Serum Albumin and Cerebro-cardiovascular Mortality During a 15-year Study in a Community-based Cohort in Tanushimaru, a Cohort of the Seven Countries Study

Yoko Umeki[1], Hisashi Adachi[2], Mika Enomoto[3], Ako Fukami[3], Sachiko Nakamura[3], Yume Nohara[3], Erika Nakao[3], Akiko Sakaue[3], Tomoko Tsuru[3], Nagisa Morikawa[3], Yoshihiro Fukumoto[3]

[1]Kurume University Graduate School of Medicine, [2]Department of Community Medicine, Kurume University School of Medicine, [3]Department of Internal Medicine, Division of Cardio-Vascular Medicine, Kurume University School of Medicine

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Address for correspondence;

Hisashi Adachi, M.D., PhD.

Department of Community Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, 830-0011, Japan

TEL:+81-942-31-7586, FAX:+81-942-31-7896

E-mail: hadac@med.kurume-u.ac.jp

ABSTRACT

Objective There is little long-term data on the association between the serum albumin levels and mortality in community-based populations. We aimed to determine whether the serum albumin level is an independent risk factor for all-cause and cause-specific death in a community-based cohort study in Japan.

Methods In 1999, we performed a periodic epidemiological survey over a 15-year period in a population of 1,905 healthy subjects (783 males, 1,122 females) who were older than 40 years of age and who resided in Tanushimaru, a rural community, in Japan. Over the course of the study, we periodically examined the blood chemistry of the study subjects, including their serum albumin levels. Their baseline serum albumin levels were categorized into quartiles.

Results The baseline albumin levels were significantly associated with age (inversely), body mass index (BMI), diastolic blood pressure, lipid profiles (high density lipoprotein-cholesterol [HDL-c], low-density lipoprotein-cholesterol [LDL-c] and triglycerides) and estimated

glomerular filtration rate (eGFR). After adjusting for confounders, a Cox proportional hazards regression analysis demonstrated that a low serum albumin level was an independent predictor of all-cause death (hazard ratio [HR]: 0.39, 95% confidence interval [CI]: 0.24-0.65), cancer death (HR: 0.43, 95% CI: 0.18-0.99), death from infection (HR: 0.21, 95% CI: 0.06-0.73) and cerebro-cardiovascular death (HR: 0.19, 95% CI: 0.06-0.63). The HRs for all-cause and cerebro-cardiovascular death in the highest quartile vs. the lowest quartile of albumin after adjusting for confounders were 0.59 (95%CI:0.39-0.88) and 0.15 (95%CI: 0.03-0.66), respectively.

Conclusions The serum albumin level was thus found to be a predictor of all-cause and cerebro-cardiovascular death in a general population.

Key words: albumin; mortality; epidemiology

Introduction

Although it has been reported that low levels of serum albumin are associated with greater all-cause mortality in the general population (1,2), the impact of serum albumin and nutrient intake on long-term mortality remains scant. The normal serum concentration of albumin in healthy adults is ≥4.0g/dL, while hypoalbuminemia is defined as a serum albumin level of ≤3.4g/L (3). A meta-analysis by Vincent et al. (4) suggested that hypoalbuminemia is a powerful, reproducible and independent risk factor that predicts a poor outcome in patients with acute illness. This finding has been consistently and pervasively observed. In older veteran patients, hypoalbuminemia at 3 months after hospital discharge was associated with a poor long-term prognosis (5). However, most reports have been limited to cause-specific death (6-9) and in-hospital outcomes (10,11).

A study of patients with end-stage renal disease revealed that low serum albumin levels in the presence of vascular disease might not reflect the nutritional state (6). Hence, a reference standard of nutritional measurement should be used to determine the presence of protein

malnutrition. Cooper et al. (7) also suggested that protein malnutrition and hypoalbuminemia are independent predictors of morbidity and mortality, whereas hypoalbuminemia predicts vascular morbidity (7), supporting the hypothesis that hypoalbuminemia is pathogenically associated with vascular disease, but the effect is dissociated from protein malnutrition in patients with end-stage renal disease.

To elucidate whether serum albumin levels and protein malnutrition are associated with cause-specific death and dietary habits, it is necessary to examine this relationship in a large number of subjects with confirmed dietary habits from a general population. We therefore investigated the relationships between serum albumin levels and protein malnutrition and cause-specific death in a general population in Japan.

Materials and Methods

Subjects

In 1999, we performed an epidemiological survey in Tanushimaru, a small

65 rural community in southwestern Japan. This was a cohort of the Seven
66 Countries Study (12). As previously reported, the demographic background
67 of the subjects in this area is similar to that of the general Japanese
68 population (13). Subjects with a known history of myocardial infarction
69 (n=2), stroke (n=3), cancer (n=5) or abnormal Q waves (n=5) (Minnesota
70 codes I-1,2) (14) were excluded from the study. Finally, serum albumin
71 data were obtained from 1,905 subjects (783 males, 1,122 females) of 40 to
72 95 years of age (40-49 years, n=282 [male, n=102; female, n=180]; 50-59
73 years, n=450 [male, n=178; female, n=272]; 60-69 years, n=623 [male,
74 n=259; female, n=364]; 70-79 years, n=464 [male, n=206; female, n=258];
75 ≥80 years, n=86 [male, n=48; female, n=38]), over a 15-year period (Figure
76 1). The respondents accounted for 48.2% of the men and 62.0% of the
77 women in Tanushimaru who were older than 40 years of age (total target
78 population: 3,463). The follow-up rate was 95.1%.
79
80 ***Data collection***
81 The subjects' medical history, alcohol intake, smoking habit, and
82 current medications for hypertension, dyslipidemia, and diabetes were

ascertained by questionnaire. The alcohol intake and smoking habit were classified according to whether or not the respondent was a current habitual user. The height and weight were measured, and the body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2), as an index of obesity. Blood pressure (BP) was measured twice with the subject in the supine position. The second BP measurement was taken after 5 deep breaths and the 5th-phase diastolic pressure was used for the analysis. Blood was drawn from the antecubital vein for the determination of the fasting plasma glucose (FPG) and hemoglobin A_{1c} level (HbA_{1c} [NGSP]), the lipid profiles (total cholesterol, high density lipoprotein-cholesterol [HDL-c], low density lipoprotein-cholesterol [LDL-c] and triglycerides) and uric acid levels. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation modified with a Japanese coefficient: eGFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) = $194 \times age^{-0.287} \times$ serum $creatinine^{-1.094}$ (if female $\times$ 0.739) (15). The serum albumin level was measured according to standard methods in a commercial laboratory (Kyodo Igaku Laboratory, Fukuoka, Japan).

101

102 ***Nutritional data***

103 Dietary habits were evaluated by a food frequency questionnaire
104 (13,15). This questionnaire was modified for Japanese individuals by
105 using an adaptation of ARIC study's food frequency questionnaire (17). It
106 consisted of 105 items. Habitual nutrient intake was estimated based on
107 reports of the average portion size of foods and the frequency at which
108 they were consumed over the previous year. Due to the difference between
109 Caucasian and Japanese diets, we added items and changed the portion
110 size of the foods consumed. The following items were added according to
111 the Japanese standard food composition tables (18); thick wheat flour
112 noodles (udon), buchwheat noodles (soba), soybean paste (miso), soybean
113 curd (tofu), fermented soybeans (natto), mushrooms, seaweed, and
114 Japanese sweet. The following items were deleted due to the low
115 frequency at which they were consumed, hamburger, hot dog, French fries,
116 brown bread, peanut butter, and low calorie carbonated water.

117 The frequency of intake and weights of the frequency were
118 classified into the following 9 categories: 1) once per day (×1.0); 2) two or

119 three times per day (×2.5); 3) four to six times per day (×5); 4) more than
120 six times per day (×7); 5) once per week (×0.14); 6) two to four times per
121 week (×0.43); 7) five or six times per week (×0.79); 8) one to three times
122 per month (×0.066); and 9) never (×0) (17).

123 The dietary data that were used for validation were compared with
124 the results of the Japanese National Nutrition Survey in 1999 (19). The
125 results of the National Nutrition Survey in 1999 are shown in parentheses.
126 The total daily energy intake of the study subjects in the present study was
127 1,945 kcal (vs. 1988 kcal), the percentages of the daily calorie intake from
128 carbohydrate, protein and fat were 58% (vs. 59%), 19% (vs. 16%), and
129 23% (vs. 24%), respectively. Thus, the eating pattern in the subjects of the
130 present study was similar to that reported in the results of the National
131 Nutrition Survey.

132 We followed up the participants annually for 15 years (from 1999 to
133 2014). The causes of death were determined based on a review of
134 obituaries, medical records, death certificates, hospital charts, and
135 interviews with primary care physicians, the families of the deceased and
136 other witnesses. The information was coded independently in accordance

137 with the rules of the Seven Countries Study (12), and using the World
138 Health Organization's 10th Revision of the International Statistical
139 Classification of Diseases and Related Health Problems (WHO-ICD) (20).
140 The follow-up data that were collected prior to the end of December 2014
141 were used in this study.

142 The present study was approved by the Ukiha and Tanushimaru
143 Branches of the Japan Medical Association, the local citizens' committee
144 of Tanushimaru, and by the Research Ethics Committee of the Kurume
145 University School of Medicine (Process No. 9908/1999). The study
146 conformed to the principles of the declaration of Helsinki. All of the
147 participants provided written informed consent.

148

149 ***Statistical analysis***

150 Natural logarithmic transformations were performed for the
151 triglyceride values because of their skewed distribution. The triglyceride
152 values after the analysis using log (natural)-transformed values are
153 presented in the original scale (Tables 1 and 2). In Tables 1 and 2, the
154 triglyceride values are presented as the geometric mean and range. Sex
155 (men=0, women=1), smoking habits (non-smoker and former smoker=0,

156 current smoker=1), alcohol intake (non-drinker and former drinker=0,
157 current drinker=1), and medications for hypertension, dyslipidemia, and
158 diabetes (no=0, yes=1) were used as dummy variables. The mean serum
159 albumin levels were classified into the following quartiles: ≤4.2g/dl,
160 4.2-4.4g/dl, 4.4-4.6g/dl, and ≥4.6g/dl. The mean parameters, stratified by
161 the albumin levels quartiles were compared using an analysis of variance.
162 The survival curves for all-cause death for each albumin quartile were
163 estimated and compared using the Kaplan-Meier method and the log-rank
164 statistic, respectively. For the categorical parameters, the χ^2 test was used
165 to test differences among groups. Uni- and multivariate regression
166 analyses were performed using Cox's proportional hazards model to
167 determine the factors associated with mortality from all causes, cancer,
168 infection and cerebro-cardiovascular disease. In order to obtain hazard
169 ratios (HRs) for all-cause, cancer, infection and cerebro-cardiovascular
170 disease death, stratified by the serum albumin level quartiles, we
171 performed a Cox proportional hazards regression analysis after adjusting
172 for confounding factors. P values of <0.05 were considered to indicate

173 statistical significance. All of the statistical analyses were performed using
174 the SAS software program (version 9.3, SAS Institute, Cary, NC).
175
176 **Results**
177 There were 343 deaths (male, n=201; female, n=142). The causes
178 of death were as follows: cancer, n=102 (29.7%); cerebro-cardiovascular
179 disease, n=48 (14.0%); infection, n=45 (13.1%); other causes, n=69
180 (20.1%); and unknown, n=79 (23.0%). The 102 cancer deaths included,
181 malignancies of the digestive system (n=34), liver, bile duct and pancreas
182 (n=29), malignancies of the respiratory system (n=19), hematological
183 malignancies (n=10), and other types of malignancy (n=10). The serum
184 albumin levels were missing from the reports of 1 subject who died of
185 cerebro-cardiovascular disease, 3 who died of cancer, and 2 who died of
186 infection. Eventually, the data of 337 subjects (male, n=196; female,
187 n=141) were analyzed (Figure 1).
188 Table 1 shows the baseline characteristics of the 1,905 subjects
189 stratified by the serum albumin quartiles. The baseline characteristics of
190 age ($p<0.001$; inversely), total cholesterol ($p<0.001$), HDL-c ($p<0.001$),
191 LDL-c ($p<0.001$), log-transformed triglycerides ($p<0.001$) and eGFR

192 ($p<0.05$) were significantly associated with the albumin quartiles. Table 2
193 shows the baseline nutrient characteristics of the 1,905 subjects stratified
194 by the serum albumin quartiles. The baseline nutrient intake levels were
195 not significantly associated with the serum albumin quartiles.

196 Three hundred forty-three of the 1,905 subjects died (male, n=201;
197 female, n=142) during the 15-year follow-up period. In a Cox proportional
198 hazards model with all-cause and cause-specific death as the outcome
199 variable, a low albumin level was found to be a predictor of all-cause
200 death (hazard ratio [HR], 0.39; 95% confidence interval [CI], 0.24-0.65;
201 $p<0.001$], cancer death (HR, 0.43; 95% CI, 0.18-0.99; $p=0.049$], death
202 from infection (HR, 0.21; 95% CI, 0.06-0.73; $p=0.014$) and
203 cerebro-cardiovascular disease-related death (HR, 0.19; 95% CI,
204 0.06-0.63; $p=0.006$) (Table 3). In a Cox proportional hazards model with
205 all-cause and cause-specific death as the outcome variable, with the
206 exception of vegetable protein (for cancer death), none of the baseline
207 nutrient levels was significantly associated with the outcome (Table 4).

208 We categorized the baseline albumin levels into quartiles and
209 calculated the HRs for all-cause, cancer, infection and

210 cerebro-cardiovascular disease death using the lowest quartile as the
211 reference value (Table 5). The HRs for all-cause and
212 cerebro-cardiovascular disease death in the lowest quartile vs. the highest
213 serum albumin quartile (after adjusting for age, sex, HDL-c, LDL-c,
214 triglycerides and eGFR) were 0.59 (95%CI: 0.39-0.88) and 0.15 (95%CI:
215 0.03-0.66), respectively.

216 In the Kaplan-Meier curves for the cumulative survival rate,
217 stratified by the serum albumin quartiles, the lowest quartile group showed
218 the worst all-cause mortality in a significant albumin level-dependent
219 manner (log rank statistic=22.9; $p<0.0001$) among the four groups (Figure
220 2).

221

222 **Discussion**

223 The novel findings of the present study were that the serum
224 albumin level was a predictor of all-cause and cerebro-cardiovascular
225 disease death, even in healthy individuals, during a 15-year follow-up
226 period, and that the serum albumin level was not correlated with nutrition
227 intake. Although two previous reports (1,2) from Japanese investigators

228 demonstrated a strong association between the serum albumin level and
229 all-cause death in a community-based population, the present study
230 reported that the serum albumin level was associated with both all-cause
231 and cerebro-cardiovascular disease death.

232 To determine the causes of death, we used a review of obituaries,
233 medical records, death certificates, hospital charts, and interviews with
234 primary care physicians, family members of the deceased and other
235 witnesses. The information was carefully independently coded in
236 accordance with the rules of the Seven Countries Study (12).

237 Among the participants of the present study (mean albumin level;
238 4.4g/dL), only 89 subjects (4.7%) showed an albumin level of ≤4.0g/dL.
239 Based on the findings of this study, we hypothesize that nutritional
240 deficiencies (low albumin and low cholesterol) may be important in the
241 pathology of all-cause and cause-specific death. It is interesting to note that
242 hypoalbuminemia has been associated with hemorrhagic stroke in previous
243 studies (21,22), and that hypoalbuminemia has been associated with
244 cause-specific death and diseases in many studies including our own study
245 (23-31). It has been demonstrated that low albumin levels are associated

246 with long-term protein-energy deprivation, liver disease (30) and renal
247 disease (6,7,25), acute illness (4,9,10,11,26,29) and chronic illness (8,25),
248 inflammation (31) and poor physical function (24). There is increasing
249 evidence to show that low albumin levels are associated with cancer (27).
250 However, whether low levels of albumin are associated with the nutritional
251 status and disease of healthy participants has not previously been
252 elucidated. Four subjects died within 1 year of enrollment due to
253 hepatocyte cell carcinoma (3.6 months), subarachnoid hemorrhage (4.8
254 months), lung cancer (6 months), and an accident (7.2 months). The low
255 albumin levels in the 2 subjects who died of cancer might have affected the
256 results; however, we were not able to exclude the cases, because their
257 records did not describe any history of cancer treatment nor was there any
258 information to suggest that they were undergoing cancer treatment at the
259 start of the study period.

260 Hypoalbuminemia, which can be associated with various diseases,
261 is frequently observed in hospitalized patients (24-31). Regardless of the
262 cause, hypoalbuminemia has a strong impact on mortality and morbidity.
263 However, in the present study, the enrolled subjects were free from

apparent cerebro-cardiovascular disease at the start of the study period and their serum albumin levels were within the normal range. Furthermore, after adjusting for confounding factors, the Cox proportional hazards regression analysis demonstrated that a low albumin level was an independent predictor of all-cause death and cerebro-cardiovascular disease death (Table 5). In contrast, the subjects with the highest albumin level had the lowest mortality rate, indicating that a small variation within the normal range of the albumin level could have an effect on future all-cause mortality, even in a general population. The precise mechanism underlying the relationship between serum albumin levels and mortality should be clarified in future studies.

Next, we focused on the association between nutrient intake and all-cause/cause-specific death, vegetable protein intake was the only nutrient-related factor that was found to be associated with mortality (Table 4). The previous reports did not consider the nutrient intake of their study populations (1,2,21-31). Our findings suggest that it is likely that the nutrient intake of the healthy subjects in the present study did not directly influence their serum albumin levels. Malabsorption or protein loss might

282 be considered when an imbalance is detected between a subject's albumin
283 level and his or her nutrient intake. This issue should be clarified in future
284 studies.
285
286 ***Study limitations***
287 The present study is associated with several limitations. First, in
288 order to exclude subjects with cardiovascular diseases, we carefully
289 examined their medical history, and performed physical examinations. The
290 subjects with Q waves on an electrocardiogram were excluded. However,
291 it is possible that some subjects with asymptomatic cardiovascular
292 diseases might have been included. Second, we were not able to exclude
293 subjects with undetected cancer. Third, the total number of deaths from
294 cerebro-cardiovascular disease and cancers was small, which limited the
295 statistical power of the outcome. Fourth, we used a single baseline
296 measurement to predict the all-cause and cause-specific death. Fifth, we
297 did not have data on chronic hepatitis in the present study. Thus, some
298 subjects' serum albumin levels might have been influenced by chronic
299 hepatitis. Finally, the pathophysiological mechanism underlying the

association between low albumin levels and all-cause death was not revealed from our observational study.

Conclusions

In conclusion, the present study demonstrated that the serum albumin level was an independent predictor of all-cause and cerebro-cardiovascular disease death in the Japanese general population.

The authors declare no Conflict of Interest (COI) in association with the present study.

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Table 1. Baseline characteristics of the 1,905 subjects stratified by serum albumin quartiles

Characteristics	Quartiles of albumin (g/dl)				p for trend
	Q1 (Lowest)	Q2	Q3	Q4 (Highest)	
Total, n	476	476	476	477	
Albumin (g/dl)	4.1 ± 0.2	4.3 ± 0.1	4.5 ± 0.1	4.7 ± 0.1	<0.0001
Age (years)	66.4 ± 11.2	63.6 ± 10.6	61.6 ± 10.8	59.1 ± 10.1	<0.0001
Sex (%males)	213 (44.7)	186 (39.1)	180 (37.8)	204 (42.8)	0.107
BMI (kg/m^2)	23.0 ± 3.4	23.0 ± 3.0	23.3 ± 3.0	23.1 ± 3.1	0.495
Systolic BP (mmHg)	140.8 ± 21.7	139.0 ± 21.2	140.4 ± 22.7	140.2 ± 20.7	0.584
Diastolic BP (mmHg)	81.2 ± 11.8	81.6 ± 12.2	82.2 ± 12.5	82.9 ± 12.0	0.144
Total cholesterol (mg/dl)	187.9 ± 35.3	196.4 ± 32.7	203.0 ± 32.0	211.8 ± 34.0	<0.0001
HDL-cholesterol (mg/dl)	53.7 ± 12.6	55.9 ± 14.0	55.4 ± 13.2	58.2 ± 15.9	<0.0001
LDL-cholesterol (mg/dl)	114.4 ± 31.3	119.0 ± 30.8	123.9 ± 30.7	128.5 ± 34.1	<0.0001
TG (mg/dl)*[range]	89 [28-392]	95 [33-963]	101 [29-843]	109 [28-1284]	<0.0001
eGFR ($ml/ml^{-1}/1.73m^{-2}$)	56.5 ± 13.4	57.1 ± 12.5	57.8 ± 12.7	58.9 ± 13.0	0.027
Uric acid (mg/dl)	5.0 ± 1.5	4.9 ± 1.3	4.9 ± 1.3	5.1 ± 1.5	0.351
HbA_{1c} (%)	5.2 ± .8	5.2 ± .8	5.2 ± .6	5.2 ± .7	0.590
Alchol intake (%yes)	107 (22.5)	100 (21.0)	92 (19.3)	122 (25.6)	0.116
Current smoking (%yes)	87 (18.3)	78 (16.4)	69 (14.5)	92 (19.3)	0.205
Medications					
Hypertension (%yes)	101 (21.2)	95 (20.0)	87 (18.3)	92 (19.3)	0.716
Hyperlipidemia (%yes)	24 (5.0)	23 (4.8)	18 (3.8)	26 (5.5)	0.661
Diabetes (%yes)	19 (4.0)	11 (2.3)	17 (3.6)	12 (2.5)	0.377

Data are means ±standard deviations, geometric mean, range, or percent.

*Variables represented in the original scale after analysis using log (natural) transformed values.

Abbreviations;

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filter; TG, triglycerides.

Table 2. Baseline means of nutrient intake stratified by albumin quartiles

Characteristics	Quartiles of albumin (g/dl)				p for trend
	Q1 (Lowest)	Q2	Q3	Q4 (Highest)	
Total, n	476	476	476	477	
Albumin (g/dl)	4.1 ± 0.2	4.3 ± 0.1	4.5 ± 0.1	4.7 ± 0.1	<0.0001
Energy (kcal/day)*[range]	1858 [817-4339]	1882 [869-4962]	1850 [913-4097]	1897 [849-4443]	0.486
Total protein (g/day)	91.3 ± 33.6	92.4 ± 32.6	92.0 ± 32.1	93.1 ± 35.3	0.870
Animal protein (g/day)	38.7 ± 18.1	40.2 ± 18.6	38.8 ± 18.7	39.2 ± 19.5	0.586
Vegetable protein (g/day)	52.6 ± 23.4	52.2 ± 22.0	53.2 ± 22.6	53.9 ± 24.0	0.693
Total fat (g/day)	49.1 ± 19.3	51.2 ± 20.1	50.5 ± 19.9	50.5 ± 21.7	0.425
Animal fat (g/day)	22.9 ± 12.9	24.2 ± 13.3	23.2 ± 13.3	23.7 ± 13.9	0.394
Vegetable fat (g/day)	26.3 ± 10.1	27.0 ± 10.5	27.3 ± 10.7	26.8 ± 11.2	0.462
Carbohydrate (g/day)	333.9 ± 103.4	331.1 ± 106.9	331.0 ± 100.2	340.3 ± 110.0	0.484
Saturate fat (g/day)	11.1 ± 4.8	11.6 ± 5.0	11.5 ± 5.0	11.6 ± 5.4	0.449
Monounsaturated fat (g/day)	14.5 ± 6.3	15.1 ± 6.6	14.9 ± 6.4	14.9 ± 6.9	0.557
Polyunsaturated fat (g/day)	10.7 ± 4.0	11.0 ± 4.4	11.1 ± 4.0	10.8 ± 4.5	0.450
N-6 fatty acid (mg/day)					
Linoleic acid (mg/day)	8144.0 ± 3128.2	8474.7 ± 3509.5	8461.6 ± 3180.8	8261.4 ± 3445.7	0.345
Arachidonic acid (mg/day)	126.5 ± 56.0	127.3 ± 58.0	122.8 ± 56.3	125.3 ± 58.1	0.650
N-3 fatty acid (mg/day)					
Linolenic acid (mg/day)	1281.4 ± 615.1	1336.0 ± 685.4	1319.6 ± 624.5	1278.9 ± 652.0	0.434
EPA (mg/day)	311.7 ± 192.0	306.6 ± 182.2	322.3 ± 247.3	330.8 ± 223.0	0.299
DHA (mg/day)	493.6 ± 281.7	486.1 ± 266.8	507.5 ± 361.8	518.2 ± 318.7	0.387

Data are means ±standard deviations, geometric mean, range, or percent.

*Variables represented in the original scale after analysis using log (natural) transformed values.

Abbreviations;

EPA; Eicosapentaenoic acid,

DHA; Docosahexaenoic acid

Table 3. Cox proportional hazards model with all-cause and cause-specific deaths as the outcome variables

All-cause deaths (n=337)

parameter	β	SE	HR (95%CI)	p value
Albumin (g/dl)	-0.931	0.256	0.39 (0.24 - 0.65)	<0.0001
Age(years)	0.099	0.007	1.10 (1.09 - 1.12)	<0.0001
Total cholesterol (mg/dl)	-0.004	0.010	1.00 (0.98 - 1.02)	0.718
HDL-cholesterol (mg/dl)	0.007	0.012	1.01 (0.98 - 1.03)	0.544
LDL-cholesterol(mg/dl)	-0.006	0.010	0.99 (0.98 - 1.01)	0.557
Triglycerides (mg/dl)*	0.218	0.319	1.24 (0.67 - 2.32)	0.493
eGFR ($ml/ml^{-1}/1.73m^{-2}$)	-0.004	0.005	1.00 (0.99 - 1.01)	0.463
Cancer deaths (n=99)				
parameter	β	SE	HR (95%CI)	p value
Albumin (g/dl)	-0.855	0.435	0.43 (0.18 - 0.99)	0.049
Age(years)	0.078	0.012	1.08 (1.06 - 1.11)	<0.0001
Total cholesterol (mg/dl)	-0.059	0.036	0.94 (0.88 - 1.01)	0.105
HDL-cholesterol (mg/dl)	0.049	0.038	1.05 (0.98 - 1.13)	0.198
LDL-cholesterol (mg/dl)	0.048	0.036	1.05 (0.98 - 1.13)	0.184
Triglycerides (mg/dl)*	1.512	0.891	4.54 (0.79 - 26.03)	0.090
eGFR ($ml/ml^{-1}/1.73m^{-2}$)	0.015	0.009	1.02 (1.01 - 1.03)	0.084
Infection deaths (n=43)				
parameter	β	SE	HR (95%CI)	p value
Albumin (g/dl)	-1.584	0.644	0.21 (0.06 - 0.73)	0.014
Age (years)	0.133	0.018	1.14 (1.1 - 1.19)	<0.0001
Total cholesterol (mg/dl)	0.001	0.033	1.00 (0.94 - 1.07)	0.973
HDL-cholesterol (mg/dl)	0.017	0.035	1.02 (0.95 - 1.09)	0.628
LDL-cholesterol (mg/dl)	-0.009	0.032	0.99 (0.93 - 1.05)	0.771
Triglycerides (mg/dl)*	0.077	0.888	1.08 (0.19 - 6.15)	0.931
eGFR ($ml/ml^{-1}/1.73m^{-2}$)	0.009	0.013	1.01 (0.98 - 1.04)	0.503
CVD deaths (n=47)				
parameter	β	SE	HR (95%CI)	p value
Albumin (g/dl)	-1.643	0.599	0.19 (0.06 - 0.63)	0.006
Age(years)	0.091	0.016	1.10 (1.06 - 1.13)	<0.0001
Total cholesterol (mg/dl)	0.014	0.017	1.01 (0.98 - 1.05)	0.412
HDL-cholesterol (mg/dl)	-0.002	0.021	1.00 (0.96 - 1.04)	0.943
LDL-cholesterol (mg/dl)	-0.022	0.016	0.98 (0.95 - 1.01)	0.162
Triglycerides (mg/dl)*	-0.330	0.623	0.72 (0.21 - 2.44)	0.597
eGFR ($ml/ml^{-1}/1.73m^{-2}$)	-0.016	0.012	0.98 (0.96 - 1.01)	0.984

Abbreviations;
CVD, Cerebro-cardiovascular diseases; CI, confidence interval; HR, hazard ratio;
eGFR, estimated glomerular filter

Table 4. Cox proportional hazards model with all-cause and cause-specific deaths as the nutrient intakes

All-cause deaths (n=337)

Vaiables	β	SE	HR (95%CI)	p value
Sex (men=0,women=1)	-0.837	0.140	0.433 (0.329 - 0.569)	<0.0001
Age (years)	0.116	0.007	1.123 (1.108 - 1.139)	<0.0001
Energy (kcal/day)	0.000	0.000	1.000 (0.999 - 1.000)	0.402
Animal protein (g/day)	-0.009	0.011	0.991 (0.971 - 1.012)	0.422
Vegetable protein (g/day)	0.016	0.009	1.016 (0.999 - 1.034)	0.066
Animal protein/vegetable protein	0.481	0.342	1.617 (0.828 - 3.159)	0.159
Animal fat (g/day)	-0.005	0.013	0.995 (0.969 - 1.021)	0.699
Vegetable fat (g/day)	0.020	0.013	1.020 (0.994 - 1.047)	0.135
Animal fat/vegetable fat	0.273	0.253	1.314 (0.800 - 2.158)	0.282
Carbohydrate (g/day)	-0.001	0.002	0.999 (0.995 - 1.004)	0.723
EPA (mg/day)	-0.001	0.002	0.999 (0.995 - 1.002)	0.451
DHA (mg/day)	0.001	0.001	1.001 (0.998 - 1.003)	0.587
Cancer deaths (n=99)				
Vaiables	β	SE	HR (95%CI)	p value
Sex (men=0,women=1)	-0.571	0.234	0.565 (0.357 - 0.894)	0.015
Age (years)	0.099	0.011	1.104 (1.080 - 1.128)	<0.0001
Energy (kcal/day)	0.000	0.001	1.000 (0.999 - 1.002)	0.644
Animal protein (g/day)	-0.018	0.017	0.982 (0.949 - 1.016)	0.295
Vegetable protein (g/day)	0.028	0.014	1.028 (1.001 - 1.056)	0.045
Animal protein/vegetable protein	0.324	0.605	1.382 (0.422 - 4.526)	0.593
Animal fat (g/day)	-0.006	0.022	0.994 (0.953 - 1.037)	0.778
Vegetable fat (g/day)	0.009	0.021	1.009 (0.968 - 1.051)	0.680
Animal fat/vegetable fat	0.374	0.415	1.453 (0.644 - 3.280)	0.368
Carbohydrate (g/day)	-0.004	0.004	0.996 (0.989 - 1.004)	0.330
EPA (mg/day)	-0.001	0.002	0.999 (0.996 - 1.003)	0.743
DHA (mg/day)	0.001	0.001	1.001 (0.998 - 1.004)	0.517
Infection deaths (n=43)				
Vaiables	β	SE	HR (95%CI)	p value
Sex (men=0,women=1)	-1.591	0.396	0.204 (0.094 - 0.442)	0.000
Age (years)	0.142	0.020	1.153 (1.109 - 1.198)	<0.0001
Energy (kcal/day)	-0.001	0.001	0.999 (0.997 - 1.001)	0.465
Animal protein (g/day)	-0.013	0.032	0.987 (0.926 - 1.052)	0.692
Vegetable protein (g/day)	-0.006	0.027	0.994 (0.943 - 1.048)	0.831
Animal protein/vegetable protein	0.193	1.060	1.213 (0.152 - 9.684)	0.855
Animal fat (g/day)	0.042	0.040	1.043 (0.964 - 1.128)	0.299
Vegetable fat (g/day)	0.013	0.040	1.014 (0.937 - 1.097)	0.739
Animal fat/vegetable fat	-0.608	0.842	0.544 (0.105 - 2.834)	0.470
Carbohydrate (g/day)	0.001	0.007	1.001 (0.989 - 1.015)	0.820
EPA (mg/day)	-0.009	0.005	0.991 (0.981 - 1.001)	0.068
DHA (mg/day)	0.006	0.003	1.006 (0.999 - 1.013)	0.080
CVD deaths (n=47)				
Vaiables	β	SE	HR (95%CI)	p value
Sex (men=0,women=1)	-0.780	0.342	0.458 (0.234 - 0.896)	0.023
Age (years)	0.118	0.017	1.126 (1.088 - 1.164)	<0.0001
Energy (kcal/day)	-0.001	0.001	0.999 (0.997 - 1.001)	0.314
Animal protein (g/day)	0.032	0.024	1.032 (0.984 - 1.082)	0.193
Vegetable protein (g/day)	0.002	0.023	1.002 (0.958 - 1.048)	0.933
Animal protein/vegetable protein	0.202	0.799	1.224 (0.256 - 5.855)	0.801
Animal fat (g/day)	-0.013	0.032	0.987 (0.928 - 1.051)	0.692
Vegetable fat (g/day)	0.036	0.034	1.037 (0.970 - 1.108)	0.288
Animal fat/vegetable fat	0.176	0.653	1.192 (0.331 - 4.289)	0.788
Carbohydrate (g/day)	0.003	0.006	1.003 (0.991 - 1.016)	0.581
EPA (mg/day)	-0.002	0.005	0.998 (0.989 - 1.008)	0.751
DHA (mg/day)	-0.001	0.003	0.999 (0.992 - 1.005)	0.700

Abbereviations;
EPA; Eicosapentaenoic acid,
DHA; Docosahexaenoic acid

Table 5. Hazard ratios of all-cause, cancer, infection and CVD mortality using the lowest quartile as the reference

	Quartiles of albumin (g/dl)			
	Q1 (Lowest) (≦4.2)	Q2 (4.2-4.4)	Q3 (4.4-4.6)	Q4 (Highest) (≧4.6)
All-cause deaths				
Total,n	476	476	476	477
No.of deaths	145	82	65	45
Model 1	1.00	0.53 (0.39 - 0.71) **	0.40 (0.29 - 0.55) **	0.28 (0.20 - 0.40) **
Model 2	1.00	0.66 (0.49 - 0.89) *	0.63 (0.46 - 0.87) *	0.56 (0.38 - 0.81) *
Model 3	1.00	0.68 (0.50 - 0.93) *	0.65 (0.46 - 0.93) *	0.59 (0.39 - 0.88) *
Cancer deaths				
Total,n	476	476	476	477
No.of deaths	39	24	20	16
Model 1	1.00	0.57 (0.34 - 0.95)	0.47 (0.27 - 0.80) *	0.36 (0.20 - 0.64) *
Model 2	1.00	0.69 (0.42 - 1.15)	0.67 (0.39 - 1.16)	0.62 (0.34 - 1.14)
Model 3	1.00	0.84 (0.50 - 1.43)	0.70 (0.37 - 1.31)	0.81 (0.42 - 1.57)
Infection deaths				
Total,n	476	476	476	477
No.of deaths	24	8	5	6
Model 1	1.00	0.30 (0.14 - 0.67) *	0.18 (0.07 - 0.48) *	0.21 (0.09 - 0.52) *
Model 2	1.00	0.39 (0.18 - 0.88) *	0.34 (0.13 - 0.89) *	0.53 (0.21 - 1.34)
Model 3	1.00	0.37 (0.16 - 0.84) *	0.35 (0.13 - 0.97) *	0.44 (0.16 - 1.26)
CVD deaths				
Total,n	476	476	476	477
No.of deaths	23	13	9	2
Model 1	1.00	0.52 (0.26 - 1.02)	0.35 (0.16 - 0.76) *	0.08 (0.02 - 0.32) **
Model 2	1.00	0.65 (0.33 - 1.29)	0.54 (0.25 - 1.18)	0.14 (0.03 - 0.61) *
Model 3	1.00	0.65 (0.32 - 1.31)	0.62 (0.27 - 1.40)	0.15 (0.03 - 0.66) *

Abbreviation;
CVD; Cerebro-cardiovascular diseases
Model 1; crude, Model 2; age and sex adjusted, Model 3; adjusted for age, sex, HDL-cholesterol, LDL-cholesterol, triglycerides and estimatied glomerular filter.
*p<0.01 vs. Q1, **p<0.001 vs.Q1

Figure Legends

Figure 1: A flow diagram of the assessment of the study subjects.

Figure 2: Cumulative survival curves stratified by serum albumin quartiles in subjects estimated by the Kaplan-Meier method. There was a significant trend across the quartiles ($p<0.0001$ by log-rank test).

Q1: Albumin $\leq$4.2g/dl (n=476)

Q2: Albumin 4.2-4.4g/dl (n=476)

Q3: Albumin 4.4-4.6g/dl (n=476)

Q4: Albumin $\geq$4.6g/dl (n=477)

Figure 1

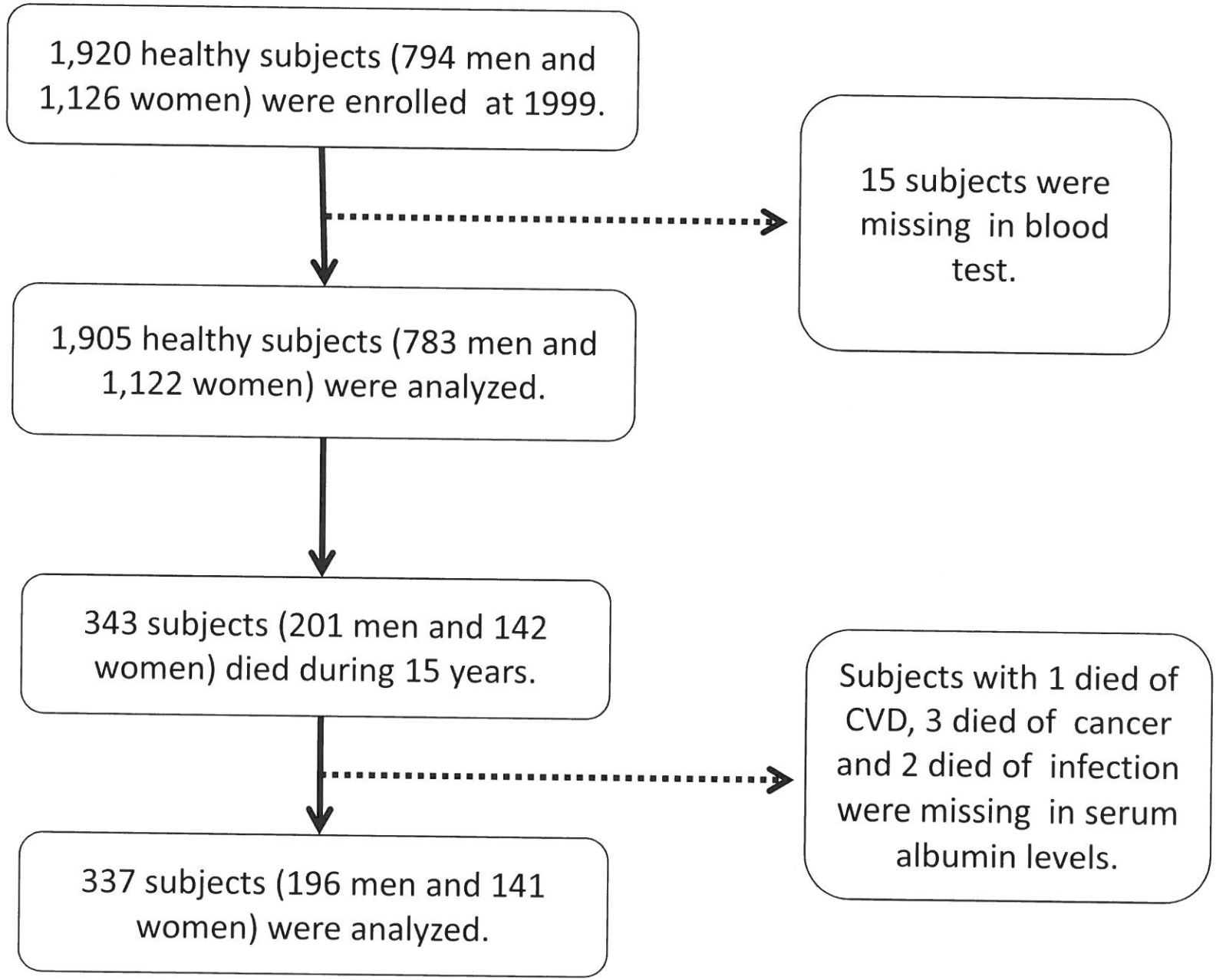
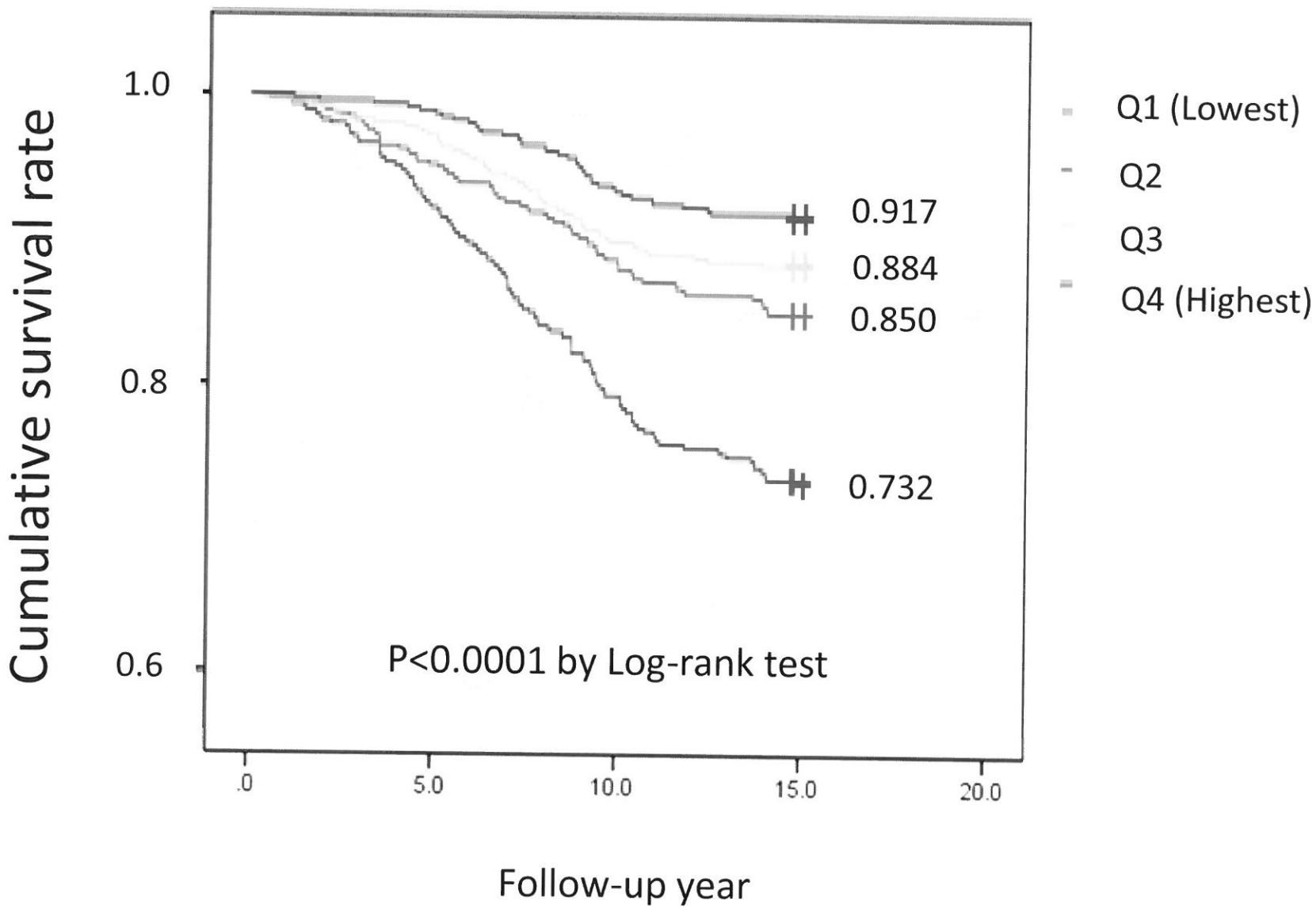

1,920 healthy subjects (794 men and 1,126 women) were enrolled at 1999.
15 subjects were missing in blood test.
1,905 healthy subjects (783 men and 1,122 women) were analyzed.
343 subjects (201 men and 142 women) died during 15 years.
Subjects with 1 died of CVD, 3 died of cancer and 2 died of infection were missing in serum albumin levels.
337 subjects (196 men and 141 women) were analyzed.

Figure 2


Cumulative survival rate
1.0
0.8
0.6
0.917
0.884
0.850
0.732
P<0.0001 by Log-rank test
.0
5.0
10.0
15.0
20.0
Follow-up year
Q1 (Lowest)
Q2
Q3
Q4 (Highest)