/min/1.73 m^2 in T_1, 90 mL/min/1.73 m^2 in T_2, and 90 mL/min/1.73 m^2 in T_3 (P for ANCOVA = 0.163) (data not shown).

Table 1. Characteristics of study participants according to serum (EPA+DHA):AA tertile in the Sasayama study, 2012–2013

	Tertile of (EPA+DHA):AA		
	T_1 (0.338–0.925)	T_2 (0.928–1.301)	T_3 (1.302–3.188)
Number of participants	184	181	184
Sex, % males	44.0	38.1	47.3
Age, years	53 (8)	58 (6)	59 (5)
BMI, kg/m^2	22.7 (2.9)	23.0 (3.5)	23.2 (3.4)
Systolic blood pressure, mm Hg	121 (16)	128 (19)	130 (19)
Diastolic blood pressure, mm Hg	73 (11)	76 (11)	78 (11)
Hypertension, %	20.7	39.2	39.7
Glucose, mg/dL	95	98	99
Diabetes, %	7.6	7.7	9.8
Total cholesterol, mg/dL	210 (34)	221 (35)	220 (38)
LDL cholesterol, mg/dL	127 (31)	134 (33)	133 (34)
HDL cholesterol, mg/dL	63 (14)	64 (15)	63 (17)
Medication for dyslipidemia, %	19.6	14.9	13.6
Current smoking, %	24.5	13.3	15.2
Current drinking, %	52.2	51.9	55.4
Past or present history of CVD, %	3.3	5.0	3.3
C-reactive protein, mg/L	0.3	0.4	0.4
Fish intake, g/week	114 (5)	256 (2)	344 (2)
Serum N-3 PUFA,[a] μg/mL	203.3 (48.3)	278.9 (57.9)	377.8 (80.3)
Serum Long chain n-3 PUFA,[b] μg/mL	180.7 (45.0)	252.0 (56.1)	348.9 (78.8)
Serum EPA, μg/mL	42.2 (15.7)	68.0 (24.9)	111.2 (42.0)
Serum DHA, μg/mL	120.6 (28.8)	162.1 (34.0)	210.5 (42.5)
Serum α-linolenic acid, μg/mL	22.6 (7.8)	26.9 (10.6)	29.0 (10.6)
Serum n-6 PUFA,[c] μg/mL	1190.3 (190.0)	1181.5 (203.5)	1140.1 (199.6)
Serum AA, μg/mL	224.9 (47.2)	207.7 (45.0)	197.2 (42.1)
Serum linoleic acid, μg/mL	907.1 (163.1)	918.2 (183.0)	894.5 (178.3)
Serum creatinine, mg/dL	0.76 (0.21)	0.74 (0.15)	0.76 (0.17)
$eGFR_{cre}$, mL/min/1.73 m^2	74 (13)	73 (12)	73 (14)
Serum cystatin C, mg/L	0.85 (0.18)	0.83 (0.12)	0.85 (0.13)
$eGFR_{cys}$, mL/min/1.73 m^2	91 (18)	89 (15)	88 (15)

AA, arachidonic acid; BMI, body mass index; CVD, cerebral and cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PUFA, polyunsaturated fatty acid.
Values are means (standard deviations), except glucose and high-sensitivity C-reactive protein levels, which are presented as medians.
Fish intake is presented as geometric mean (SD).
[a]Serum n-3 PUFA: sum of α-linolenic acid, EPA, DHA, and docosapentaenoic acid.
[b]Serum n-6 PUFA: sum of linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, and AA.
[c]Long-chain n-3 PUFA: sum of EPA, DHA, and docosapentaenoic acid.

Table 5 shows the standardized coefficients of EPA+DHA and other PUFA concentrations in relation to $eGFR_{cre}$ and $eGFR_{cys}$ in multivariate-adjusted linear regression analysis in all participants. None of the presented PUFA concentrations, including EPA+DHA, were significantly associated with $eGFR_{cre}$; however, EPA+DHA concentration was significantly associated with $eGFR_{cys}$.

DISCUSSION

In the present study, the higher serum (EPA+DHA):AA tertile was significantly associated with higher fish intake in Japanese community-dwelling men and women. Furthermore, especially in men, higher serum (EPA+DHA):AA was significantly associated with higher $eGFR_{cys}$. In all participants, $eGFR_{cre}$ non-significantly increased according to an increase of (EPA+DHA):AA.

To our knowledge, the present study is the first to investigate the relationships between serum long-chain n-3 and n-6 PUFA profile and $eGFR_{cys}$. Because the ratios of n-3 PUFA to AA are considered to be markers for balance of anti-inflammatory and proinflammatory action by n-3 PUFAs and AA,[5] previous studies among Japanese patients have investigated the relationships between cardiac events and the ratio of n-3 PUFAs to AA. Among patients undergoing coronary angioplasty or hemodialysis, lower EPA:AA ratios were associated with higher risk of acute coronary syndrome,[16] and lower (EPA+DHA):AA ratios were associated with higher incidence of cardiovascular disease.[5] However, the relationships between serum long-chain n-3 and n-6 PUFA profiles and GFR estimated by Japanese-specific equations have not been investigated in community-dwellers.

In Western populations, only a few previous studies have investigated the relationships between PUFAs and renal function in community-dwellers. Gopinath et al showed that dietary intake of long-chain n-3 PUFA was inversely associated with the prevalence of CKD in a cross-sectional study of 2600 community-dwellers in Australia.[15] Lauretani

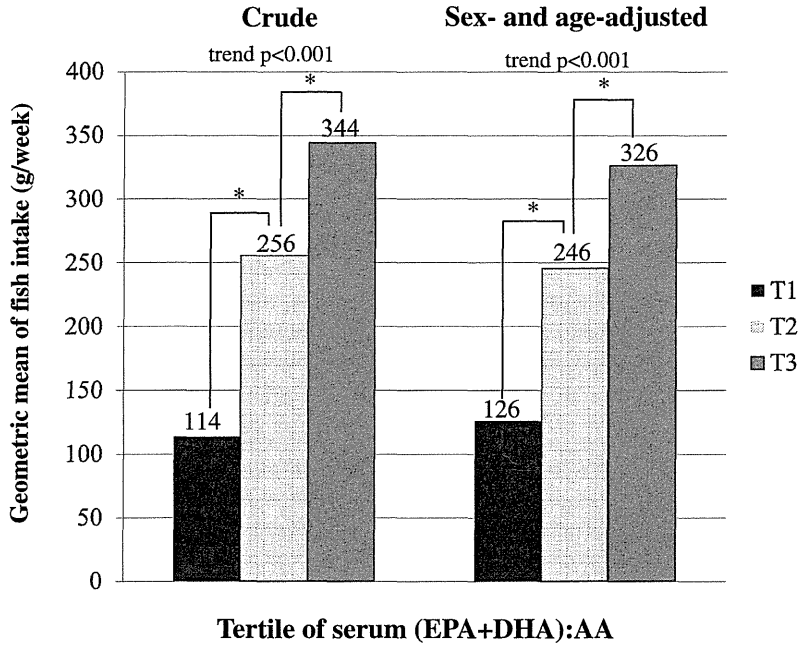

Figure. Tertile of serum (EPA+DHA):AA and geometric mean of fish intake. *Bonferroni correction for multiple post-hoc comparisons. Significance between the presented tertiles: $P < 0.05$.

Table 2. Multivariate-adjusted $eGFR_{cre}$ according to tertile of serum (EPA+DHA):AA ratio in the Sasayama study, 2012–2013

	Tertile of serum (EPA+DHA):AA			*P* value[a]
	T_1	T_2	T_3	
Men				
Number of participants	78	80	79	
Range of (EPA+DHA):AA ratio	0.409–0.904	0.908–1.318	1.323–3.188	
Sex- and age-adjusted mean $eGFR_{cre}$ (95% CI)	72.1 (68.8–75.4)	72.2 (69.1–75.4)	75.2 (72.0–78.5)	0.322
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 1)[b]	71.3 (68.0–74.6)	72.5 (69.4–75.6)	75.8 (72.6–79.0)	0.139
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 2)[c]	71.3 (68.0–74.6)	72.5 (69.4–75.6)	75.8 (72.6–79.0)	0.147
Women				
Number of participants	103	104	105	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.929–1.282	1.283–2.777	
Sex- and age-adjusted mean $eGFR_{cre}$ (95% CI)	73.2 (70.7–75.7)	73.2 (70.9–75.5)	73.0 (70.7–75.3)	0.989
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 1)[b]	73.2 (70.6–75.7)	73.2 (70.9–75.5)	73.1 (70.7–75.4)	0.998
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 2)[c]	73.2 (70.6–75.7)	73.1 (70.8–75.5)	73.1 (70.8–75.5)	1.000
Men and women combined				
Number of participants	184	181	184	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.928–1.301	1.301–3.188	
Sex- and age-adjusted mean $eGFR_{cre}$ (95% CI)	72.7 (70.7–74.7)	73.1 (71.2–75.0)	73.7 (71.8–75.6)	0.776
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 1)[b]	72.3 (70.3–74.2)	73.3 (71.4–75.2)	74.0 (72.1–75.9)	0.507
Multivariate-adjusted mean $eGFR_{cre}$ (95% CI) (Model 2)[c]	72.2 (70.2–74.2)	73.3 (71.4–75.1)	74.0 (72.1–75.9)	0.476

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate.
[a]*P* value for ANCOVA.
[b]Multivariate-adjusted (Model 1): eGFR adjusted for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, current smoking, and current drinking.
[c]Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.

et al showed that participants with higher plasma n-3 PUFA concentration had a significantly lower risk of developing CKD and mortality in a cohort study of 931 community-dwellers.[1] The results of these previous studies are consistent with those of the present study.

On the other hand, there has been a series of conflicting reports regarding the benefit of fish oil preparations containing n-3 PUFA given to patients with a variety of disease. Hsu et al showed that frequent intake of fish and vegetables correlated significantly with decreased creatinine and marginally with increased GFR estimated by Cre in a cohort study of patients with type 2 diabetes in Taiwan.[17] According to a meta-analysis of clinical trials by Miller III et al, the decline of GFR was slower in participants with n-3 PUFA supplementation

Table 3. Multivariate-adjusted $eGFR_{cys}$ according to tertile of serum (EPA+DHA):AA ratio in the Sasayama study, 2012–2013

	Tertile of serum (EPA+DHA):AA			*P* value[a]
	T_1	T_2	T_3	
Men				
Number of participants	78	80	79	
Range of (EPA+DHA):AA ratio	0.409–0.904	0.908–1.318	1.323–3.188	
Sex- and age-adjusted mean $eGFR_{cys}$ (95% CI)	82.9 (79.5–86.3)	89.5 (86.2–92.7)	90.7 (87.4–94.1)	0.003
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 1)[b]	83.9 (80.5–87.2)	88.2 (85.0–91.3)	91.0 (87.8–94.3)	0.015
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 2)[c]	84.0 (80.6–87.3)	88.2 (85.1–91.4)	90.9 (87.7–94.1)	0.017
Women				
Number of participants	103	104	105	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.929–1.282	1.283–2.777	
Sex- and age-adjusted mean $eGFR_{cys}$ (95% CI)	89.6 (86.8–92.3)	91.2 (88.7–93.8)	91.2 (88.6–93.8)	0.654
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 1)[b]	89.3 (86.6–91.9)	91.5 (89.1–93.9)	91.2 (88.8–93.7)	0.466
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 2)[c]	89.2 (86.6–91.8)	91.4 (89.0–93.8)	91.4 (89.0–93.7)	0.454
Men and women combined				
Number of participants	184	181	184	
Range of (EPA+DHA):AA ratio	0.338–0.925	0.928–1.301	1.301–3.188	
Sex- and age-adjusted mean $eGFR_{cys}$ (95% CI)	86.9 (84.7–89.0)	90.7 (88.6–92.8)	90.7 (88.5–92.7)	0.023
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 1)[b]	86.9 (84.9–89.0)	90.6 (88.7–92.6)	90.6 (88.7–92.6)	0.021
Multivariate-adjusted mean $eGFR_{cys}$ (95% CI) (Model 2)[c]	86.9 (84.9–88.9)	90.5 (88.6–92.4)	90.8 (88.9–92.7)	0.016

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate.
[a]*P* value for ANCOVA.
[b]Multivariate-adjusted (Model 1): eGFR adjusted for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, current smoking, and current drinking.
[c]Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.

Table 4. Multivariate-adjusted eGFR in T_1 and T_2+T_3 of serum (EPA+DHA):AA ratio in the Sasayama study, 2012–2013

	Tertile of serum (EPA+DHA):AA		*P* value[a]
	T_1 (0.338–0.925)	T_2+T_3 (0.928–3.188)	
Number of participants	184	365	
Mean $eGFR_{cre}$ (ml/min/1.73 m^2)			
Sex- and age-adjusted	72.7 (70.8–74.7)	73.4 (72.0–74.8)	0.598
Multivariate-adjusted (Model 1)[b]	72.3 (70.3–74.3)	73.6 (72.3–75.0)	0.291
Multivariate-adjusted (Model 2)[c]	72.3 (70.3–74.2)	73.6 (72.3–75.0)	0.282
Mean $eGFR_{cys}$ (ml/min/1.73 m^2)			
Sex- and age-adjusted	86.9 (84.7–89.0)	90.7 (89.2–92.1)	0.006
Multivariate-adjusted (Model 1)[b]	86.9 (84.9–89.0)	90.6 (89.2–92.0)	0.005
Multivariate-adjusted (Model 2)[c]	86.9 (84.9–88.9)	90.6 (89.3–92.0)	0.004

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate.
[a]*P* value for ANCOVA.
[b]Multivariate-adjusted (Model 1): eGFR adjusted for age, sex, BMI, hypertension, diabetes, HDL-C, LDL-C, medication for dyslipidemia, current smoking, and current drinking.
[c]Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.
Multivariate-adjusted (Model 2): eGFR adjusted for variables in model 1 plus log-transformed high-sensitivity C-reactive protein.

Table 5. Multivariate-adjusted linear regression models[a] between eGFR and serum PUFAs, including EPA+DHA, in the Sasayama study, 2012–2013

	Independent variables			
	$eGFR_{cre}$		$eGFR_{cys}$	
	Standardized coefficients	*P* value	Standardized coefficients	*P* value
Model 1				
Serum EPA+DHA	−0.012	0.815	0.097	0.025
Serum α-linolenic acid	0.088	0.191	0.015	0.797
Serum AA	0.008	0.862	−0.025	0.541
Serum linoleic acid	−0.144	0.064	−0.016	0.803
Model 2				
Serum EPA+DHA	0.014	0.770	0.096	0.015
Serum n-6 PUFA[b]	−0.055	0.313	−0.022	0.622

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFR, glomerular filtration rate; PUFA, polyunsaturated fatty acids.
[a]Relationships between presented fatty acids and eGFR were evaluated by linear regression model after adjusting for age, sex, BMI, presence of hypertension and diabetes mellitus, serum HDL- and LDL- cholesterol level, medication for dyslipidemia, current smoking and drinking, and log-transformed C-reactive protein.
[b]Serum n-6 PUFA: sum of linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid.

than in control participants, but this effect was not significant, and they concluded that n-3 PUFA supplementation did not ameliorate the decline in GFR. However, they also noted that differences in methods of assessing GFR, such as GFR measured or estimated by serum Cre or 24-h urine Cre clearance, limited the ability to draw conclusions.[18] Furthermore, serum Cre level is affected by various factors, such as muscle mass and diet.[10]

In the present study, the difference between $eGFR_{cys}$ and $eGFR_{cre}$ in relation to EPA+DHA:AA was especially apparent in men. Thus, muscle mass could be an important factor influencing the relationship between serum EPA+DHA:AA and $eGFR_{cre}$, and $eGFR_{cys}$ might be more useful than $eGFR_{cre}$ when investigating the relationship between eGFR and PUFA profiles. In addition, in the previous studies,[1,15,17,18] ratios of long-chain n-3 and n-6 PUFAs, such as (EPA+DHA):AA,