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Associations of Serum Folate and Homocysteine Concentrations with All-Cause, Cardiovascular Disease, and Cancer Mortality in Men and Women in Korea: the Cardiovascular Disease Association Study

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A B S T R A C T

Background: Evidence on the association of serum folate and homocysteine concentrations with risk of mortality in the general population is unclear.
Objectives: This study aimed to examine the associations of serum folate and homocysteine concentrations with all-cause, CVD, and cancer mortality risk in Korean men and women aged ≥40 y.
Methods: In this population-based prospective cohort study, serum folate and homocysteine concentrations were measured in a subset of participants enrolled between 2005 and 2012. A total of 21,260 participants were linked to mortality data from the survey date to 31 December 2019. Cox proportional hazards models and restricted cubic splines were used to identify the associations of serum folate and homocysteine concentrations with mortality.
Results: During a median follow-up of 12.3 y, 2501, 549, and 842 deaths were attributed to all-cause, CVD, and cancer, respectively. The prevalence of folate deficiency and hyperhomocysteinemia were higher in men than in women. In men, a nonlinear inverse association was observed between serum folate concentrations and all-cause mortality. Men in the third quartile of serum folate concentrations exhibited a lower risk of all-cause mortality (HR: 0.85; 95% CI: 0.73, 0.99) than those in the lowest quartile. Serum homocysteine concentration was positively associated with all-cause and CVD mortality. Men and women in the highest compared with those in the lowest serum homocysteine quartile showed a higher risk of CVD mortality (HR: 1.60; 95% CI: 1.07, 2.39; and HR: 1.79; 95% CI: 1.11, 2.89, respectively). Hyperhomocysteinemia combined with folate deficiency was associated with increased all-cause, CVD, and cancer-related mortality rates.
Conclusions: Higher serum homocysteine and lower serum folate concentrations were associated with an increased risk of all-cause, CVD, and cancer-related mortality in Korean adults. The finding of a nonlinear inverse relationship between serum folate concentration and mortality in men warrants further investigation.

Keywords: serum folate, homocysteine, mortality, cohort study, epidemiology

Introduction

Folates, which are water-soluble B vitamins, include naturally occurring food folate and synthetic folic acid used in fortified foods and dietary supplements [1]. Adequate folate intake is vital for cell division and homeostasis because of its role in 1-carbon metabolism involved in DNA synthesis and methylation reactions [1, 2]. Therefore, folate and metabolically related B vitamins are essential throughout life, particularly in the early stages of human development. Although the association between folate status and health outcomes beyond neural tube defects has not been fully explored, current epidemiologic evidence suggests that folate deficiency may be associated with an increased risk of chronic diseases, such as CVDs, cancer, and neurologic disorders [3, 4].

The potential benefit of folate in reducing CVD risk may be achieved either through its homocysteine-lowering effects or through its independent effects on blood vessels [5]. In the general population, folate and vitamin B12 deficiencies are the most common causes of elevated levels of homocysteine, a

Abbreviations: CAVAS, Cardiovascular Disease Association Study; KDCA, Korea Disease Control and Prevention Agency; KNIH, Korea National Institute of Health; KoGES, Korean Genome and Epidemiology Study.

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potentially cytotoxic sulfur-containing amino acid formed by demethylation of methionine [6]. Observational studies have shown that elevated homocysteine levels are associated with an increased risk of CVD and mortality [7–9]. Experimental studies have shown several potential cellular mechanisms by which increased homocysteine levels may play a role in endothelial dysfunction and atherosclerosis, such as induction of inflammation, oxidative stress, and endoplasmic reticulum stress [10, 11]. However, the causal relationship between hyperhomocysteinemia and CVD risk is still unclear because most homocysteine-lowering intervention trials on the effect of folic acid and vitamin B supplementation on CVD outcomes have yielded largely null results [12–14].

Observational studies have provided limited and conflicting evidence regarding the association between folate levels and mortality. A few recent US studies, conducted after the introduction of folate fortification, found a J-shaped relationship between serum folate concentrations and all-cause and CVD mortality [15, 16]. Therefore, concerns have been raised about the potential adverse effects of excessive folic acid intake, although adverse events have not been reported [17]. Moreover, although a meta-analysis of case–control studies suggested that elevated homocysteine and low folate concentrations are associated with an increased overall risk of cancer [18], few longitudinal studies have been conducted on the role of homocysteine and folate in cancer outcomes. Furthermore, data on the relationship between circulating folate and homocysteine concentrations and all-cause and cause-specific mortality are lacking in Asian populations.

This study aimed to examine the association of serum folate and homocysteine concentrations with risk of all-cause, CVD, and cancer mortality in a large prospective cohort of Korean men and women aged ≥40 y, in a setting in which folic acid fortification is not mandatory.

Material and Methods

The Cardiovascular Disease Association Study (CAVAS) is an ongoing prospective cohort study established as part of the Korean Genome and Epidemiology Study (KoGES) conducted by the Korea National Institute of Health (KNIH) [19]. The CAVAS was designed to investigate risk factors for CVD in Korean adults, aged ≥40 y, living in rural areas. Between 2005 and 2012, 28,337 men and women were recruited from 11 counties. Blood samples were collected under fasting conditions during the baseline survey. The separated plasma and serum samples were stored at −185°C in the National Biobank of Korea until use [20]. All participants provided informed consent, and ethical approval was obtained from the institutional review boards of the KoGES group collaborators and the Korea Disease Control and Prevention Agency (KDCA). Follow-up surveys have been conducted in 6 of the 11 counties, with a baseline sample size of 21,714 participants. Of them, serum samples from 21,265 participants were used to generate additional biomarker data, including folate and homocysteine, between 2018 and 2019. In participants with serum folate ($n = 21,265$) or homocysteine ($n = 21,253$) results, those with missing data on mortality ($n = 3$), or who died within 1 mo ($n = 2$) were excluded, leaving 21,260 participants (8169 men and 13,091 women) in the analysis, of whom 21,248 participants were included in the homocysteine analyses. This study was approved by the institutional review board of KDCA (2021-04-02-2C-A, 2018-03-05-4C-A, and 2017-05-03-4C-A).

Measurement of serum folate and homocysteine concentrations

The serum samples were sent to Seoul Clinical Laboratories for testing. Serum folate concentrations were measured using an electrochemiluminescence immunoassay (Elecsys Folate III, Cobas e602; Roche Diagnostics), with interassay coefficients of variability ranging from 2.62% to 6.16%, depending on the serum folate concentration. Serum homocysteine concentrations were measured using an enzymatic assay on a Cobas c702 analyzer (Roche Diagnostics), with interassay coefficients of variability ranging from 1.78% to 2.64%. Folate deficiency was defined as a serum folate concentration of <10 nmol/L (4 ng/mL) [21], and hyperhomocysteinemia was defined as a serum homocysteine concentration of > 15μmol/L [22].

Ascertainment of mortality

Data on deaths were obtained by linking the CAVAS data to the Cause of Death Statistics in Statistics Korea to 31 December 2019. The cause of death was classified according to the International Statistical Classification of Diseases and Related Health problems, 10th Revision [23]. The primary outcomes were death from all causes, CVD (I00–I99), and cancer (C00–C97). CVD mortality was subdivided into heart disease (I20–I51) and stroke (I60–I69). The 2 most common cancer types in the cohort were gastrointestinal cancer (C15-C26) and respiratory cancer (C30–C39). The follow-up time was calculated from baseline to the date of death or the end of follow-up (December 31, 2019).

Covariate assessment

Data were collected using interviewer-administered questionnaires, physical examination, and subsequent laboratory testing [19]. Sociodemographic and lifestyle factors and a medical history were obtained using questionnaires. Participants were asked about the frequency and amount of alcohol consumed over the past year, and the average daily alcohol consumption was calculated (ethanol in g/d). Participants were also asked about their smoking history, and pack-years of smoking were calculated by multiplying the number of packs of cigarettes smoked per day by the number of years smoked. Participants were asked about their use of dietary supplements, such as single vitamins and multivitamins, during the past year. Dietary intake over the past year was assessed using a validated food frequency questionnaire [24]. The mean daily intake of energy and nutrients were calculated using the Food Composition Table of Korea [25]. Participants were also asked whether they exercised regularly (enough to work up sweating), and if yes, the minutes of exercise per week. Anthropometric and blood pressure measurements and fasting blood samples were obtained using standardized procedures. Serum concentrations of fasting glucose, albumin, and creatinine were analyzed at baseline, and cystatin C was measured in 2018 and 2019. BMI was calculated as body weight divided by height squared (kg/m^2). The estimated glomerular filtration rate was calculated using the creatinine-cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation [26]. Information on self-reported physician-diagnosed diseases was obtained using a

questionnaire. In this study, hypertension was defined as a previous diagnosis, systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg. Diabetes was defined as a previous diagnosis or fasting glucose level of ≥126 mg/dL. CVD was defined as a previous diagnosis of angina, myocardial infarction, or cerebrovascular disease. Cancer cases at baseline, except for nonmelanoma skin cancer, were identified by linkage to the Korea Central Cancer Registry.

Statistical analysis

The baseline characteristics of the participants were summarized by sex. Differences in baseline characteristics by sex were assessed using the χ^2 test for categorical variables and Student *t* test or Mann–Whitney *U* test for continuous variables. The Spearman correlation coefficient was used to assess the relationship between serum folate and homocysteine concentrations. Cox proportional hazards models were used to estimate adjusted HRs and 95% CIs of the association of serum folate and homocysteine concentrations with risk of all-cause, CVD, and cancer mortality. Analyses were conducted for men and women combined and separately to provide evidence on the overall and sex-specific associations between biomarkers and mortality. Serum concentrations of folate and homocysteine were analyzed as quartiles and diagnostic cut points, using the lowest category as the reference group. Multivariable model 1 was stratified by age group (40–59, 60–69, or ≥70 y) and sex and adjusted for age at baseline (continuous, years). Multivariable model 2 was additionally adjusted for baseline year (continuous, years), education level (below elementary school, elementary school, middle school, high school, or above high school for men; below elementary school, elementary school, middle school, above middle school for women), alcohol consumption (none, <5, 5–14.9, 15–29.9, ≥30 g/d for men; none, <5, ≥5 g/d for women), smoking status (none, <20, 20–29.9, or ≥30 pack-y for men; none, <10, ≥10 pack-y for women), dietary supplement use (multivitamins, B vitamins, or folic acid; yes or no), physical activity (none, ≤median (3.5 h/wk), > median), BMI (continuous, kg/m^2), history of diabetes (yes or no), hypertension (yes or no), CVDs (yes or no), cancer (yes or no), serum albumin concentration (continuous, g/dL), and estimated glomerular filtration rate (<60 or ≥60 mL/min/1.73 m^2). Serum homocysteine and folate concentrations were mutually adjusted (continuous, log-transformed). *P* values for trend were calculated by assigning the median value of each quartile of exposures into the model as a continuous variable. The proportional hazard assumption was tested using time-dependent interaction terms, and no strong evidence of violation of the assumption was observed. Furthermore, the nonlinearity of dose-response associations was examined using restricted cubic splines with 4 knots (5th, 35th, 65th, and 95th percentiles) in a fully adjusted model using the 5th percentile as the reference point [27, 28].

The interaction between serum folate and homocysteine concentrations was assessed using a log-likelihood ratio test to compare models with and without the interaction term

TABLE 1
Baseline characteristics of study participants by sex

Characteristics	All (*N* = 21,260)	Men (*n* = 8169)	Women (*n* = 13,091)	*P*
Age (y)	58.9 ± 9.7	59.7 ± 9.5	58.5 ± 9.8	<0.001
Education levels				
Below middle school	11,796 (55.6)	3410 (41.9)	8386 (64.2)	<0.001
Middle school or above	9401 (44.4)	4730 (58.1)	4671 (35.8)	
BMI (kg/m^2)	24.4 ± 3.2	24.2 ± 3.0	24.6 ± 3.3	<0.001
Physical activity (h/wk)	1.6 ± 3.5	1.8 ± 3.8	1.5 ± 3.3	<0.001
Current alcohol drinkers	8897 (41.9)	5300 (64.9)	3597 (27.5)	<0.001
Ever-smokers	6466 (30.5)	5880 (72.0)	586 (4.5)	<0.001
Serum concentrations				
Folate (nmol/L)	18.4 (13.4–24.8)	15.8 (11.4–21.7)	20.1 (14.9–26.4)	<0.001
<10	2112 (9.9)	1401 (17.2)	711 (5.4)	<0.001
Homocysteine (μmol/L)	10.1 (8.3–12.4)	11.8 (9.9–14.3)	9.1 (7.7–11.0)	<0.001
>15	2446 (11.5)	1683 (20.6)	763 (5.8)	<0.001
Daily dietary intake				
Energy (kcal)	1639.9 ± 505.2	1769.3 ± 516.6	1559.1 ± 480.7	<0.001
Fiber (g/1000 kcal)	3.2 ± 1.3	3.1 ± 1.2	3.3 ± 1.3	<0.001
Folate (μg/1000 kcal)	114.2 ± 51.1	107.5 ± 45.6	118.3 ± 53.8	<0.001
Multivitamin/vitamin B/folic acid use[1]	2941 (14.6)	997 (12.9)	1944 (15.7)	<0.001
Disease history				
Hypertension[2]	7080 (33.4)	2734 (33.5)	4346 (33.2)	0.675
Diabetes[3]	2542 (12.0)	1159 (14.2)	1383 (10.6)	<0.001
CVD[4]	1554 (7.3)	631 (7.7)	923 (7.1)	0.065
Cancer[5]	601 (2.8)	271 (3.3)	330 (2.5)	0.001
eGFR[6] < 60 mL/min/1.73 m^2	937 (4.4)	339 (4.2)	598 (4.6)	0.150

Data are expressed as the mean ± standard deviation, median (interquartile range), or number (%).
eGFR, estimated glomerular filtration rate.
[1] Vitamin B users (25 men and 52 women) or folic acid users (4 women).
[2] Hypertension was defined by self-reported previous diagnosis and/or systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥90 mm Hg.
[3] Diabetes was defined by a self-reported previous diagnosis and/or a fasting glucose level of ≥126 mg/dL.
[4] CVD was defined as a self-reported history of angina, myocardial infarction, or cerebrovascular disease.
[5] Cancer cases except nonmelanoma skin cancer were identified through linkage with the Korea Central Cancer Registry.
[6] eGFR was calculated using the equation from the creatinine-cystatin C-based Chronic Kidney Disease Epidemiology Collaboration.

(hyperhomocysteinemia × folate deficiency) in the model. In the subgroup analyses of other risk factors, potential effect modifiers of the association between subgroups were tested. Furthermore, sensitivity analyses were performed, restricting the analysis to participants without a history of CVD or cancer at baseline, aged <80 y, and with a follow-up duration of at least 3 y. When we examined the associations of serum folate and homocysteine concentrations with non–CVD mortality, the results were found to be similar to those of all-cause mortality and were, therefore, not presented.

A 2-tailed *P* value of <0.05 was considered to be statistically significant. All analyses were conducted using SAS version 9.4 (SAS Institute).

Results

During the 246,521 person-y of follow-up, 2501 deaths were documented, with 549 and 842 deaths attributed to CVD and cancer, respectively. The mean (SD) age at baseline was 58.9 (9.7) y, and the median follow-up time was 12.3 y. The baseline characteristics of the men and women are summarized in Table 1. The prevalence of folate deficiency and hyperhomocysteinemia was higher in men (17% and 21%, respectively) than in women (5% and 6%, respectively). The median serum folate and homocysteine concentrations were 15.8 nmol/L and 11.8 μmol/L, respectively, in men, and 20.1 nmol/L and 9.1 μmol/L, respectively, in women. The mean dietary folate intake (per 1000 kcal) and the prevalence of multivitamin, B vitamin, or folic acid use was higher in women than in men. Moreover, the proportions of smokers, alcohol drinkers, and participants with a history of diabetes or cancer were higher in men than in women. The serum folate concentration was negatively correlated with the serum homocysteine concentration (Spearman correlation coefficient: −0.41 in men and −0.34 in women). The baseline participant characteristics according to the diagnostic cut point of serum folate and homocysteine concentrations are given in Supplemental Table 1. Regardless of sex, participants with hyperhomocysteinemia were more likely to be older, be smokers, and present with a chronic disease and impaired renal function and were less likely to engage in regular physical activity and use dietary supplements. Similar characteristics were observed in the participants with folate deficiency.

The HRs and 95% CIs of serum folate concentration for all-cause and cause-specific mortality are summarized in Table 2. Inverse associations between serum folate concentrations and mortality were attenuated after adjustment for potential confounders, especially after adjusting for the serum homocysteine concentration. In fully adjusted models, participants with a serum folate concentration in the third quartile exhibited a lower risk of all-cause mortality than those with a serum folate concentration in the lowest quartile, in men (HR: 0.85; 95% CI: 0.73, 0.99) and overall (HR: 0.87; 95% CI: 0.77, 0.98). Consistently, significant nonlinear inverse associations were observed between serum folate concentrations and all-cause mortality in the dose-response analyses (Figure 1A, B). In men, the all-cause mortality rate decreased with increasing serum folate concentration and leveled off at approximately 20 nmol/L. No significant linear or nonlinear associations were observed between serum folate concentrations and cause-specific mortality (data not shown). Positive associations were observed between serum homocysteine concentrations and all-cause and cause-specific mortality (Table 2). The HRs (95% CIs) comparing extreme quartiles of serum homocysteine concentration were 1.47 (1.24, 1.75) and 1.46 (1.17, 1.82) for all-cause mortality and 1.60 (1.07, 2.39) and 1.79 (1.11, 2.87) for CVD mortality in men and women, respectively. In addition, significant linear relationships were observed for all-cause (Figure 1D–F) and cause-specific mortality (data not shown), except for cancer mortality in women.

When we further examined the associations of serum folate and homocysteine concentrations with heart disease or stroke mortality, significant associations were limited to stroke mortality (Supplemental Table 2). In men, comparing the highest with the lowest quartile, serum folate (HR: 0.47; 95% CI: 0.25, 0.87) and homocysteine (HR: 2.48; 95% CI: 1.22, 5.04) concentrations were significantly associated with stroke mortality. In women, those in the third quartile of serum homocysteine concentrations exhibited a 2-fold higher risk of stroke mortality than those in the first quartile. Moreover, men in the third quartile of serum folate concentrations showed a lower risk of respiratory cancer mortality (HR: 0.60; 95% CI: 0.38, 0.96) than those in the lowest quartile. A suggestive positive association was observed between serum hocmocysteine concentrations and gastrointestinal cancer mortality in men.

The prevalence of folate deficiency and hyperhomocysteinemia was much higher in men (8.1%) than in women (1.4%) (Table 3). A significant interaction was observed between folate deficiency and hyperhomocysteinemia for all-cause mortality in women (*P*-interaction: 0.01). Compared with women with normal serum folate and homocysteine concentrations, women with a folate deficiency and hyperhomocysteinemia exhibited significantly higher all-cause mortality (HR: 1.74, 95% CI: 1.28, 2.37). The highest HRs for CVD mortality in men, and for cancer mortality in women, were observed in participants with both folate deficiency and hyperhomocysteinemia, although no significant interaction was observed in cause-specific mortality.

The association between hyperhomocysteinemia and all-cause mortality differed by age and chronic disease status in men and current alcohol drinking status in women (*P*-interaction < 0.05 for all) (Table 4). In men, the positive association between serum homocysteine concentrations and all-cause mortality appeared to be stronger in older participants and those with chronic disease at baseline and tended to be stronger in those with a BMI of <25 kg/m^2 and dietary supplement users. In women, the significant positive association between serum homocysteine concentrations and all-cause mortality was limited to current alcohol drinkers. No significant interaction was found between folate deficiency and several risk factors for all-cause mortality (Supplemental Table 3).

Diagnostic cut point analyses showed consistent and robust associations of serum folate and homocysteine concentrations with all-cause and CVD mortality (Supplemental Table 4). The results were generally robust when the analyses were restricted to participants without a history of CVD or cancer at baseline, <80 y, and with a follow-up duration of at least 3 y.

TABLE 2
Association of serum folate and homocysteine concentrations with all-cause, CVD, and cancer mortality

Serum concentrations	Persons at risk (*n*)	Person-years	Deaths (*n*)	All-cause mortality		Deaths (*n*)	CVD mortality		Deaths (*n*)	Cancer mortality	
				Model 1[1]	Model 2[2]		Model 1[1]	Model 2[2]		Model 1[1]	Model 2[2]
Folate (nmol/L)											
All (N = 21,260)											
Q1 (<13.4)	5308	58,998	884	Reference	Reference	202	Reference	Reference	291	Reference	Reference
Q2 (13.4–18.4)	5331	62,038	585	0.81 (0.73, 0.90)	0.94 (0.84, 1.05)	135	0.81 (0.65, 1.00)	0.94 (0.74, 1.17)	197	0.84 (0.70, 1.01)	0.97 (0.80, 1.17)
Q3 (18.5–24.7)	5312	63,115	501	0.75 (0.67, 0.84)	0.87 (0.77, 0.98)	106	0.68 (0.54, 0.87)	0.81 (0.63, 1.05)	165	0.76 (0.63, 0.92)	0.87 (0.71, 1.07)
Q4 (≥24.8)	5309	62,370	531	0.82 (0.73, 0.91)	0.99 (0.88, 1.12)	106	0.69 (0.54, 0.88)	0.88 (0.68, 1.14)	189	0.89 (0.74, 1.07)	1.07 (0.87, 1.31)
P-trend				<0.001	0.794		0.001	0.249		0.197	0.615
Men (n = 8169)											
Q1 (<11.4)	2043	21,952	471	Reference	Reference	95	Reference	Reference	166	Reference	Reference
Q2 (11.4–15.7)	2040	23,280	336	0.72 (0.63, 0.83)	0.87 (0.75, 1.00)	72	0.77 (0.57, 1.05)	0.91 (0.66, 1.26)	128	0.77 (0.61, 0.97)	0.92 (0.72, 1.17)
Q3 (15.8–21.6)	2041	23,591	322	0.69 (0.60, 0.80)	0.85 (0.73, 0.99)	57	0.61 (0.44, 0.86)	0.78 (0.55, 1.11)	112	0.67 (0.53, 0.85)	0.83 (0.64, 1.07)
Q4 (≥21.7)	2045	23,623	336	0.68 (0.59, 0.78)	0.87 (0.74, 1.01)	56	0.57 (0.41, 0.80)	0.78 (0.54, 1.12)	129	0.72 (0.57, 0.91)	0.93 (0.72, 1.20)
P-trend				<0.001	0.128		0.001	0.142		0.009	0.602
Women (n = 13,091)											
Q1 (<14.9)	3283	37,604	324	Reference	Reference	89	Reference	Reference	85	Reference	Reference
Q2 (14.9–20.0)	3254	38,442	250	0.91 (0.77, 1.07)	0.97 (0.82, 1.15)	65	0.91 (0.66, 1.25)	0.98 (0.71, 1.36)	80	1.06 (0.78, 1.44)	1.09 (0.79, 1.49)
Q3 (20.1–26.3)	3279	39,226	223	0.87 (0.74, 1.04)	0.97 (0.81, 1.16)	65	1.01 (0.73, 1.39)	1.18 (0.84, 1.67)	61	0.85 (0.61, 1.18)	0.87 (0.62, 1.23)
Q4 (≥26.4)	3275	38,803	239	0.92 (0.78, 1.09)	1.03 (0.86, 1.24)	50	0.76 (0.54, 1.08)	0.87 (0.60, 1.26)	81	1.11 (0.82, 1.51)	1.12 (0.80, 1.56)
P-trend				0.338	0.728		0.176	0.602		0.680	0.668
Homocysteine (μmol/L)											
All (N = 21,248)											
Q1 (<8.3)	5310	65,893	290	Reference	Reference	45	Reference	Reference	113	Reference	Reference
Q2 (8.3–10.0)	5325	63,906	450	1.02 (0.88, 1.19)	1.08 (0.93, 1.26)	101	1.48 (1.04, 2.11)	1.53 (1.07, 2.19)	157	0.94 (0.73, 1.20)	1.01 (0.79, 1.29)
Q3 (10.1–12.4)	5302	60,870	659	1.14 (0.99, 1.32)	1.20 (1.04, 1.39)	150	1.70 (1.20, 2.39)	1.68 (1.19, 2.39)	220	0.99 (0.78, 1.26)	1.06 (0.83, 1.35)
Q4 (≥12.5)	5311	55,699	1099	1.57 (1.36, 1.80)	1.58 (1.35, 1.84)	252	2.38 (1.70, 3.34)	2.23 (1.55, 3.21)	351	1.29 (1.02, 1.63)	1.35 (1.04, 1.75)
P-trend				<0.001	<0.001		<0.001	<0.001		0.001	0.003
Men (n = 8163)											
Q1 (<9.9)	2037	24,686	269	Reference	Reference	46	Reference	Reference	100	Reference	Reference
Q2 (9.9–11.7)	2042	24,278	288	0.96 (0.81, 1.13)	1.02 (0.86, 1.20)	57	1.09 (0.74, 1.61)	1.11 (0.75, 1.65)	112	1.01 (0.77, 1.33)	1.03 (0.78, 1.35)
Q3 (11.8–14.2)	2043	22,648	386	1.21 (1.04, 1.42)	1.29 (1.10, 1.52)	65	1.16 (0.79, 1.69)	1.17 (0.79, 1.74)	150	1.29 (1.00, 1.67)	1.28 (0.98, 1.67)
Q4 (≥14.3)	2041	20,761	519	1.55 (1.33, 1.80)	1.47 (1.24, 1.75)	111	1.85 (1.30, 2.64)	1.60 (1.07, 2.39)	172	1.41 (1.10, 1.82)	1.25 (0.94, 1.67)
P-trend				<0.001	<0.001		<0.001	0.011		0.002	0.088
Women (n = 13,085)											
Q1 (<7.7)	3282	41,024	139	Reference	Reference	26	Reference	Reference	53	Reference	Reference
Q2 (7.7–9.0)	3248	39,647	175	0.89 (0.71, 1.12)	0.96 (0.77, 1.21)	29	0.73 (0.43, 1.24)	0.79 (0.46, 1.34)	71	1.07 (0.75, 1.54)	1.14 (0.79, 1.64)
Q3 (9.1–10.9)	3289	38,345	252	1.07 (0.86, 1.32)	1.15 (0.93, 1.43)	75	1.50 (0.96, 2.36)	1.56 (0.98, 2.47)	74	0.97 (0.67, 1.39)	1.07 (0.74, 1.55)
Q4 (≥11.0)	3266	34,979	470	1.44 (1.17, 1.76)	1.46 (1.17, 1.82)	139	1.83 (1.18, 2.84)	1.79 (1.11, 2.87)	109	1.11 (0.78, 1.58)	1.27 (0.86, 1.87)
P-trend				<0.001	<0.001		<0.001	0.001		0.598	0.265

Values are given as hazard ratio (95% confidence interval).
[1] Model 1 stratified by age group and sex, if applicable, and adjusted for age at baseline.
[2] Model 2 was additionally adjusted for baseline year, education level, alcohol consumption, smoking, BMI, physical activity, and dietary supplement use; a history of diabetes, hypertension, CVD, and cancer; serum albumin concentration; and estimated glomerular filtration rate. Serum folate and homocysteine concentrations were mutually adjusted in model 2.

Discussion

This study showed that folate sufficiency was associated with a lower risk of all-cause mortality with a nonlinear inverse association in men and higher homocysteine concentrations were associated with an increased risk of all-cause and CVD mortality in men and women. The associations of serum folate and homocysteine concentrations with CVD mortality were primarily driven by their effects on stroke mortality. In the combined analysis, participants with both folate deficiency and hyperhomocysteinemia showed a significantly higher risk of all-cause, CVD, and cancer-related mortality than those with normal levels.

Previous studies regarding the relationship between circulating folate concentrations and mortality risk have shown inconsistent results. Although several epidemiologic studies have found an association between low folate concentrations and all-cause [29–34], CVD [30, 32, 33], and cancer [30, 35] mortality, some studies have found no association [36–43]. US studies have found positive [16, 44–47] or nonlinear [15, 16, 30, 32, 48] associations between folate status and mortality, before and after mandatory fortification of cereal grain products with folic acid was introduced in 1998 [49]. Consistent with several previous studies, we found that folate deficiency was associated with higher all-cause and CVD-related mortality and found a nonlinear inverse relationship between serum folate concentrations and all-cause mortality. A cohort study of US adults also found nonlinear inverse associations between serum folate concentrations and all-cause, CVD-related, and cancer-related mortality [30]. In a cohort study of Japanese adults, serum folate concentration had an inverse association with all-cause mortality for folate concentrations of <25 nmol/L [50].

Positive associations between RBC folate concentrations and all-cause and CVD mortality have been reported in US adults with diabetes [44, 46] and hypertension [45, 47] and in adults at high risk of CVD [16]. Because serum folate is an indicator of recent folate intake and RBC folate reflects a long-term folate status [3], differences in indicators may have contributed to inconsistent findings. In this study, the association between serum folate concentrations and mortality did not differ according to chronic disease status at baseline, but the inverse association was only significant in those without chronic disease. Further research is warranted regarding the effects of long-term high levels of circulating folate on mortality, with consideration of chronic disease status.

In this study, the reduced risk of CVD mortality in men with normal folate concentrations was primarily driven by a reduction in stroke-related mortality. The mechanism underlying the association between folate concentration and mortality remains unclear; however, our findings are plausible given previous research. Population-based data from the United States and Canada suggest a substantial improvement in stroke mortality since the introduction of folic acid fortification [51]. Moreover, in meta-analyses of randomized controlled trials, preventive benefits of folic acid were found for risk of stroke and CVD [12, 14]. In this study, the association between serum folate concentrations and mortality remained significant even after adjustment for serum homocysteine concentrations. In addition to the role of folate in homocysteine metabolism, folate may have beneficial effects on vascular endothelial function by increasing nitric oxide bioavailability and scavenging superoxide radicals [5, 52].

In a previous meta-analysis, the benefits of folic acid for stroke and CVD prevention were more pronounced in participants with lower baseline blood folate concentrations [12]. The potential benefits of folate may be more evident in populations with folate deficiency, and relatively low folate concentrations in men compared with that in women may partially explain the sex differences in our findings. Consistent with this study, men recorded lower median serum folate concentrations than women (12.5 vs. 17.9 nmol/L) in the seventh Korea National Health and Nutrition Examination Survey, 2016–2018 [53]. In a previous Canadian study, the prevalence of folate deficiency was higher in women than in men, and a significant inverse association between serum folate concentration and coronary heart disease mortality was observed only in women [54]. However, a few studies have found a significant sex-specific association between folate and mortality, without apparent sex differences in circulating folate concentrations [35, 55]. Further research is needed to clarify the sex-specific role of serum folate on health outcomes.

Folate and vitamin B12 deficiencies are the most common causes of elevated homocysteine concentrations and interact with other biological and environmental factors in a sex-dependent manner [56, 57]. Circulating homocysteine concentrations are typically higher in men than in women because of sex differences in creatine synthesis, estrogen levels, and environmental factors such as smoking status and disease prevalence [6, 56, 57]. Consistent with our findings, a previous meta-analysis found that elevated homocysteine concentrations were an independent predictor of all-cause and CVD mortality [8] and a dose-response meta-analysis found a linear positive relationship between homocysteine concentrations and all-cause mortality [9]. In the meta-analyses, a significant positive association between homocysteine concentrations and all-cause mortality was observed in women but not in men [8, 9]. However, owing to the limited number of sex-specific studies, further studies are needed to draw sex-specific conclusions. In this study, significant positive associations were observed in the percentile and dose-response analyses in women. The threshold for the effect of elevated homocysteine concentrations may be lower in women than in men, and this warrants further investigation.

Moreover, in this study, the association between hyperhomocysteinemia and mortality was stronger in men with older age and comorbidities and in women who drink alcohol. Most studies on the relationship between homocysteine and mortality have been conducted in older adults, and there is limited evidence of effect modification by other factors [9]. A Dutch study reported a suggestive interaction between hyperhomocysteinemia and diabetes with mortality risk [58], where hyperhomocysteinemia was found to be a stronger predictor for mortality in diabetic than in nondiabetic participants. An interaction of hyperhomocysteinemia with alcohol consumption is biologically plausible because alcohol is known to interfere with 1-carbon metabolism [6]. In addition, we observed that the association between hyperhomocysteinemia and all-cause mortality was stronger in those who reported the use of multivitamins, B vitamins, or folic acid than in nonusers. Although the causal relationship is unclear, our results suggest that the benefits of B

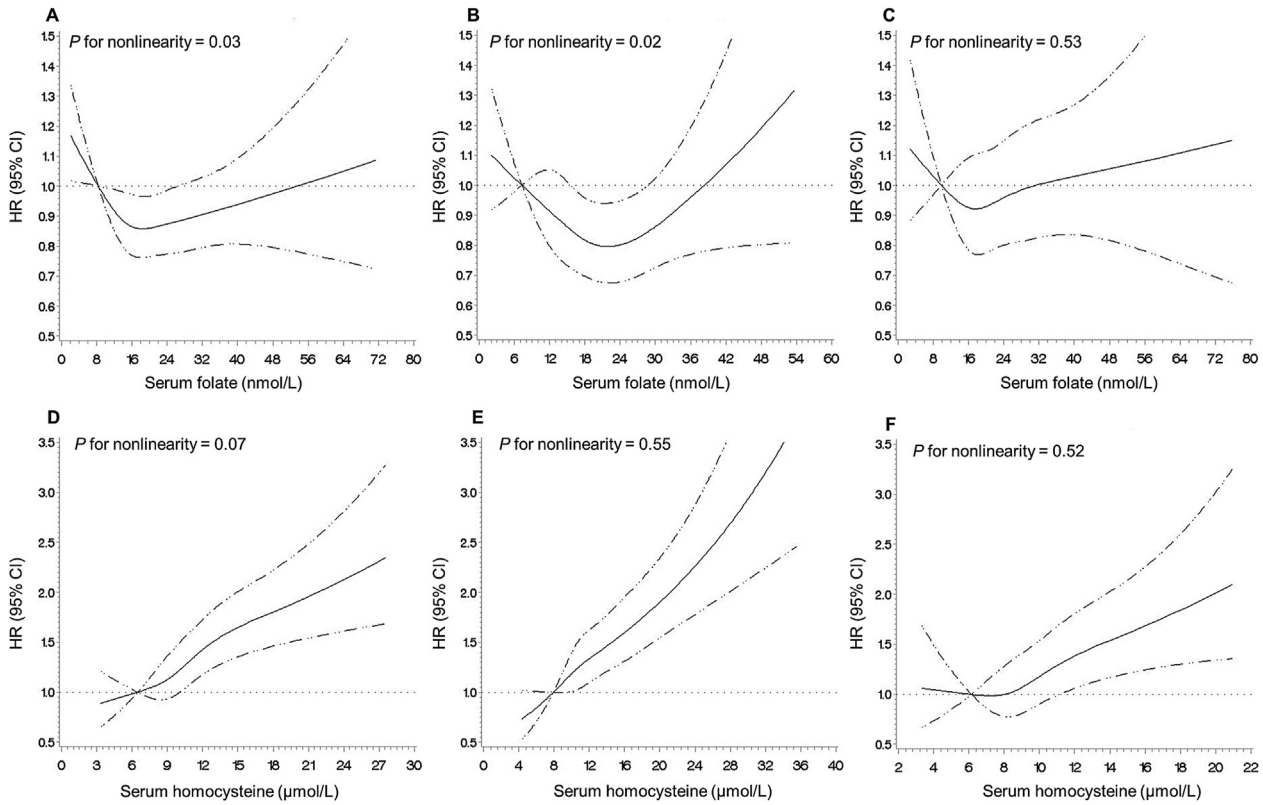

FIGURE 1. Restricted cubic spline curves of the dose-response relationship of serum folate and homocysteine concentrations with all-cause mortality. All: (A) n = 21,047 and (D) n = 21,035; men: (B): n = 8087 and (E) n = 8081; and women: (C) n = 12,959 and (F) n = 12,953. The results are stratified by age group and sex, if applicable, and adjusted for age, baseline year, education level, alcohol consumption, smoking, BMI, physical activity, and dietary supplement use; a history of diabetes, hypertension, CVD, and cancer; serum albumin concentration; and estimated glomerular filtration rate. The results are HRs for serum folate, and homocysteine concentrations are mutually adjusted. The dashed lines indicate the confidence intervals for the restricted cubic spline models. The graphs are truncated at the 99th percentile of serum folate and homocysteine values.

vitamin supplementation for hyperhomocysteinemia may not be substantial and thus warrants further investigation.

Moreover, consistent with the results of the folate analyses, the associations were more pronounced for stroke mortality. Observational studies have suggested that elevated homocysteine levels are associated with a higher risk of ischemic heart disease and stroke [7]. A recent Mendelian randomization study reported a suggestive causal effect of circulating homocysteine and B vitamins in stroke, but these associations disappeared after multiple testing corrections [59]. Further studies are warranted to determine whether folate and homocysteine concentrations are causally associated with CVD outcomes, especially for stroke.

In this study, higher homocysteine concentrations were associated with increased risk of deaths due to all cancers and gastrointestinal cancer. A moderate concentration of serum folate was associated with a reduced risk of respiratory cancer mortality in men. Furthermore, folate deficiency with hyperhomocysteinemia was associated with increased cancer mortality in men and women. Folate deficiency may be involved in cancer initiation through DNA breaks caused by uracil incorporation and DNA hypomethylation [3, 60]. In addition to the association between folate deficiency and hyperhomocysteinemia, the production of homocysteine-mediated free radicals and homocysteine thiolactone may lead to cancer [61]. However, the evidence regarding the association between cancer-related mortality and serum homocysteine [62, 63] and folate concentrations [15, 35, 48, 64] is limited and inconsistent. Moreover, a meta-analysis of randomized controlled trials of folic acid supplementation to lower homocysteine concentrations showed no significant effects on overall cancer incidence or mortality [65]. Further studies are warranted to investigate the role of folate and homocysteine concentrations in cancer incidence and mortality.

The strengths of this present study include the large sample size, long-term follow-up, and standardized measurement methods. To our knowledge, this is the first study to examine the associations of serum folate and homocysteine concentrations with all-cause and cause-specific mortality in Korean adults. Moreover, the sex-specific findings may provide a deeper understanding and insights to guide future research. However, this study has several limitations. First, serum folate and homocysteine concentrations were measured at a single time point and may not accurately reflect participants' long-term concentrations. Misclassification of exposure may have attenuated the associations. However, elevated homocysteine concentrations, a functional indicator of folate deficiency, were consistent with the results of the folate analyses. Second, there is a possibility of residual confounding. Detailed information on supplement use was lacking, and serum concentrations of other B vitamins were not measured. Finally, the generalizability of the findings may be limited because the study was conducted in adults living in selected rural areas.

TABLE 3
Associations between combinations of folate deficiency and hyperhomocysteinemia and mortality risk

Serum concentrations	Persons at risk (*n*)	Persons at risk, %	Deaths (*n*), *n*/1000 person-years	All-cause mortality, HR (95% CI)[1]	Deaths (*n*), *n*/1000 person-years	CVD mortality, HR (95% CI)[1]	Deaths (*n*), *n*/1000 person-years	Cancer mortality, HR (95% CI)[1]
All ($N = 21,248$)								
Normal folate and homocysteine	17,540	82.6	1709 (8.2)	Reference	359 (1.7)	Reference	591 (2.8)	Reference
Normal folate and hyperhomocysteinemia	1596	7.5	356 (21.8)	1.26 (1.11, 1.42)	86 (5.3)	1.39 (1.07, 1.79)	109 (6.7)	1.21 (0.97, 1.50)
Folate deficiency and normal homocysteine	1262	5.9	194 (13.7)	1.33 (1.14, 1.54)	48 (3.4)	1.67 (1.23, 2.27)	66 (4.7)	1.24 (0.96, 1.60)
Folate deficiency and hyperhomocysteinemia	850	4.0	239 (28.2)	1.60 (1.38, 1.85)	55 (6.5)	1.89 (1.39, 2.57)	75 (8.8)	1.42 (1.10, 1.84)
P-interaction				0.71		0.39		0.80
Men ($n = 8163$)								
Normal folate and homocysteine	5743	70.4	861 (12.8)	Reference	148 (2.2)	Reference	329 (4.9)	Reference
Normal folate and hyperhomocysteinemia	1019	12.5	253 (24.5)	1.37 (1.18, 1.60)	54 (5.2)	1.63 (1.17, 2.28)	87 (8.4)	1.25 (0.98, 1.60)
Folate deficiency and normal homocysteine	737	9.0	155 (18.9)	1.50 (1.26, 1.79)	33 (4.0)	1.94 (1.32, 2.84)	55 (6.7)	1.37 (1.02, 1.83)
Folate deficiency and hyperhomocysteinemia	664	8.1	193 (29.2)	1.62 (1.37, 1.91)	44 (6.6)	2.13 (1.49, 3.06)	63 (9.5)	1.35 (1.02, 1.80)
P-interaction				0.06		0.16		0.29
Women ($n = 13,085$)								
Normal folate and homocysteine	11,797	90.2	848 (6.0)	Reference	211 (1.5)	Reference	262 (1.9)	Reference
Normal folate and hyperhomocysteinemia	577	4.4	103 (17.2)	1.04 (0.83, 1.30)	32 (5.3)	1.07 (0.70, 1.62)	22 (3.7)	1.01 (0.64, 1.61)
Folate deficiency and normal homocysteine	525	4.0	39 (6.6)	0.91 (0.66, 1.26)	15 (2.5)	1.32 (0.77, 2.25)	11 (1.8)	0.87 (0.47, 1.60)
Folate deficiency and hyperhomocysteinemia	186	1.4	46 (24.7)	1.74 (1.28, 2.37)	11 (5.9)	1.50 (0.80, 2.81)	12 (6.4)	1.90 (1.04, 3.49)
P-interaction				0.01		0.89		0.10

[1] The results are stratified by age group and sex, if applicable, and adjusted for age, baseline year, education level, alcohol consumption, smoking, BMI, physical activity, and dietary supplement use; a history of diabetes, hypertension, CVD, and cancer; serum albumin concentration; and estimated glomerular filtration rate.

TABLE 4
Association between serum homocysteine concentration and all-cause mortality in various subgroups

Serum homocysteine concentration (μmol/L)	Men (n = 8163)				Women (n = 13,085)			
	Deaths (n), n/1000 person-years		All-cause mortality, HR (95% CI)[1]	P-interaction	Deaths (n), n/1000 person-years		All-cause mortality, HR (95% CI)[1]	P-interaction
Subgroup	≤15	>15	>15 vs. ≤15		≤15	>15	>15 vs. ≤15	
Age (y)								
<65	377 (7.0)	92 (9.2)	1.04 (0.81, 1.35)	0.04	245 (2.3)	19 (5.8)	1.15 (0.65, 2.03)	0.34
≥65	639 (29.5)	354 (51.0)	1.37 (1.17, 1.60)		642 (16.3)	130 (28.3)	1.13 (0.91, 1.40)	
BMI (kg/m^2)								
<25	740 (16.3)	344 (33.0)	1.35 (1.16, 1.56)	0.08	556 (6.6)	98 (22.8)	1.16 (0.90, 1.48)	0.77
≥25	276 (9.2)	101 (15.6)	1.15 (0.88, 1.51)		331 (5.3)	51 (14.3)	1.21 (0.85, 1.70)	
Physical activity								
No	761 (15.2)	345 (29.1)	1.31 (1.12, 1.52)	0.69	695 (6.9)	128 (20.8)	1.18 (0.94, 1.46)	0.65
Yes	254 (10.0)	101 (19.9)	1.40 (1.07, 1.83)		191 (4.2)	21 (12.3)	1.11 (0.66, 1.84)	
Current alcohol drinking								
No	423 (16.5)	187 (32.4)	1.26 (1.03, 1.55)	0.67	699 (6.7)	111 (18.0)	1.06 (0.84, 1.33)	0.01
Yes	592 (11.9)	259 (23.2)	1.34 (1.13, 1.59)		188 (4.6)	38 (22.6)	1.57 (1.05, 2.36)	
Smoking status								
Never	209 (9.3)	76 (19.9)	1.19 (0.87, 1.63)	0.65	789 (5.6)	122 (17.1)	1.19 (0.96, 1.48)	0.47
Ever	807 (15.2)	369 (28.2)	1.33 (1.15, 1.54)		96 (16.6)	27 (37.8)	1.13 (0.68, 1.87)	
Chronic diseases at baseline[2]								
No	493 (11.5)	148 (18.2)	1.16 (0.94, 1.42)	0.02	356 (4.1)	41 (13.8)	1.16 (0.81, 1.67)	0.64
Yes	522 (16.2)	298 (33.9)	1.44 (1.21, 1.70)		530 (9.0)	107 (21.9)	1.16 (0.91, 1.48)	
Multivitamin/vitamin B/folic acid use								
No	844 (13.7)	387 (26.6)	1.23 (1.07, 1.42)	0.06	717 (6.2)	124 (18.8)	1.17 (0.94, 1.46)	0.67
Yes	98 (10.2)	33 (22.0)	2.50 (1.54, 4.05)		97 (4.5)	11 (14.0)	1.02 (0.50, 2.09)	

[1] The results are stratified by age group and sex, if applicable, and adjusted for age, baseline year, education level, alcohol consumption, smoking, BMI, physical activity, and dietary supplement use; a history of diabetes, hypertension, CVD, and cancer; serum albumin concentration; and estimated glomerular filtration rate. The results are HRs for serum folate, and homocysteine concentrations are mutually adjusted.

[2] Chronic diseases included a history of hypertension, diabetes, CVD, and cancer.

In summary, this large prospective cohort study of Korean adults suggests that lower serum folate and higher homocysteine concentrations are associated with increased risk of all-cause, CVD, and cancer mortality. Sex-specific analyses showed that the association between serum folate concentrations and mortality risk in men was nonlinear. The associations of serum folate and homocysteine concentrations with CVD mortality were primarily driven by stroke mortality, warranting further investigation.

Author disclosures

The authors report no conflicts of interest.

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The authors' responsibilities were as follows—SS, HYP: contributed to the study concept and design; BMS, HYP: contributed to the data acquisition; SS: performed the statistical analysis and drafted the manuscript; and all authors: critically reviewed and revised the manuscript and read and approved the final version of the manuscript.

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Data Availability

Data are available on request owing to privacy/ethical restrictions. The data that support the findings of this study are available on request at [https://nih.go.kr/], with the permission of the National Institute of Health, Korea Disease Control and Prevention Agency.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2023.01.023.

References

[1] L.B. Bailey, J.F. Gregory III, Folate metabolism and requirements, J Nutr 129 (4) (1999) 779–782.
[2] C. Wagner, Biochemical role of folate in cellular metabolism, Clin Res Regul Aff 18 (3) (2001) 161–180.
[3] L.B. Bailey, P.J. Stover, H. McNulty, M.F. Fenech, J.F. Gregory III, J.L. Mills, et al., Biomarkers of nutrition for development-folate review, J Nutr 145 (7) (2015) 1636S–1680S.
[4] Y. Bo, Y. Zhu, Y. Tao, X. Li, D. Zhai, Y. Bu, et al., Association between folate and health outcomes: an umbrella review of meta-analyses, Front Public Health 8 (2020), 550753.
[5] S.J. Moat, D. Lang, I.F. McDowell, Z.L. Clarke, A.K. Madhavan, M.J. Lewis, et al., Folate, homocysteine, endothelial function and cardiovascular disease, J Nutr Biochem 15 (2) (2004) 64–79.
[6] J. Schneede, H. Refsum, P.M. Ueland, Biological and environmental determinants of plasma homocysteine, Semin Thromb Hemost 26 (3) (2000) 263–279.
[7] Homocysteine Studies Collaboration, Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis, JAMA 288 (16) (2002) 2015–2022.
[8] H.Y. Peng, C.F. Man, J. Xu, Y. Fan, Elevated homocysteine levels and risk of cardiovascular and all-cause mortality: a meta-analysis of prospective studies, J Zhejiang Univ Sci B 16 (1) (2015) 78–86.
[9] R. Fan, A. Zhang, F. Zhong, Association between homocysteine levels and all-cause mortality: a dose-response meta-analysis of prospective studies, Sci Rep 7 (1) (2017) 4769.
[10] J. Zhou, R.C. Austin, Contributions of hyperhomocysteinemia to atherosclerosis: causal relationship and potential mechanisms, Biofactors 35 (2) (2009) 120–129.
[11] R.C. Austin, S.R. Lentz, G.H. Werstuck, Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease, Cell Death Differ 11 (Suppl 1) (2004) S56–S64.
[12] Y. Li, T. Huang, Y. Zheng, T. Muka, J. Troup, F.B. Hu, Folic acid supplementation and the risk of cardiovascular diseases: a meta-analysis of randomized controlled trials, J Am Heart Assoc 5 (8) (2016), e003768.
[13] A.J. Marti-Carvajal, I. Sola, D. Lathyris, M. Dayer, Homocysteine-lowering interventions for preventing cardiovascular events, Cochrane Database Syst Rev 8 (8) (2017) CD006612.
[14] D.J. Jenkins, J.D. Spence, E.L. Giovannucci, Y-i Kim, R. Josse, R. Vieth, et al., Supplemental vitamins and minerals for CVD prevention and treatment, J Am Coll Cardiol 71 (22) (2018) 2570–2584.
[15] Y. Cui, H.-L. Zhou, M.-H. Wei, W.-J. Song, D.-S. Di, R.-Y. Zhang, et al., Multiple vitamin co-exposure and mortality risk: a prospective study, Clin Nutr 41 (2) (2022) 337–347.
[16] X. Xu, W. Wei, W. Jiang, Q. Song, Y. Chen, Y. Li, et al., Association of folate intake with cardiovascular-disease mortality and all-cause mortality among people at high risk of cardiovascular-disease, Clin Nutr 41 (1) (2022) 246–254.
[17] M. Eichholzer, O. Tönz, R. Zimmermann, Folic acid: a public-health challenge, Lancet 367 (9519) (2006) 1352–1361.
[18] D. Zhang, X. Wen, W. Wu, Y. Guo, W. Cui, Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis: meta-analysis of 83 case-control studies involving 35,758 individuals, PLoS One 10 (5) (2015), e0123423.
[19] Y. Kim, B.-G. Han, KoGES Group, Cohort profile: the Korean Genome and Epidemiology Study (KoGES) Consortium, Int J Epidemiol 46 (2) (2017), e20.
[20] S.Y. Cho, E.J. Hong, J.M. Nam, B. Han, C. Chu, O. Park, Opening of the national biobank of Korea as the infrastructure of future biomedical science in Korea, Osong Public Health Res Perspect 3 (3) (2012) 177–184.
[21] World Health Organization, Serum and red blood cell folate concentrations for assessing folate status in populations, World Health Organization, 2015.
[22] P.M. Ueland, H. Refsum, S.P. Stabler, M.R. Malinow, A. Andersson, R.H. Allen, Total homocysteine in plasma or serum: methods and clinical applications, Clin Chem 39 (9) (1993) 1764–1779.
[23] World Health Organization, International statistical classification of diseases and related health problems, 10th revision, 5th ed., World Health Organization, Geneva, 2015.
[24] Y. Ahn, E. Kwon, J.E. Shim, M.K. Park, Y. Joo, K. Kimm, et al., Validation and reproducibility of food frequency questionnaire for Korean genome epidemiologic study, Eur J Clin Nutr 61 (12) (2007) 1435–1441.
[25] The Korean Nutrition Society, Food Composition Table, The Korean Nutrition Society, Seoul, 2000.
[26] L.A. Inker, N.D. Eneanya, J. Coresh, H. Tighiouart, D. Wang, Y. Sang, et al., New creatinine-and cystatin C–based equations to estimate GFR without race, N Engl J Med 385 (19) (2021) 1737–1749.
[27] F.E. Harrell, Regression modeling strategies with applications to linear models, logistic and ordinal regression, and survival analysis, 2nd ed., Springer, New York, 2015.
[28] R. Li, E. Hertzmark, M. Louie, L. Chen, D. Spiegelman, The sas lgtphcurv9 macro, Channing Laboratory, Boston, 2011.
[29] B. Gopinath, V.M. Flood, E. Rochtchina, A. Thiagalingam, P. Mitchell, Serum homocysteine and folate but not vitamin B12 are predictors of CHD mortality in older adults, Eur J Prev Cardiol 19 (6) (2012) 1420–1429.
[30] Y. Peng, B. Dong, Z. Wang, Serum folate concentrations and all-cause, cardiovascular disease and cancer mortality: a cohort study based on 1999–2010 National Health and Nutrition Examination Survey (NHANES), Int J Cardiol 219 (2016) 136–142.
[31] A. Rotstein, A. Kodesh, Y. Goldberg, A. Reichenberg, S.Z. Levine, Serum folate deficiency and the risks of dementia and all-cause mortality: a national study of old age, Evid Based Ment Health 25 (2) (2022) 63–68.
[32] Y. Liu, T. Geng, Z. Wan, Q. Lu, X. Zhang, Z. Qiu, et al., Associations of serum folate and vitamin B12 Levels with cardiovascular disease

mortality among patients with type 2 diabetes, JAMA Netw Open 5 (1) (2022), e2146124.
[33] S. Nkemjika, E. Ifebi, L.T. Cowan, I. Chun-Hai Fung, F. Twum, F. Liu, et al., Association between serum folate and cardiovascular deaths among adults with hypertension, Eur J Clin Nutr 74 (6) (2020) 970–978.
[34] T. Taverner, F.L. Crowe, G.N. Thomas, K. Gokhale, R. Thayakaran, K. Nirantharakumar, et al., Circulating folate concentrations and risk of peripheral neuropathy and mortality: a retrospective cohort study in the U.K, Nutrients 11 (10) (2019) 2443.
[35] E. Rossi, J. Hung, J.P. Beilby, M.W. Knuiman, M.L. Divitini, H. Bartholomew, Folate levels and cancer morbidity and mortality: prospective cohort study from Busselton, Western Australia, Ann Epidemiol 16 (3) (2006) 206–212.
[36] A. Zeitlin, W.H. Frishman, C.J. Chang, The association of vitamin b 12 and folate blood levels with mortality and cardiovascular morbidity incidence in the old old: the Bronx aging study, Am J Ther 4 (7–8) (1997) 275–281.
[37] J. Marniemi, J. Järvisalo, T. Toikka, I. Räihä, M. Ahotupa, L. Sourander, Blood vitamins, mineral elements and inflammation markers as risk factors of vascular and non-vascular disease mortality in an elderly population, Int J Epidemiol 27 (5) (1998) 799–807.
[38] J. Hung, J.P. Beilby, M.W. Knuiman, M. Divitini, Folate and vitamin B-12 and risk of fatal cardiovascular disease: cohort study from Busselton, Western Australia, BMJ 326 (7381) (2003) 131.
[39] S. González, J.M. Huerta, S. Fernández, A.M. Patterson, C. Lasheras, Homocysteine increases the risk of mortality in elderly individuals, Br J Nutr 97 (6) (2007) 1138–1143.
[40] A.D. Dangour, E. Breeze, R. Clarke, P.S. Shetty, R. Uauy, A.E. Fletcher, Plasma homocysteine, but not folate or vitamin B-12, predicts mortality in older people in the United Kingdom, J Nutr 138 (6) (2008) 1121–1128.
[41] C.J. Bates, M.A. Mansoor, K.D. Pentieva, M. Hamer, G.D. Mishra, Biochemical risk indices, including plasma homocysteine, that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of people aged 65 years and over, Br J Nutr 104 (6) (2010) 893–899.
[42] Y.-C. Huang, M.-S. Lee, M.L. Wahlqvist, Prediction of all-cause mortality by B group vitamin status in the elderly, Clin Nutr 31 (2) (2012) 191–198.
[43] N. Mendonça, C. Jagger, A. Granic, C. Martin-Ruiz, J.C. Mathers, C.J. Seal, et al., Elevated total homocysteine in all participants and plasma vitamin B12 concentrations in women are associated with all-cause and cardiovascular mortality in the very old: the Newcastle 85+ Study, J Gerontol A Biol Sci Med Sci 73 (9) (2018) 1258–1264.
[44] B. Kyte, E. Ifebi, S. Shrestha, S. Charles, F. Liu, J. Zhang, High red blood cell folate is associated with an increased risk of death among adults with diabetes, a 15-year follow-up of a national cohort, Nutr Metab Cardiovasc Dis 25 (11) (2015) 997–1006.
[45] F. Twum, N. Morte, Y. Wei, S. Nkemjika, F. Liu, J. Zhang, Red blood cell folate and cardiovascular deaths among hypertensive adults, an 18-year follow-up of a national cohort, Hypertens Res 43 (9) (2020) 938–947.
[46] H. Xiong, X. Li, S. Cheng, P. Chen, S. Guo, X. Huang, et al., Folate status and mortality in US adults with diabetes: a nationally representative cohort study, Front Cardiovasc Med 9 (2022), 802247.
[47] L. Zhou, H. Huang, X. Wen, Y. Chen, J. Liao, F. Chen, et al., Associations of serum and red blood cell folate with all-cause and cardiovascular mortality among hypertensive patients with elevated homocysteine, Front Nutr 9 (2022), 849561.
[48] Q. Yang, R.M. Bostick, J.M. Friedman, W.D. Flanders, Serum folate and cancer mortality among U.S. adults: findings from the Third National Health and Nutritional Examination Survey linked mortality file, Cancer Epidemiol Biomarkers Prev 18 (5) (2009) 1439–1447.
[49] Food and Drug Administration, Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid, Federal Register (1996) 8781–8797.
[50] S. Chen, T. Honda, J. Hata, S. Sakata, Y. Furuta, D. Yoshida, et al., High serum folate concentrations are associated with decreased risk of mortality among Japanese adults, J Nutr 151 (3) (2021) 657–665.
[51] Q. Yang, L.D. Botto, J.D. Erickson, R.J. Berry, C. Sambell, H. Johansen, et al., Improvement in stroke mortality in Canada and the United States, 1990 to 2002, Circulation 113 (10) (2006) 1335–1343.
[52] A.E. Stanhewicz, W.L. Kenney, Role of folic acid in nitric oxide bioavailability and vascular endothelial function, Nutr Rev 75 (1) (2016) 61–70.
[53] Ministry of Health and Welfare, Korea Disease Control and Prevention Agency, Korea Health Statistics 2018: Korea National Health and Nutrition Examination Survey (KNHANES Ⅶ-3), Ministry of Health and Welfare, Korea, 2019.
[54] H.I. Morrison, D. Schaubel, M. Desmeules, D.T. Wigle, Serum folate and risk of fatal coronary heart disease, JAMA 275 (24) (1996) 1893–1896.
[55] A. de Bree, W.M. Verschuren, H.J. Blom, M. Nadeau, F.J. Trijbels, D. Kromhout, Coronary heart disease mortality, plasma homocysteine, and B-vitamins: a prospective study, Atherosclerosis 166 (2) (2003) 369–377.
[56] A. Stanisławska-Sachadyn, J.V. Woodside, K.S. Brown, I.S. Young, L. Murray, H. McNulty, et al., Evidence for sex differences in the determinants of homocysteine concentrations, Mol Genet Metab 93 (4) (2008) 355–362.
[57] O. Nygård, S.E. Vollset, H. Refsum, I. Stensvold, A. Tverdal, J.E. Nordrehaug, et al., Total plasma homocysteine and cardiovascular risk profile: the Hordaland Homocysteine Study, JAMA 274 (19) (1995) 1526–1533.
[58] E.K. Hoogeveen, P.J. Kostense, C. Jakobs, J.M. Dekker, G. Nijpels, R.J. Heine, et al., Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes, Circulation 101 (13) (2000) 1506–1511.
[59] S. Yuan, A.M. Mason, P. Carter, S. Burgess, S.C. Larsson, Homocysteine, B vitamins, and cardiovascular disease: a Mendelian randomization study, BMC Med 19 (1) (2021) 97.
[60] B.C. Blount, M.M. Mack, C.M. Wehr, J.T. MacGregor, R.A. Hiatt, G. Wang, et al., Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage, Proc Natl Acad Sci U S A 94 (7) (1997) 3290–3295.
[61] L.L. Wu, J.T. Wu, Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker, Clin Chim Acta 322 (1–2) (2002) 21–28.
[62] S.E. Vollset, H. Refsum, A. Tverdal, O. Nygård, J.E. Nordrehaug, G.S. Tell, et al., Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study, Am J Clin Nutr 74 (1) (2001) 130–136.
[63] W. Zhao, Y. Lin, H. He, H. Ma, W. Yang, Q. Hu, et al., Association between hyperhomocysteinaemia and the risk of all-cause and cause-specific mortality among adults in the USA, Br J Nutr (2022) 1–12.
[64] E.S. Ford, T.E. Byers, W.H. Giles, Serum folate and chronic disease risk: findings from a cohort of United States adults, Int J Epidemiol 27 (4) (1998) 592–598.
[65] R. Clarke, J. Halsey, S. Lewington, E. Lonn, J. Armitage, J.E. Manson, et al., Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals, Arch Intern Med 170 (18) (2010) 1622–1631.