Circulating concentrations of biomarkers and metabolites related to vitamin status, one-carbon and the kynurenine pathways in US, Nordic, Asian, and Australian populations[1–3]

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ABSTRACT

Background: Circulating concentrations of biomarkers that are related to vitamin status vary by factors such as diet, fortification, and supplement use. Published biomarker concentrations have also been influenced by the variation across laboratories, which complicates a comparison of results from different studies.

Objective: We robustly and comprehensively assessed differences in biomarkers that are related to vitamin status across geographic regions.

Design: The trial was a cross-sectional study in which we investigated 38 biomarkers that are related to vitamin status and one-carbon and tryptophan metabolism in serum and plasma from 5314 healthy control subjects representing 20 cohorts recruited from the United States, Nordic countries, Asia, and Australia, participating in the Lung Cancer Cohort Consortium. All samples were analyzed in a centralized laboratory.

Results: Circulating concentrations of riboflavin, pyridoxal 5′-phosphate, folate, vitamin B-12, all-*trans* retinol, 25-hydroxyvitamin D, and α-tocopherol as well as combined vitamin scores that were based on these nutrients showed that the general B-vitamin concentration was highest in the United States and that the B vitamins and lipid soluble vitamins were low in Asians. Conversely, circulating concentrations of metabolites that are inversely related to B vitamins involved in the one-carbon and kynurenine pathways were high in Asians. The high B-vitamin concentration in the United States appears to be driven mainly by multivitamin-supplement users.

Conclusions: The observed differences likely reflect the variation in intake of vitamins and, in particular, the widespread multivitamin-supplement use in the United States. The results provide valuable information about the differences in biomarker

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Keywords: biomarker, Lung Cancer Cohort Consortium, one-carbon metabolism, tryptophan metabolism, vitamin status

INTRODUCTION

The quantitative measurement of circulating biomarker concentrations has been used in various studies that have investigated nutritional status, vitamin status, and lifestyle factors in relation to mortality and morbidities such as cancer and cardiovascular disease.

Circulating concentrations of vitamins and associated metabolites are related to vitamin intakes (1–3), which vary across the globe because of factors such as diet, lifestyle, vitamin-enrichment and food-fortification practices, and supplement use. In some countries, food fortification with various vitamins has been implemented to correct identified deficiencies or to reduce disease risk. In the United States, fortification has become widespread; margarine and milk have been voluntarily fortified with vitamins A and D since the 1930s (4, 5), while enrichment of flour and cereals with thiamin, riboflavin, and niacin since the 1940s (5) and with folic acid since 1998 (5) has been mandatory. In comparison, other countries generally have a much more restrictive approach to vitamin fortification and have implemented voluntary rather than mandatory food-fortification strategies. In addition to the mandatory fortification strategies in the United States, food manufacturers often add various vitamins to different food products (6–8), occasionally at very high concentrations (9), on a discretionary basis and at times as a marketing approach to promote product sales. The individual use of vitamin supplements adds to these geographical differences in vitamin intake from enriched and fortified foods.

Metabolism of the amino acids methionine (10) and tryptophan (11) are dependent on various B vitamins serving as cofactors. Thus, intakes (12, 13) and circulating concentrations (14, 15) of B vitamins can also influence the concentrations of these amino acids and their downstream metabolites.

The performance of different analytical methods used to quantify biomarkers also varies (16, 17), which has further contributed to the inherent challenge of comparing results between studies. The use of a centralized laboratory can overcome such difficulties.

Based on the European analyses that showed inverse relations between circulating vitamin B-6 [pyridoxal 5′-phosphate (PLP)][39] and methionine and lung cancer risk (18), the Lung Cancer Cohort Consortium (LC3) was established to prospectively investigate associations between vitamin B-6, one-carbon metabolites, and related biomarkers and lung cancer in a large number of cohorts across different geographic regions. In the current investigation, we describe circulating concentrations of 38 biomarkers that are related to vitamin status, one-carbon metabolism (OCM), and tryptophan metabolism (through the kynurenine pathway) in the 5314 healthy control subjects from the 20 participating cohorts of the LC3, which has a total of 10,728 participants from the United States, Nordic region, Asia, and Australia. The inclusion of 7 circulating vitamins allowed for the construction of composite vitamin scores to describe general vitamin status across geographic regions. All samples underwent identical biochemical analyses with the use of the same analytic assays in a single laboratory.

METHODS

Study design and population

Information on the participating cohorts, including cohort acronyms, is shown in **Supplemental Methods**. Study participants included the 5364 healthy control subjects from the LC3. The consortium consisted of 20 cohorts: 11 cohorts from the United States, 4 cohorts from the Nordic region (Norway, Sweden, and Finland), 4 cohorts from Asia (Chinese populations residing in Shanghai or Singapore), and 1 cohort from Australia. Each cohort contributed 81–513 control participants. Blood samples (serum or plasma) were collected from 1974 to 2010 (**Figure 1**). We excluded 50 participants with missing biomarker

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[3] Supplemental Methods, Supplemental Tables 1–8, and Supplemental Figures 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[39] Abbreviations used: BVS, B-vitamin score; FVS, fat-soluble vitamin score; HK, 3-hydroxykynurenine; LC3, Lung Cancer Cohort Consortium; MetSO, methionine sulfoxide; MMA, methylmalonic acid; OCM, one-carbon metabolism; PCA, principal component analysis; PLP, pyridoxal 5′-phosphate; tHcy, total homocysteine; TVS, total vitamin score; XA, xanthurenic acid; 25(OH)D, 25-hydroxyvitamin D.

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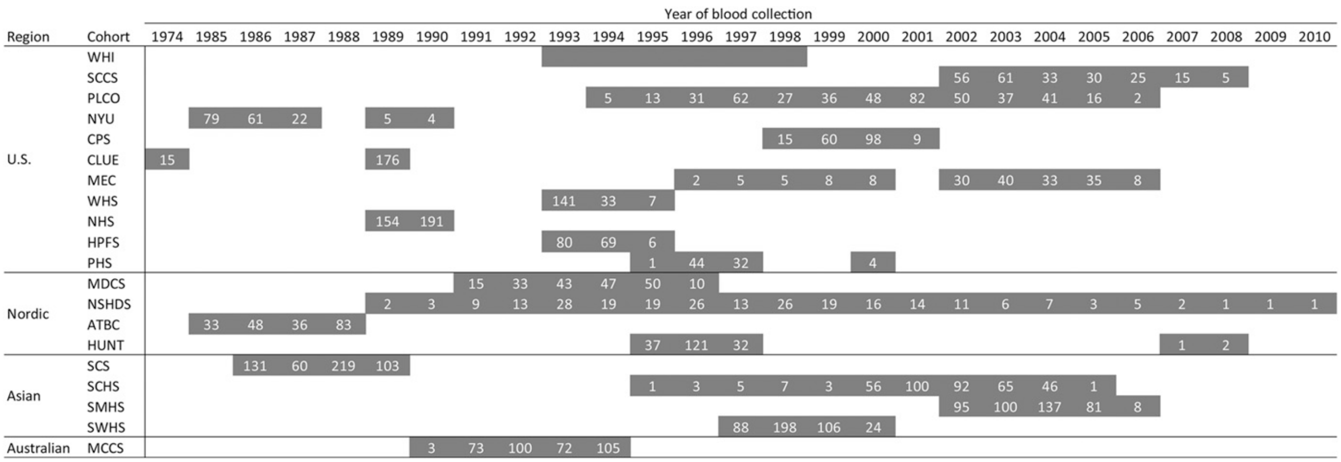

FIGURE 1 Year of blood sample collection. Numbers in each cell indicate the number of samples included from each cohort that year; for the WHI cohort, this information was not available. ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CLUE, Campaign Against Cancer and Stroke and Campaign Against Cancer and Heart Disease; CPS, American Cancer Society Cancer Prevention Study-II Nutrition Cohort; HPFS, Health Professionals Follow-Up Study; HUNT, Nord-Trøndelag Health Study; MCCS, Melbourne Collaborative Cohort Study; MDCS, Malmö Diet and Cancer Study; MEC, Multiethnic Cohort; NHS, Nurses' Health Study; NSHDS, Northern Sweden Health and Disease Study Cohort; NYU, New York University Women's Health Study; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCCS, Southern Community Cohort Study; SCHS, Singapore Chinese Health Study; SCS, Shanghai Cohort Study; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study; U.S., United States; WHI, Women's Health Initiative; WHS, Women's Health Study.

concentrations in plasma or serum samples, which provided a study population of 5314 participants with a complete data set (**Supplemental Figure 1**). Demographic data for the total study population and each geographic region are shown in **Table 1** and, for each cohort, in **Supplemental Table 1**. All participants gave written informed consent to participate in the study. The research was approved by the institutional review board of the International Agency for Research of Cancer and each participating cohort.

Multivitamin-supplement use and smoking

Data regarding the self-reported use of multivitamin-supplements (defined as supplements that contained ≥3 vitamins) were obtained from questionnaires and were coded as current or no-current use for 12 cohorts and as ever or never for 4 (United States) cohorts (Supplemental Table 1). In the United States, circulating vitamin concentrations were similar between subjects who reported current use of multivitamin supplements and those who reported ever use of multivitamin supplements (data not shown). Similar vitamin concentrations were also found for those reporting no-current use compared with those reporting never use (data not shown). Therefore, we combined subjects who reported current and ever use into multivitamin-supplement users and those who reported no-current or never use into nonusers. No information on multivitamin-supplement use was available for this study from 2 Nordic and 2 Asian cohorts and for a varying number of participants in several of the other cohorts (Supplemental Table 1). Smoking was classified via self-reports as never, former, or current smoker.

Biochemical analyses

All plasma and serum samples were stored at ≤−80°C from the time of collection until shipment to the Bevital laboratory (www.bevital.no) for biochemical analyses. Plasma concentrations of methionine, total homocysteine (tHcy), cystathionine, total cysteine, serine, glycine, sarcosine, methylmalonic acid (MMA), tryptophan, and kynurenine were measured with the use of gas chromatography–tandem mass spectrometry (19). Methionine sulfoxide (MetSO), choline, betaine, dimethylglycine, creatinine, arginine, asymmetric dimethylarginine, symmetric dimethylarginine, homoarginine (20), PLP, pyridoxal, 4-pyridoxic acid, riboflavin, kynurenic acid, anthranilic acid, 3-hydroxykynurenine (HK), xanthurenic acid (XA), 3-hydroxyanthranilic acid, quinolinic acid, cotinine (21), all-*trans* retinol (vitamin A), 25-hydroxyvitamin D_2 [25(OH)D_2], 25(OH)D_3, α-tocopherol, and γ-tocopherol (22) were analyzed with the use of liquid-chromatography–tandem mass spectrometry. Folate (23) and vitamin B-12 (24) were determined by microbiological methods, whereas C-reactive protein was analyzed with the use of an immunomatrix-assisted laser-desorption ionization–mass spectrometry (25). A plasma sample was included as a quality control in all batches.

We modeled the seasonality of circulating 25(OH)D_3 separately for each cohort with the use of a function that included 2 pairs of sine and cosine functions of the day of blood collection. The sum of 25(OH)D_2 and season-adjusted 25(OH)D_3 was combined into season-adjusted total 25(OH)D, which was used as a measure of vitamin D status. Because methionine may be oxidized to MetSO during sample storage (26), we used total methionine (i.e., methionine plus MetSO) as a measure of circulating methionine concentrations. The kynurenine-to-tryptophan ratio was calculated as kynurenine (expressed in nmol/L) divided by tryptophan (expressed as μmol/L), and the PAr was calculated as 4-pyridoxic acid:(PLP plus pyridoxal) (27).

Statistical methods

Because most biomarkers were not normally distributed, crude circulating biomarker concentrations are reported as geometric means (5th and 95th percentiles). Values of cotinine, which is a marker of recent nicotine exposure, less than the limit of detection (1 nmol/L) were set to 1 nmol/L (which is well below the

TABLE 1
Baseline characteristics of study population by geographic region[1]

	Region						United States by MV use		
	Total	United States	Nordic	Asian	Australian	*P*	No	Yes	*P*
n	5314	2397	835	1729	353		1440	840	
Sex, F, *n* (%)	2422 (45.6)	1406 (58.7)	360 (43.1)	515 (29.8)	141 (39.9)	<0.001	797 (55.3)	555 (66.1)	<0.001
Age,[2] y	62.0 (47.0–75.0)	68.0 (53.0–79.0)	59.9 (44.6–70.8)	63.0 (46.5–75.0)	61.0 (45.0–67.4)	<0.001	64.0 (47.8–77.0)	64.1 (47.0–79.0)	0.012
Education, *n* (%)						<0.001			<0.001
Less than high school	1655 (31.4)	215 (9.1)	365 (44.2)	843 (49.0)	232 (65.7)		160 (11.6)	53 (6.5)	
Completed high school	781 (14.8)	374 (15.8)	143 (17.3)	227 (13.2)	37 (10.5)		234 (16.9)	127 (15.5)	
Vocational school	912 (17.3)	435 (18.3)	167 (20.2)	277 (16.1)	33 (9.3)		273 (19.7)	140 (17.1)	
Some college	715 (13.6)	390 (16.4)	120 (14.5)	196 (11.4)	9 (2.5)		234 (16.9)	141 (17.2)	
College graduate	496 (9.4)	319 (13.5)	22 (2.7)	113 (6.6)	42 (11.9)		195 (14.1)	106 (13.0)	
Graduate studies	636 (12.1)	564 (23.8)	8 (1.0)	64 (3.7)	0 (0.0)		289 (20.9)	251 (30.7)	
Unknown	73 (1.4)	73 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)		46 (3.3)	11 (1.3)	
Smoking status, *n* (%)						<0.001			0.015
Never	1286 (24.2)	569 (23.7)	107 (12.8)	561 (32.4)	49 (13.9)		323 (22.4)	228 (27.1)	
Former	1517 (28.5)	1006 (42.0)	190 (22.8)	176 (10.2)	145 (41.1)		607 (42.1)	343 (40.8)	
Current	2511 (47.3)	822 (34.3)	538 (64.4)	992 (57.4)	159 (45.0)		511 (35.5)	269 (32.0)	
BMI,[3] kg/m^2	24.7 (19.3–33.4)	25.8 (20.6–35.6)	25.8 (20.2–33.2)	24.1 (18.9–32.6)	27.5 (21.2–35.6)	<0.001	25.8 (20.1–35.6)	25.1 (20.1–34.2)	0.011
MV use, all participants, *n* (%)						<0.001	—	—	—
Never	527 (9.9)	527 (22.0)	0 (0.0)	0 (0.0)	0 (0.0)		—	—	
Ever	329 (6.2)	329 (13.7)	0 (0.0)	0 (0.0)	0 (0.0)		—	—	
No current	2149 (40.4)	914 (38.1)	150 (18.0)	779 (45.1)	306 (86.7)		—	—	
Current	671 (12.6)	511 (21.3)	55 (6.6)	58 (3.4)	47 (13.3)		—	—	
Missing	1638 (30.8)	116 (4.8)	630 (75.4)	892 (51.6)	0 (0.0)		—	—	
MV use in subjects with available data, *n* (%)						<0.001	—	—	—
No (never + no current)	2676 (72.8)	1441 (63.2)	150 (73.2)	779 (93.1)	306 (86.7)		—	—	
Yes (ever + current)	1000 (27.2)	840 (36.8)	55 (26.8)	58 (6.9)	47 (13.3)		—	—	

[1] *P* values were determined with the use of a Kruskal-Wallis, ANOVA, or chi-square test. MV, multivitamin supplement.
[2] All values are means (5th–95th percentiles).
[3] All values are medians (5th–95th percentiles). BMI was calculated as weight divided by the square of height.

concentrations in both passive and active smokers) before being log transformed. Geometric means (95% CIs) by region were estimated with the use of mixed models that were adjusted for age, sex, and smoking status (former compared with never; current compared with never) with the cohort as a random effect. Between-region spreads of adjusted biomarker geometric means were calculated as CVs (SD divided by the mean of the geometric means, expressed as %). In the US region, we also investigated biomarker concentrations after stratification by multivitamin-supplement use (by combining current and ever compared with no-current and never), and prefolate fortification compared with postfolate fortification (1998). The effect of multivitamin-supplement use was not investigated for non-US populations because such information was only available for a low number of these participants. Geometric means (95% CIs) by cohort were estimated by adjusting for age, sex, and smoking status (former compared with never; current compared with never) with the use of generalized linear models.

We investigated proportional differences at each fifth percentile of biomarker distributions across the regions (United States, Nordic region, Asia, and Australia) by quantile regression (28). The results were plotted graphically as the percentage of differences between regions (with the Unites States as the reference) compared with the metabolite concentrations at each quantile cutoff. These models were adjusted for age (years) at blood sampling, sex, smoking (former compared with never; current compared with never) and cohort.

Patterns in biomarker concentrations across cohorts were investigated by performing a principal component analysis (PCA) on a matrix that contained centered and standardized cohort geometric mean biomarker concentrations from the generalized linear models. To ensure vitamin B-6 was weighted in the same way as other biomarkers were weighted, we included only one of the analyzed vitamin B-6 forms [i.e., PLP, which is the most commonly used vitamin B-6 marker (29)] in the PCA.

ANOVA was used for comparisons of normally distributed variables, and the Kruskal-Wallis test was used for comparisons of variables that were not normally distributed. Categorical variables were compared by using the chi-square test.

We combined individual circulating vitamin concentrations, which were log transformed, centered and standardized to have a mean of 0 and SD of 1, into 3 different vitamin scores to obtain measures of general vitamin status. Thus, the B-vitamin score (BVS) was obtained as the mean of the transformed concentrations of riboflavin, PLP, folate, and vitamin B-12. The fat-soluble vitamin score (FVS) included vitamin A, 25(OH)D, and α-tocopherol. The total vitamin score (TVS) combined all 7 vitamins. Each combined vitamin score was again standardized to have a mean of 0 and SD of 1. Only the vitamin E form α-tocopherol was included in the FVS and TVS because this is the form that is usually used for the assessment of vitamin E status (30). Vitamin scores across regions, cohorts, and US multivitamin-supplement users and nonusers were estimated by adjusting for age, sex, and smoking as previously described for biomarker concentrations.

Statistical tests were 2-sided, and significance was determined at the 0.05 level. Statistical analyses were performed by using SPSS version 22 for Windows software (SPSS Inc.) and R version 3.2.3 software (http://www.r-project.org; The R Foundation) [using the lm function, the packages lme4 (31), lmerTest, quantreg, and prcomp].

RESULTS

Demographics

Demographic data are given in Table 1 and Supplemental Table 1. Overall, the proportion of women was 45.6%. The geometric mean age differed across regions [from 59.9 y (Nordic) to 68.0 y (United States); $P < 0.001$]. Of the total population, 47.3% were current smokers, ranging from 34.3% of subjects in the United States to 64.4% of subjects in the Nordic region.

For 30.8% of the total population, information on multivitamin-supplement use was missing mainly because such data were not available for this study from several Nordic and Asian cohorts (Table 1). Of subjects with this information available, 27.2% reported the use of such supplements, whereas 72.8% reported no use of such supplements. The prevalence of multivitamin-supplement use was 36.8% in the United States, 26.8% in the Nordic region (data available for only 205 participants), 6.9% in Asians, and 13.3% in Australians.

All samples from the United States were collected from 1985 to 2008 except for a small number of samples (n = 15) from the Campaign Against Cancer and Stroke and the Campaign Against Cancer and Heart Disease, which were collected in 1974. For the US-based Women's Health Initiative cohort, information on the year of blood sampling for each participant was not available because of confidentiality concerns. Samples were collected from Nordic cohorts during 1985–2010, from Asian cohorts during 1986–2006, and from the Australian cohort during 1990–1994 (Figure 1). **Supplemental Table 2** shows the crude geometric mean (5th and 95th percentile) biomarker concentrations for the total population and each geographic region, whereas **Supplemental Tables 3** and **4** show these data for individual cohorts.

PCA using cohort geometric means

The first 2 PCs (**Figure 2**) explained a total of 53% (32% and 21%, respectively) of the variation in cohort geometric means obtained from mixed models. Vitamins (with the exception of γ-tocopherol) were grouped together with negative loadings on PC2 in the loading plot (Figure 2A). The functional B-vitamin markers (tHcy, MMA, cystathionine, HK, and HK:XA) and components of OCM were located opposite the vitamin group. The kynurenines (except HK) were also grouped together.

When the loading plots (Figure 2A) and score plots (Figure 2B) that were formed from the first 2 PCs were compared, the US cohorts were generally located in the same direction from the origin as the vitamin group and opposite the location of the functional B-vitamin markers. Asian cohorts were located in the same direction as the functional B-vitamin markers and OCM group and opposite the location of the US cohorts, whereas the Nordic and Australian cohorts were located closer to the center of the score plot.

PC3 and PC4 (which explained 12% and 8% of the variation in the data set, respectively) did not provide additional information about general vitamin concentrations or reveal other clear biomarker patterns and, thus, were not investigated further.

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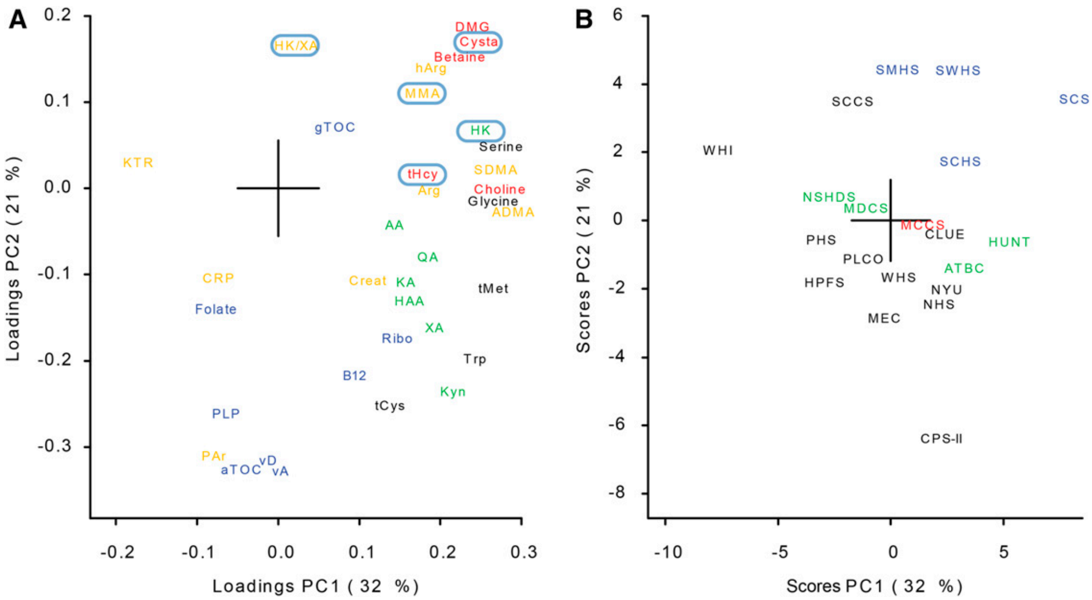

FIGURE 2 The first 2 PCs (PC1 and PC2) obtained from the principal component analysis based on centered and standardized geometric mean biomarker concentrations from all cohorts. The loading plot (A) shows the following colors and corresponding biomarkers: blue, vitamins; black, amino acids; red, one-carbon metabolites; green, kynurenines; and orange, other biomarkers. Functional B-vitamin markers are marked by light-blue ellipses. The score plot (B) shows the following colors and corresponding cohorts: black, United States; green, Nordic; blue, Asian; and red, Australian. AA, anthranilic acid; ADMA, asymmetric dimethylarginine; Arg, arginine; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; aTOC, α-tocopherol; B12, vitamin B-12; CLUE, Campaign Against Cancer and Stroke and Campaign Against Cancer and Heart Disease; CPS-II, American Cancer Society Cancer Prevention Study-II Nutrition Cohort; Creat, creatinine; CRP, C-reactive protein; Cysta, cystathionine; DMG, dimethylglycine; gTOC, γ-tocopherol; HAA, 3-hydroxyanthranilic acid; hArg, homoarginine; HK, 3-hydroxykynurenine; HPFS, Health Professionals Follow-Up Study; HUNT, Nord-Trøndelag Health Study; KA, kynurenic acid; KTR, kynurenine:tryptophan ratio; Kyn, kynurenine; MCCS, Melbourne Collaborative Cohort Study; MDCS, Malmö Diet and Cancer Study; MEC, Multiethnic Cohort; MMA, methylmalonic acid; NHS, Nurses' Health Study; NSHDS, Northern Sweden Health and Disease Study Cohort; NYU, New York University Women's Health Study; PAr, 4-pyridoxic acid:(pyridoxal 5′-phosphate plus pyridoxal); PC, principal component; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; PLP, pyridoxal 5′-phosphate; QA, quinolinic acid; Ribo, riboflavin; SCCS, Southern Community Cohort Study; SCHS, Singapore Chinese Health Study; SCS, Shanghai Cohort Study; SDMA, symmetric dimethylarginine, SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study; tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; Trp, tryptophan; vA, vitamin A; vD, total 25-hydroxyvitamin D; WHI, Women's Health Initiative; WHS, Women's Health Study; XA, xanthurenic acid.

Circulating vitamin scores

Vitamin scores across regions and cohorts are shown in **Table 2** and **Supplemental Tables 5** and **6**, respectively, and are shown graphically in **Supplemental Figure 2**. The highest mean BVS at 0.28 (95% CI: 0.10, 0.46; $P < 0.05$) was found in the United States, followed by the Australian (−0.09; 95% CI: −0.57, 0.39), Asian (−0.25; 95% CI: −0.53, 0.02), and Nordic (−0.28; 95% CI: −0.52, −0.03) regions. The FVS was highest at 0.48 (95% CI: 0.23, 0.72) in the Nordic region, followed by the Australian (0.40; 95% CI: −0.08, 0.86), US (0.06; 95% CI: −0.11, 0.25), and Asian (−0.32; 95% CI: −0.61, −0.07) regions. Across regions, the highest TVS was observed in the United States.

Biomarkers and metabolites

The adjusted geometric mean (95% CI) biomarker concentrations for the geographic regions that were obtained from the mixed models are provided in Table 2 and for each cohort in Supplemental Tables 5 and 6. Across regions, the spread (CV) in adjusted geometric mean biomarker concentration was largest for folate (39%) and γ-tocopherol (38%), whereas the CV was <10% for 17 of 38 biomarkers. Folate was highest in the United States compared with in the other regions. Functional B-vitamin markers (tHcy, MMA, cystathionine, HK, and HK:XA) were generally high in the Asian region and low in the US region, and fat-soluble vitamins A, 25(OH)D, and α-tocopherol were higher in Nordic and Australian regions than in US and Asian regions.

Methionine and downstream OCM biomarkers were generally highest in the Asian region, whereas tryptophan and kynurenines were generally lowest in the United States and highest in Asia.

Quantile regression (**Figure 3**) showed that the entire distributions of PLP and folate and the upper part of the distribution of riboflavin were elevated in the United States compared with in the other regions. For tHcy, the entire distribution was lower in the US and Nordic regions than in the Asian and Australian regions, whereas the entire distribution of cystathionine was elevated in Asians. In Asia, the upper ranges of MMA and HK were higher than those observed in other regions. Compared with other regions, the distribution of the fat-soluble vitamins A, 25(OH)D, and α-tocopherol were lower in Asia, whereas γ-tocopherol was lower in Australia.

Multivitamin-supplement use, mandatory folate fortification in the United States

For US multivitamin-supplement users and nonusers, the mean BVS was 0.80 (95% CI: 0.62, 0.97) and −0.01 (95% CI: −0.18, 0.17), respectively, the FVS was 0.41 (95% CI: 0.24, 0.57) and −0.13 (95% CI: −0.29, 0.03), respectively, and the TVS was 0.74 (95% CI: 0.59, 0.89) and −0.06 (95% CI: −0.21, 0.08), respectively

TABLE 2
Biomarker concentrations by region from mixed models[1]

	Region					United States by MV use	
	United States	Nordic	Asian	Australian	CV, %	No	Yes
n	2397	835	1729	353	—	1441	840
Vitamin score					—		
TVS	0.23 (0.07, 0.39)[2]	0.02 (−0.21, 0.24)[3]	−0.30 (−0.55, −0.05)[3,4]	0.08 (−0.35, 0.51)		−0.06 (−0.21, 0.08)	0.74 (0.59, 0.89)[5]
BVS	0.28 (0.10, 0.46)	−0.28 (−0.53, −0.03)[3,4]	−0.25 (−0.53, 0.02)[3,4]	−0.09 (−0.57, 0.39)[3]		−0.01 (−0.18, 0.17)	0.80 (0.62, 0.97)[5]
FVS	0.06 (−0.12, 0.23)	0.48 (0.24, 0.72)[5]	−0.32 (−0.59, −0.06)[3,6]	0.40 (−0.06, 0.87)		−0.13 (−0.29, 0.03)	0.41 (0.24, 0.57)[5]
Vitamin							
Vitamin B-2 (riboflavin), nmol/L	21.3 (17.5, 25.9)	17.0 (13.0, 22.3)	19.1 (14.1, 25.8)	25.2 (14.9, 42.6)	17	18.3 (15.0, 22.4)	28.0 (22.9, 34.3)[5]
PLP, nmol/L	50.1 (43.8, 57.3)	37.5 (31.1, 45.2)[3]	37.0 (30.1, 45.5)[3]	35.4 (24.8, 50.7)[3]	17	40.1 (35.7, 44.9)	72.3 (64.3, 81.3)[5]
Pyridoxal, nmol/L	17.0 (12.5, 23.1)	18.3 (11.9, 28.0)	15.9 (9.9, 25.6)	21.8 (9.5, 49.7)	14	13.1 (9.5, 17.9)	26.5 (19.3, 36.4)[5]
PA, nmol/L	31.8 (27.6, 36.6)	28.4 (23.4, 34.6)	18.0 (14.5, 22.4)[3–6]	24.0 (16.5, 34.9)	23	24.1 (21.1, 27.4)	50.3 (43.8, 57.6)[5]
Folate, nmol/L	28.5 (24.2, 33.6)	12.4 (9.8, 15.6)[3–5]	14.7 (11.4, 19.0)[3–5]	17.1 (11.0, 26.7)[3]	39	23.6 (19.9, 28.1)	38.1 (32.0, 45.4)[5]
Vitamin B-12, pmol/L	442 (409, 477)	457 (411, 508)	409 (363, 460)	407 (332, 500)	6	414 (385, 446)	491 (455, 529)[5]
Vitamin A (all-*trans* retinol), µmol/L	2.19 (2.10, 2.29)	2.37 (2.23, 2.52)	1.91 (1.79, 2.05)[3–6]	2.32 (2.06, 2.62)	9	2.15 (2.06, 2.24)	2.28 (2.18, 2.38)[5]
Vitamin D,[7] nmol/L	53.0 (49.6, 56.6)	64.3 (58.6, 70.4)[4,5]	53.7 (48.5, 59.4)[6]	58.1 (48.8, 69.3)	9	49.0 (45.8. 52.5)	61.3 (57.2. 65.8)[5]
αTOC, µmol/L	31.4 (29.4, 33.6)	34.6 (31.5, 38.0)[5]	27.0 (24.3, 30.0)[3,6,8]	36.3 (30.3, 43.5)	13	29.3 (27.5, 31.1)	35.5 (33.3, 37.8)[5]
γTOC, µmol/L	4.38 (3.58, 5.35)	2.69 (2.03, 3.55)[5]	3.88 (2.84, 5.29)	1.69 (0.98, 2.91)[4,5]	38	5.00 (4.11, 6.08)	3.44 (2.83, 4.19)[5]
One-carbon metabolites							
tMet, µmol/L	27.4 (26.4, 28.5)	27.3 (25.9, 28.8)	29.7 (28.0, 31.5)	27.5 (24.8, 30.6)	4	27.6 (26.5, 28.8)	27.4 (26.3, 28.6)
tHcy, µmol/L	11.5 (10.6, 12.4)	11.0 (9.9, 12.3)	12.9 (11.4, 14.5)	12.8 (10.4, 15.7)	8	12.0 (11.1, 13.0)	10.8 (9.9, 11.7)[5]
Cystathionine, µmol/L	0.170 (0.157, 0.184)	0.190 (0.170, 0.212)[3]	0.283 (0.250, 0.319)[3–6,8]	0.185 (0.150, 0.229)	25	0.179 (0.165. 0.195)	0.157 (0.145. 0.171)[5]
tCys, µmol/L	300 (290, 311)	300 (286, 314)	286 (272, 302)	313 (286, 342)	4	300 (289, 310)	302 (292, 313)
Serine, µmol/L	107.6 (97.0, 119.4)	122.9 (106.3, 142.0)	136.4 (116.0, 160.4)	115.9 (87.5, 153.6)	10	110 (99, 122)	105 (95, 117)[5]
Glycine, µmol/L	265 (244, 287)	253 (226, 284)	278 (245, 315)	248 (199, 309)	5	266 (245, 289)	267 (245, 290)
Choline, µmol/L	12.8 (11.4, 14.4)	11.6 (9.8, 13.6)	14.3 (11.9, 17.2)	14.3 (10.4, 19.6)	10	12.9 (11.5, 14.6)	12.9 (11.4, 14.5)
Betaine, µmol/L	37.3 (34.7, 40.2)	32.3 (29.2, 35.8)	50.1 (44.8, 56.1)[3–6,8]	34.9 (28.6, 42.5)	20	37.7 (35.0, 40.6)	36.9 (34.2, 39.8)
Dimethylglycine, µmol/L	3.7 (3.4, 3.9)	4.0 (3.6, 4.4)	5.0 (4.5, 5.6)[3–6,8]	3.8 (3.2, 4.6)	15	3.75 (3.49, 4.04)	3.49 (3.24, 3.76)[5]
Tryptophan and metabolites							
Tryptophan, µmol/L	62.6 (59.3, 66.0)	67.6 (62.8, 72.8)	70.2 (64.7, 76.2)	67.5 (58.5, 77.9)	5	62.7 (59.4, 66.2)	62.8 (59.4, 66.3)
Kynurenine, µmol/L	1.47 (1.41, 1.53)	1.55 (1.47, 1.65)	1.58 (1.48, 1.68)	1.53 (1.37, 1.71)	3	1.48 (1.42, 1.55)	1.46 (1.40, 1.52)
KA, nmol/L	41.7 (39.1, 44.5)	40.7 (37.1, 44.5)	51.5 (46.6, 56.9)[4,6]	43.9 (36.9, 52.3)	11	41.8 (39.0, 44.7)	42.1 (39.2, 45.2)
AA, nmol/L	14.6 (13.5, 15.8)	14.9 (13.4, 16.6)	13.9 (12.3, 15.7)	15.6 (12.6, 19.2)	5	14.6 (13.5, 15.7)	14.7 (13.6, 15.9)
HK, nmol/L	33.9 (31.8, 36.2)	37.2 (34.0, 40.7)	40.6 (36.8, 44.9)[3,4]	37.6 (31.6, 44.8)	7	35.4 (33.2, 37.9)	31.8 (29.7, 34.0)[5]
XA, nmol/L	10.5 (9.6, 11.4)	12.4 (11.0, 13.9)	15.1 (13.2, 17.2)[3–5]	11.0 (8.7, 13.8)	17	10.53 (9.64, 11.49)	10.52 (9.60, 11.52)
HAA, nmol/L	27.1 (24.6, 29.9)	36.0 (31.5, 41.3)[3–5]	37.5 (32.3, 43.6)[3–5]	32.1 (24.7, 41.7)	14	26.6 (24.1, 29.4)	28.3 (25.6, 31.4)[5]
QA, nmol/L	360 (344, 377)	349 (328, 373)	373 (348, 400)	357 (316, 403)	3	362 (345, 380)	355 (337, 373)
Other							
CRP, µg/L	2.26 (1.87, 2.72)	1.51 (1.17, 1.96)	1.26 (0.95, 1.68)[4,5]	2.29 (1.39, 3.77)	29	2.21 (1.83, 2.68)	2.28 (1.87, 2.79)
KTR, nmol/µmol	23.5 (23.0, 24.1)	23.0 (22.3, 23.8)	22.4 (21.7, 23.2)	22.7 (21.3, 24.1)	2	23.7 (23.1, 24.2)	23.2 (22.6, 23.8)
PAr	0.444 (0.413, 0.476)	0.500 (0.453, 0.552)	0.322 (0.289, 0.358)[3–6]	0.423 (0.351, 0.511)	18	0.429 (0.399, 0.461)	0.468 (0.434, 0.505)[5]
Creatinine, µmol/L	77.0 (74.6, 79.4)	72.4 (69.4, 75.6)	73.6 (70.2, 77.2)	71.4 (65.7, 77.5)	3	77.7 (75.2, 80.2)	76.0 (73.6, 78.6)[5]
HK:XA	3.24 (2.93, 3.58)	3.00 (2.61, 3.45)	2.69 (2.31, 3.14)	3.43 (2.63, 4.47)	10	3.36 (3.05, 3.71)	3.02 (2.73, 3.34)[5]
Cotinine, nmol/L	37 (30, 45)	48 (36, 63)	54 (41, 72)[3]	38 (23, 62)	19	38 (31, 47)	35 (28, 44)
Arginine, µmol/L	55.8 (43.2, 72.1)	97.3 (68.0, 139.0)	82.3 (55.2, 122.8)	72.4 (36.1, 145.2)	23	55.8 (43.0, 72.5)	57.1 (43.9, 74.2)

(Continued)

TABLE 2 (*Continued*)

	Region					United States by MV use	
	United States	Nordic	Asian	Australian	CV, %	No	Yes
ADMA, μmol/L	0.529 (0.514, 0.544)	0.543 (0.521, 0.565)	0.576 (0.551, 0.602)[3,4]	0.546 (0.505, 0.590)	4	0.533 (0.517, 0.549)	0.525 (0.509, 0.542)
SDMA, μmol/L	0.571 (0.547, 0.596)	0.552 (0.520, 0.585)	0.629 (0.590, 0.672)[6]	0.604 (0.539, 0.676)	6	0.576 (0.552, 0.602)	0.569 (0.544, 0.594)
Homoarginine, μmol/L	1.82 (1.74, 1.91)	1.76 (1.65, 1.87)	2.28 (2.13, 2.44)[3–6]	2.05 (1.82, 2.31)	12	1.81 (1.72, 1.89)	1.86 (1.77, 1.95)
MMA, μmol/L	0.172 (0.162, 0.182)	0.170 (0.157, 0.184)	0.207 (0.189, 0.226)[3,5,6]	0.178 (0.152, 0.207)	9	0.176 (0.166, 0.187)	0.165 (0.155, 0.176)[5]

[1] AA, anthranilic acid; ADMA, asymmetric dimethylarginine; BVS, B-vitamin score; CRP, C-reactive protein; FVS, fat-soluble vitamin score; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine:tryptophan ratio; MMA, methylmalonic acid; MV, multivitamin supplement; PA, 4-pyridoxic acid; PAr, 4-pyridoxic acid:(pyridoxal 5′-phosphate + pyridoxal); PLP, pyridoxal 5′-phosphate; QA, quinolinic acid; SDMA, symmetric dimethylarginine, tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; TVS, total vitamin score; XA, xanthurenic acid; αTOC, α-tocopherol; γTOC, γ-tocopherol.

[2] Geometric means; 95% CIs in parentheses (all such values). Values by region were estimated with the use of mixed models that were adjusted for age, sex, and smoking status (former compared with never; current compared with never) with the cohort as a random effect.

[3] Significantly different from the United States with MV use ($P < 0.05$).

[4] Significantly different from the United States ($P < 0.05$)

[5] Significantly different from United States with no MV use ($P < 0.05$).

[6] Significantly different from the Nordic region ($P < 0.05$).

[7] Sum of 25-hydroxyvitamin D_2 and season-adjusted 25-hydroxyvitamin D_3.

[8] Significantly different from Australia ($P < 0.05$).

(Table 2). US-based participants who were taking multivitamins had higher circulating concentrations throughout the concentration ranges of all vitamins (except for γ-tocopherol, which was lower), and had lower concentrations of all functional B-vitamin markers compared with US participants who did not use such supplements (**Supplemental Figure 3**).

US multivitamin-supplement users had a higher BVS than that in all other regions, a higher FVS than that of Asians, and a higher TVS than in Nordic and Asian regions ($P < 0.05$) (Table 2). PLP and folate were higher in US multivitamin-supplement users than in other regions ($P < 0.05$).

In US participants who were not using multivitamin supplements, the FVS was lower than in the Nordic region and higher than in Asia, folate was higher than in the Nordic and Asian regions, and γ-tocopherol was higher than in Asia and Australia ($P < 0.05$) (Table 2). The mean BVS tended to be slightly higher in US participants who were not using multivitamin supplements (−0.01; 95% CI: −0.18, 0.17) compared with Nordic and Asian regions [−0.28 (95% CI: −0.53, −0.03) and −0.25 (95% CI: −0.53, 0.02), respectively], although these differences were NS.

In the United States, the circulating geometric mean concentration of folate before and after the implementation of the mandatory folate fortification of flour (1998) was 23.9 nmol/L (95% CI: 19.1, 29.8 nmol/L) compared with 37.2 nmol/L (95% CI: 29.6, 46.7 nmol/L) ($P < 0.001$). No other biomarkers were different before compared with after folate fortification (data not shown). For tHcy, the geometric mean was 12.1 μmol/L (95% CI: 11.1, 13.2 μmol/L) compared with 11.5 μmol/L (95% CI: 10.5, 12.6 μmol/L) ($P = 0.84$).

Vitamin deficiency

Plasma and serum concentration cutoffs that were used to indicate vitamin deficiency were 5 nmol/L for riboflavin (14, 32), 20 nmol/L for PLP (29), 5 nmol/L for folate (33), 150 pmol/L for vitamin B-12 (33), 0.7 μmol/L for vitamin A (34), 30 nmol/L for 25(OH)D (35), and 12 μmol/L for α-tocopherol (36).

Vitamin B-6 deficiency was observed in 16.1% of the total population (**Supplemental Table 7**) and ranged from 9.4% in US participants to 23.5% in Asians. Of the total population, 9.1% were vitamin D deficient with the highest prevalence of 14.7% in the Asian region and the lowest prevalence in Australian (4.5%) and Nordic (4.8%) regions. For the other vitamins, the prevalence of deficiency was low. In US multivitamin-supplement users, the highest prevalence of deficiency was 3.3%, which was observed for vitamin B-6 and vitamin D, compared with 12.7% and 9.5%, respectively, in US participants who were not taking multivitamin supplements.

DISCUSSION

Principal findings

We report circulating concentrations of 38 biomarkers that are related to vitamin status, one-carbon metabolism, and tryptophan metabolism in 5314 healthy individuals from 20 cohorts who represented US, Nordic, Asian, and Australian regions. Biochemical analyses were performed by a single laboratory, which enabled the direct comparison of biomarker concentrations across regions and cohorts. Composite vitamin scores that were

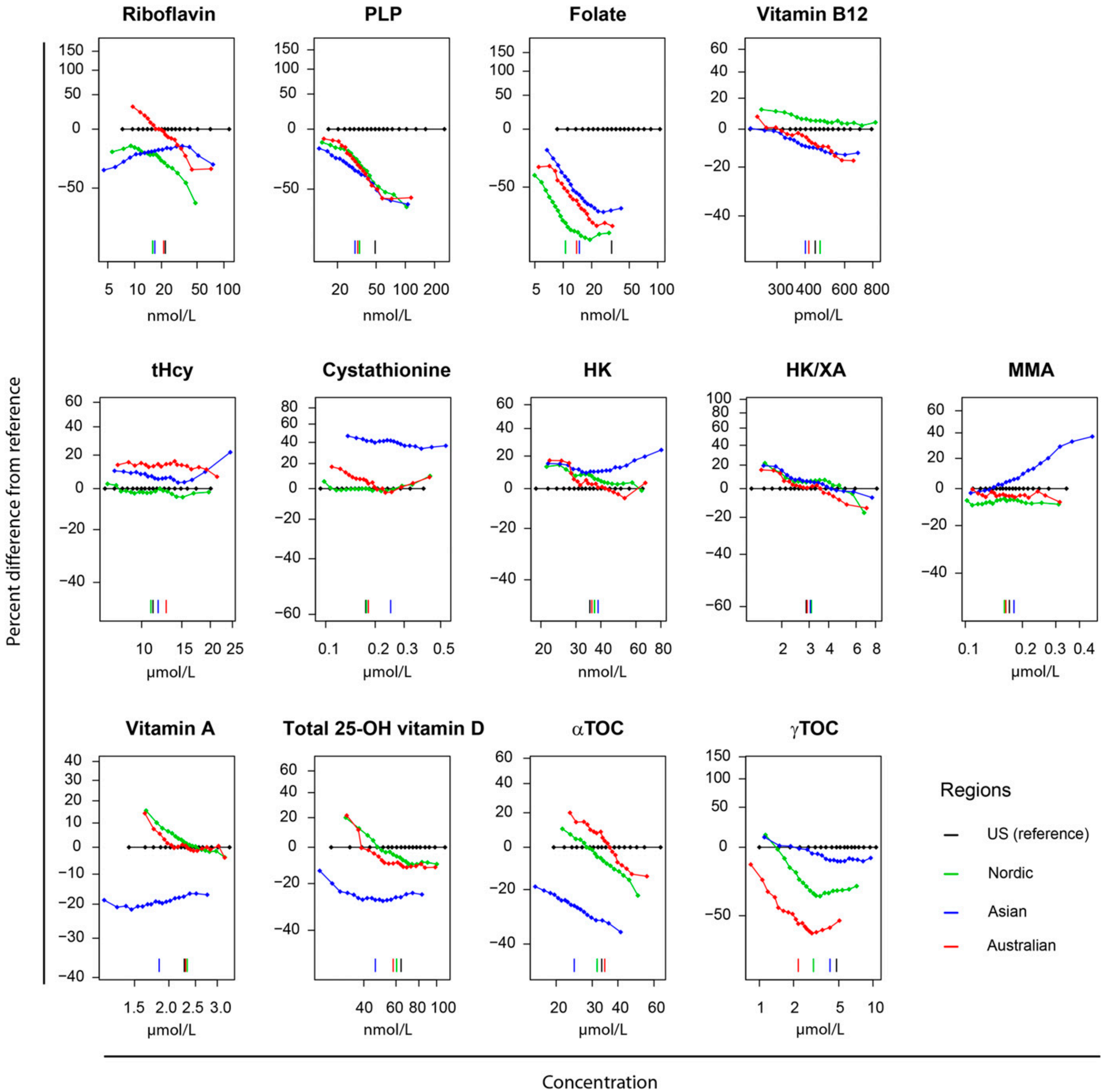

FIGURE 3 Distribution of vitamins and vitamin markers in regions from a quantile regression by 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 50th, 55th, 60th, 65th, 70th, 75th, 80th, 85th, 90th, and 95th percentiles. The models were adjusted for age, sex, smoking (former compared with never; current compared with never) and cohort. The *y* axis in each panel is scaled to show 3 SDs of the distribution of the biomarker in that panel. The vertical line in each panel indicates the 50th quantile for each group. HK, 3-hydroxykynurenine; HK/XA, 3-hydroxykynurenine:xanthurenic acid; MMA, methylmalonic acid; PLP, pyridoxal 5′-phosphate; Total 25(OH) vitamin D, 25-hydroxyvitamin D_2 plus season-adjusted 25-hydroxyvitamin D_3; tHcy, total homocysteine; US, United States; αTOC, α-tocopherol; γTOC, γ-tocopherol.

based on serum and plasma vitamin concentrations showed that the general B-vitamin status was highest in the United States, and the general vitamin concentration was low in the Asian region. We observed a high general vitamin status in multivitamin-supplement users in the United States. Differences in B-vitamin concentrations were further reflected by the concentrations of one-carbon and tryptophan metabolites, which serve as functional markers of B-vitamin status.

Vitamin status across regions

The grouping of the 7 measured vitamins, which reflected general positive intercorrelations, combined with the similar location of the US cohorts in the space spanned by the first 2 PCs from the PCA motivated the construction of circulating vitamin scores.

The grouping of US cohorts away from Nordic, Asian, and Australian cohorts in the score plot combined with the grouping of the vitamins in the loading plot probably reflected abundant fortification, enrichment, and dietary supplementation practices in the United States. For instance, the BVS was higher in the United States than in the other regions. The consumption of fortified foods strongly affects B-vitamin status in the United States (5, 8) with ready-to-eat cereals, which are a staple food in the United States, being a major source of many vitamins (37–40). Furthermore, widespread voluntary fortification practices in the United States are not bound by strict regulations and legislation, which often result in the fortification of food products above mandatory concentrations (5).

At the time of blood sampling, vitamin fortification was much less common in the other regions included in this study, which is in accordance with the lower vitamin concentrations that were observed for these regions. Within the Nordic region, Norway (41) and Sweden (42), fortification was limited to only vitamins A and D in dairy products, and the consumption of cod-liver oil provided a further source of vitamin D in Norway (43), whereas there was no fortification in Finland (44). The concentrations of vitamins B-2, B-6, folate, and B-12 in the Nordic cohorts that were included in LC3 were similar to the concentrations that were previously measured by the same laboratory in Northern European (Sweden and Denmark) (45) and Norwegian (14, 46) cohorts. In Asia, China had no vitamin fortification during the sampling period, whereas Singapore (47) started fortification with folic acid in 1998, and in Australia, only the fortification of cereals with retinol took place (48). In addition, it has been reported that intake of vitamin A is low in China (49) compared with in the United States (9, 50). Low circulating 25(OH)D in Asians (51, 52) was suggested to be related to skin pigmentation and the cultural avoidance of sun exposure (52).

Multivitamin-supplement use

The high prevalence of multivitamin-supplement use among US participants in this study (36.8%) (Table 1) is consistent with the literature (53, 54) and contributed significantly to the high B-vitamin status in this region. For populations outside of the United States in this study, multivitamin-supplement use was less common, and data were available for only a few of these participants. A prevalence of multivitamin-supplement use of 22–27% in the Nordic countries Sweden and Norway has been reported (55), similar to the 26.8% shown in the current study. Because of the limited data available, stratification by multivitamin-supplement use in Nordic, Asian, and Australian regions was not performed when modeling vitamin status. Thus, the vitamin status obtained for each of these regions was influenced by the inclusion of participants who were not using supplements as well as subjects who were using supplements. Despite the reported increases in use of vitamin supplements over the past decades (56, 57), we found that the year of blood sample collection was not related to vitamin status (data not shown). This result might be related to the considerable heterogeneity of the participating cohorts or the distribution of participants over the time span during which blood samples were collected.

Notably, US multivitamin-supplement users showed distributions in the upper concentration range for all vitamins except for γ-tocopherol, which was lower. A study of middle-aged Americans showed that supplement users, on average, had vitamin intakes that were considerably higher than the estimated average requirements for several vitamins (58). It has also been reported that, in some US subgroups, supplement use has provided vitamins that are already consumed in adequate amounts (9, 59).

B-vitamin status and one-carbon and tryptophan metabolites

Components of OCM and tryptophan metabolism may serve as functional markers of B-vitamin status with inverse relations observed for tHcy with folate and vitamin B-12 (60); for cystathionine, serine, HK, and HK:XA with vitamin B-6 (29); and for MMA with vitamin B-12 (60). The grouping of OCM biomarkers HK and HK:XA opposite the B vitamins in the loading plot was in-line with these established relations and likely reflected the functions of B vitamins as cofactors of enzymes that are involved in these metabolic pathways (61). The low concentrations of functional markers in the US cohorts were in accordance with high circulating concentrations of individual B vitamins. A notable exception was observed for tHcy, and a lack of strong inverse relations between geometric means of tHcy with folate across geographic regions in Europe has been reported (45). In contrast, in studies in homogenous populations, intakes of folate (12, 62), vitamin B-2 (12), and vitamin B-6 (12) and circulating concentrations of folate (15, 62), vitamin B-12 (15, 62), vitamin B-2 (15), and vitamin B-6 (15, 62) were inversely related to serum and plasma tHcy concentrations. A lack of inverse associations between tHcy and B-vitamin status across geographical regions may be related to ethnicity, genetics, nutritional, and other lifestyle factors that are known to influence tHcy (63).

Circulating concentrations of the tryptophan metabolite HK (3) and the HK:XA ratio (64) have been shown to be functional markers of vitamin B-6 status, and both markers were inversely related to PLP across cohorts in the current study. The HK:XA ratio has been suggested to be a better functional vitamin B-6 marker than HK alone (64), but the HK:XA was not the lowest in the United States, which was the population that showed the highest PLP concentrations. Kynurenines other than HK were also related, although more weakly than HK was, to circulating concentrations of riboflavin and PLP in a homogenous Norwegian population (65) and also across cohorts in this study. However, we did not consistently find the anticipated relations across regions. Conceivably, as with tHcy, additional factors may influence circulating concentrations of tryptophan metabolites.

Strengths and weaknesses

The study has a large sample size, particularly from the United States. Information on multivitamin-supplement use in the US cohorts enabled the categorization according to this influential behavior.

A further strength of this work is the use of a single laboratory for the analyses of all biomarkers and metabolites. Between-batch CVs of quality-control samples showed that the variability of the assays that were used was small (**Supplemental Table 8**). Inadequate sample handling or storage will increase serum and plasma concentrations of MetSO, choline, and anthranilic acid combined with decreased methionine, HK, and 3-hydroxyanthranilic acid (26, 66) and may compromise the usefulness of comparing specimens from different biobanks. We did not observe biomarker concentrations that indicated detrimental sample handling or storage in this study.

Limitations of the study also exist. Because the consortium was formed to investigate lung cancer, it was oversampled with regard to the number of smokers. However, all models were adjusted for smoking. The included Asian cohorts consisted of Chinese subjects only. Thus, the current data cannot be expected to be representative of the Asian region. The Australian cohort was rather small. Information on multivitamin-supplement use was available for relatively few participants from the Nordic and Asian regions. Information on the use of single vitamin supplements was available for very few participants and, thus, was not included in this work. Most participants were middle aged or older, and the reported data may not be representative of other age groups. Finally, information on fasting status, which influences the circulating concentrations of some biomarkers (67), was not available for all cohorts and, therefore, was not included in the analyses.

In conclusion, the present study is the first study, to our knowledge, to robustly and comprehensively investigate vitamin status as reflected by circulating biomarkers in different regions from around the globe. Through the quantification of circulating concentrations of 7 vitamins and 5 functional B-vitamin markers, we show that B-vitamin status was higher in the United States than in the Nordic region, Asia, and Australia, whereas vitamin status, in general, was low in the Asians. The high prevalence of multivitamin-supplement use is suggested to be a major determinant of the higher B-vitamin status in the United States. The presented data will be useful when investigating circulating biomarker concentrations, nutritional status, and risk of chronic diseases particularly when different regions or populations are compared.

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The authors' responsibilities were as follows—PMU, ØM, and KM: led the laboratory analysis; ØM, DT, DCM, and AU: conducted the statistical analysis; ØM, DT, and AM: drafted the first version of the manuscript; PB and Mattias Johansson: initiated and served as the overall coordinators of the LC3 that was implemented in collaboration with the main investigators in the collaborating cohorts; PB and Mattias Johansson: acquired the main funding for the LC3; GS: acquired additional funding for the LC3; ØM: had primary responsibility for the final content of the manuscript; and all authors: were involved in the data collection and interpretation, critical revisions of the manuscript, and read and approved the final version of the manuscript. PMU is member of the steering board of the nonprofit Foundation to Promote Research into Functional Vitamin B12 Deficiency. The remaining authors reported no conflicts of interest related to the study.

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Online Supplemental Material

Supplemental Methods

Brief description of the participating cohorts in the Lung Cancer Cohort Consortium (LC3)

We invited all prospective cohort studies that in 2009 were members in the US National Cancer Institute (NCI) Cohort Consortium to participate in the Lung Cancer Cohort Consortium (LC3). Additional inclusion criteria included the occurrence of at least 200 incident lung cancer cases with baseline questionnaire data and either plasma or serum samples cryopreserved at <80°C available. Twenty cohorts fulfilled those criteria and accepted to participate, resulting in a combined cohort population of over 2,000,000 participants from North America, Europe, Asia and Australia. The present work included the healthy controls, selected by 1-1 matching with lung cancer cases, from each cohort.

1) US cohorts

The Women's Health Initiative (WHI)

WHI is a long-term health study of 161,808 post-menopausal women aged 50 to 79 years at 40 clinical centers throughout the U.S. WHI comprises a Clinical Trial (CT) component (68,132 women), and an Observational Study (OS) component (93,676 women), and has included several extension studies. Some detailed descriptions of WHI have been previously presented (1, 2).The CT evaluated two forms of postmenopausal hormone therapy, a low-fat dietary pattern intervention, and calcium and vitamin D supplementation in a randomized, controlled fashion, in a partial factorial design. The hormone therapy component findings led to major reductions in the use of hormone therapy worldwide, and are thought to have led to noteworthy reductions in breast cancer incidence.

The Southern Community Cohort Study (SCCS)

The Southern Community Cohort Study (SCCS) (3) is a prospective cohort of African and non-African Americans which during 2002-2009 enrolled approximately 86,000 residents aged 40-79 years across 12 southern states. Recruitment occurred mainly at community health centers, institutions providing basic health services primarily to the medically uninsured, so that the cohort includes many adults of lower income and educational status. Each study participant completed a detailed baseline questionnaire, and nearly 90% provided a biologic specimen (approximately 45% a blood sample and 45% buccal cells). Follow-up of the cohort is conducted by linkage to national mortality registers and to state cancer registries. Included in the LC3 are 480 African American lung cancer case-control pairs.

Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO)

The PLCO study, a randomized trial aimed at evaluating the efficacy of screening in reducing cancer mortality, recruited approximately 155,000 men and women age 55 to 74 years from 1992 to 2001 (4). Screening for lung cancer among participants in the intervention arm included a chest x-ray at baseline followed by either three annual x-rays (for current or former smokers at enrollment) or two annual x-rays (for never smokers); participants in the control arm received routine health care. Screening-arm participants provided data on sociodemographic factors, smoking behavior, anthropometric characteristics, medical history, and family history of cancer, as well as blood samples annually for the first 6 years of the study (baseline [T0] and T1 through T5). Follow-up in the trial as of July 2009 was 96.7%.

The New York University Women's Health Study (NYUWHS)

The New York University Women's Health Study (NYUWHS) is a prospective cohort study of women enrolled at a mammography screening center in New York City. From March 1985 through June 1991, 14,274 women between the ages of 34 and 65 were enrolled in the study. Because the original focus of the study was endogenous hormones and breast cancer, women who had taken hormone medications in the 6 months preceding baseline enrolment were not eligible for the study.

At the time of enrolment, data on demographics, anthropometric measures, medical history, reproductive and lifestyle variables were collected through self-administered questionnaires after written informed consent was obtained.

The American Cancer Society Cancer Prevention Study-II (CPS-II) Nutrition Cohort

The CPS-II Nutrition Cohort is a prospective study of cancer incidence and mortality among 86,404 men and 97,786 women. The CPS-II Nutrition Cohort, which is described in detail elsewhere (5), was initiated in 1992 as a subgroup of CPS-II, a prospective study of cancer mortality involving approximately 1.2 million Americans begun in 1982. Participants in the CPS-II Nutrition Cohort were recruited from CPS-II members who resided in 21 states and were between the ages of 50 and 74 years. At enrollment in 1992/1993, participants completed a self-administered questionnaire that included demographic, medical, dietary, and lifestyle information. Follow-up questionnaires were sent to all living Nutrition Cohort members in 1997, and every two years after this to update exposure information and to ascertain newly diagnosed cancers. Between June 1998 and June 2001, blood samples were collected from a subset of CPS-II Nutrition Cohort participants (21,965 women and 17,411 men).

The Campaign Against Cancer and Stroke (CLUE I) and the Campaign Against Cancer and Heart Disease (CLUE II).

The CLUE studies include two large cohorts of volunteers from Washington County, Maryland that were enrolled in 1974 and 1989, respectively. CLUE I was conducted in Washington County, Maryland, in the fall of 1974. Brief health histories and blood pressures were taken and 15 ml of blood was drawn from 26,147 volunteers (23,951 were residents of Washington County) at the time of enrollment. Linkage of the records from this program to those of a private census in the summer of 1975 indicated that almost a third of the adult population of the county had participated. CLUE II was an outgrowth of CLUE I conducted from May through October in 1989. As in CLUE I, a brief health history was obtained and 20 ml of blood was drawn. A blood sample was collected from 32,894 volunteers at the time of enrollment (25,076 were residents of Washington County). Participants were also given a food frequency questionnaire to complete at home and were asked to return it with a toenail clipping of the large toe for trace metal assays. Comparisons with published figures from the 1990 Census indicted that approximately 30 percent of adult residents had participated.

The Multiethnic Cohort (MEC)

The MEC includes over 215,000 men and women aged 45-75 years at recruitment from five different racial/ethnic groups (African Americans, Japanese Americans, Native Hawaiians, Latinos and European Americans) in Hawaii and California (6). The cohort was assembled in 1993-1996 by mailing a self-administered, 26-page questionnaire to obtain extensive information on demographics, medical and reproductive histories, medication use, family history of various cancers, physical activity and diet.

Women's Health Study (WHS)

The WHS was a randomized trial of low-dose aspirin, vitamin E, and beta-carotene in the primary prevention of cardiovascular disease and cancer beginning in 1992 among 39,876 female US health professionals aged ≥45 years (7). Information on major clinical, lifestyle, and dietary factors was collected via self-reports on baseline questionnaires. Women also provided baseline bloods.

Physicians' Health Study (PHS)

The PHS I began in 1982 as a randomized trial of aspirin and beta-carotene for the primary prevention of heart disease and cancer among 22,071 male, Caucasian physicians initially aged 40 to 84 years (8), followed by the PHS II trial beginning in 1997 to evaluate beta-carotene, vitamin C, vitamin E, and a daily multivitamin on the prevention of cancer, CVD, and other endpoints. The PHS II included 14,641 men, with 7,641 participants from the PHS I plus 7,000 new physicians, for a total of 29,071 PHS participants (9). A wide range of demographic, clinical, and lifestyle factors were assessed via baseline questionnaires, along with baseline bloods.

The Nurses' Health Study (NHS)

The Nurses' Health Study (NHS) (10, 11) was established in 1976, when 121,700 married female registered nurses aged 30 to 55 years residing in 11 States in the U.S. completed and returned a self-administered questionnaire. Questionnaires have been mailed to participants in both cohorts every 2 years since baseline to collect updated information on demographics, lifestyle factors, medical history, and disease outcomes. A semi quantitative food frequency questionnaire (FFQ) was administered to obtain information on usual dietary intake over the previous year. The reproducibility and validity of the FFQs have been established (12-16). The FFQ was first administered in 1980 in the NHS, and were repeated almost every 4 years thereafter. For each food item, the questionnaire specified a common serving size and queried respondents on average intake during the previous year; responses in 9 categories ranged from almost never to 6 or more per day. Most nutritional variables measured by these FFQs have been developed, tested, and refined by our group over the past 30 years (https://regepi.bwh.harvard.edu/health/).

The follow-up rate has been greater than 90%. The institutional review board at the Brigham and Women's Hospital approved the study. As approved by the committee, return of the questionnaires was considered to imply informed consent.

Health Professionnals Follow-up Study (HPFS)

The Health Professionals Follow-up Study (HPFS) (17) is an ongoing cohort study of 51,529 U.S. male professionals who were aged 40 to 75 years at baseline in 1986. Questionnaires have been mailed to participants in both cohorts every 2 years since baseline to collect updated information on demographics, lifestyle factors, mwedical history, and disease outcomes. The follow-up rate has been greater than 90%. The institutional review board at the Harvard T.H. Chan School Public Health approved this study. As approved by the committee, return of the questionnaires was considered to imply informed consent. A semiquantitative food frequency questionnaire (FFQ) was administered to obtain information on usual dietary intake over the previous year. The FFQ was first administered in 1986 in the HPFS and was repeated almost every 4 years thereafter. The reproducibility and validity of the FFQ have been estabished (14, 18). For each food item, the questionnaire specified a common serving size and queried respondents on average intake during the previous year; responses in 9 categories ranged from almost never to 6 or more per day.

2) Nordic cohorts

The Malmö Diet and Cancer Study (MDCS)

The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort study that between 1991 and 1996 recruited men and women aged 44 to 74 years of age living in Malmö, Sweden (19). The main goal of the MDCS is to study the impact of diet on cancer incidence and mortality. In total 28098 individuals completed all baseline examinations, which consisted of dietary assessment using a modified diet history method including a 7-day food record on lunch and dinner meals, a self-administered questionnaire covering lifestyle and socioeconomic factors, anthropometric measurements and collection of blood samples.

The Northern Sweden Health and Disease Study Cohort (NSHDS)

The Northern Sweden Health and Disease Study (NSHDS) encompasses several prospective cohorts, the current study involving study participants from the Västerbotten Intervention Project (VIP), a sub-cohort within NSHDS (20). VIP is an ongoing prospective cohort and intervention study intended for health promotion of the general population of the Västerbotten County in northern Sweden. VIP was initiated in 1985 and all residents in the Västerbotten County were invited to participate by attending a health check-up at 40, 50 and 60 years of age. Participants were asked to complete a self-administered questionnaire including various demographic factors such as education, smoking habits, physical activity and diet. In addition, height and weight were measured and participants were asked to donate a fasting blood sample for future research.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC)

The ATBC Study was a randomized, double-blind, placebo-controlled, primary cancer prevention trial testing daily supplementation with α-tocopherol (50 mg/day) or β-carotene (20 mg/day), or both (21). Between 1985 and 1988, the study enrolled and randomized 29,133 50-69 year old male cigarette smokers from southwestern Finland. Study supplementation continued for 5-8 years (median 6.1 years) until death or trial closure (April 30, 1993). At baseline, participants completed questionnaires regarding general risk factors, medical history, smoking habits, and dietary intake. Height, weight, heart rate, and blood pressure were measured by trained nurses and fasting serum samples were collected and stored at –70 ^{o}C.

The Nord-Trøndelag Health Study (HUNT)

The HUNT study is a longitudinal population based study having invited all persons aged 20-100 years living in the county of Nord-Trøndelag, Norway to three data collections, HUNT1 (1984-86), HUNT2 (1995-97) and HUNT 3 (2006-08) (http://www.ntnu.edu/hunt). Comprehensive data on life style, health status, symptoms, diseases and anthropometrics have been collected through questionnaires, interviews and clinical examinations, and in HUNT2 and HUNT3 biological material as blood and urine additionally were collected and stored.

3. Asian cohorts

The Shanghai Men's Health Study (SMHS) and the Shanghai Women's Health Study (SWHS)

The SMHS and SWHS are population-based cohort studies conducted in eight communities of urban Shanghai. Their designs and methods have been described elsewhere (22, 23).

Briefly, the SWHS recruited 74,941 women during 1997-2000 (response rate: 93%) and the SMHS recruited 61,480 men during 2002-2006 (response rate: 74%). Similar methods and questionnaires were used in both studies. At baseline in-person interviews, information on sociodemographic, diet, lifestyle, occupation and medical history was obtained; height, body weight, and waist circumference were measured. Blood samples were collected from 75% of the study participants in both studies, processed within 6 hours, and stored at -70℃ until analysis.

The SMHS and SWHS have been followed up by annual record linkage with the population-based Shanghai Cancer Registry and Shanghai Vital Statistics Registry and in-person surveys every 2-3 years. Exposure information, including dietary intake, was updated in the in-person follow-up surveys. All possible matches from the linkages are checked manually and verified by home visits. Medical charts were obtained from the initial diagnostic hospitals to verify cancer diagnosis.

Death certificate data from the Shanghai Vital Statistics Unit was used to identify the primary cause of death.

The studies were approved by the Institutional Review boards of the Shanghai Cancer Institute and Vanderbilt University. Informed consent was obtained from all participants.

The Singapore Chinese Health Study (SCHS)

The design of the SCHS study has been described (24, 25). Briefly, the cohort was drawn from permanent residents or citizens of Singapore who resided in government-built housing estates (86% of the Singapore population reside in such facilities). The eligible age range for cohort enrolment was 45-74 years. We restricted study subjects to the two major dialect groups of Chinese in Singapore: the Hokkiens and the Cantonese, who originated from Fujian and Guangdong provinces in Southern China, respectively. Between April 1993 and December 1998, 63,257 subjects (approximately 85% of eligible subjects) were enrolled into the cohort study. At recruitment, each study subject was interviewed in person by a trained interviewer using a structured questionnaire that emphasized current diet assessed via a validated, 165-item food frequency questionnaire. The questionnaire also requested information on demographics, lifetime use of tobacco, incense use, current physical activity, usual sleep duration, reproductive history (women only), occupational exposure, medical history, and family history of cancer.

Beginning in April 1994, a random 3% sample of cohort participants were asked to provide blood or buccal cell (if request for blood sample was denied), and spot urine samples. Eligibility for this biospecimen subcohort was extended to all surviving cohort participants starting in January 2000. By April 2005, all surviving cohort subjects had been contacted for biospecimen donation. Approximately 60% of eligible cohort participants donated biospecimens.

The cohort has been passively followed for death and cancer occurrence through regular record linkage with the population-based Singapore Cancer Registry and the Singapore Registry of Births and Deaths. Migration out of Singapore, especially among housing estate residents, is negligible. As of latest update, only 55 individuals from this cohort were known to be lost to follow-up due to migration and other reason.

The Shanghai Cohort Study (SCS)

The SCS study is a residential cohort of 18,244 men in Shanghai, China, assembled during 1986-89 when subjects were between the ages of 45 and 64 years. Approximately 80% of eligible men participated in the study. At the time of recruitment, each cohort subject was interviewed in-person by a trained nurse interviewer using a structured questionnaire that included background information, history of tobacco and alcohol use, current diet, and medical history (26, 27).

At the completion of the interview, the nurse collected a 10 ml blood and a single void urine specimen from the study participant. Blood and urine samples were kept in insulated boxes with ice (0-2^{o}C). The serum was separated from blood specimen within 3-4 hours after collection. Two sets of serum (2 ml and 1 ml, respectively) and two sets of urine samples (10 ml each) per subject have been stored at -80^{o}C.

The cohort has been followed for the occurrence of cancer and death through routine ascertainment of new cases from the population-based Shanghai Cancer Registry and Shanghai Vital Statistics Units. To maximize the cancer findings and minimize the loss of follow-up, we have recontacted each surviving cohort member annually. Retired nurses visit the last known address of each living cohort member and record details of the interim health history of the cohort member. As of December 31, 2014, cumulatively 612 (3.4%) original subjects were lost to follow-up (i.e., persons we have no record of death and we have been unable to locate through our annual follow-up recontacts), and 574 (3.1%) refused to our continued follow-up interview (their cancer and vital status has been continually updated through record linkage analyses) after 26 years of follow-up since the beginning of the study.

4. **Australian cohort**

<u>The Melbourne Collaborative Cohort Study (MCCS)</u>

The MCCS is a prospective cohort study of 41,514 participants (17,045 men and 24,469 women) aged 27-88 years at recruitment (28); 99.3% of whom were aged 40-69 years. Recruitment occurred between 1990 and 1994. Southern European migrants to Australia (including 5,411 Italians and 4,525 Greeks) were over-sampled to extend the range of lifestyle exposures and to increase genetic variation.

Subjects were recruited via Electoral Rolls (registration to vote is compulsory for adults in Australia), advertisements, and community announcements in local media. Comprehensive lists of Italian and Greek surnames were used to target southern European migrants in phonebooks and electoral rolls. Passive follow-up of the cohort has been conducted by record linkage to

Electoral Rolls, electronic phonebooks, the Victorian Cancer Registry and death records; as well as national cancer and death records to identify events outside of Victoria.

At recruitment participant's height and weight were measured, blood samples collected and questionnaires covering lifestyle (diet, smoking, physical activity and alcohol consumption), demographics and medical history completed.

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SUPPLEMENTAL TABLE 1

Baseline characteristics of study participants by cohort

Region	US										
Cohort[1]	WHI	SCCS	PLCO	NYUWHS	CPS-II	CLUE	MEC	WHS	NHS	HPFS	PHS
Number, n	241	226	450	171	182	191	174	181	345	155	81
Sex (female), n (%)	241 (100.0)	79 (35.0)	140 (31.1)	171 (100.0)	99 (54.4)	85 (44.5)	65 (37.4)	181 (100.0)	345 (100.0)	0 (0.0)	0 (0.0)
Age (years, mean (5th-95th percentile))	68.0 (55.0, 79.0)	53.8 (44.0, 71.0)	67.0 (59.0, 75.0)	56.0 (41.0, 64.0)	70.0 (62.0, 79.0)	60.0 (43.0, 75.0)	73.5 (59.0, 83.0)	57.0 (47.0, 70.0)	61.0 (48.2, 68.0)	67.0 (50.7, 76.3)	70.0 (56.0, 81.0)
Education, n (%)											
Less than high school	15 (6.2)	99 (43.8)	4 (0.9)	6 (3.7)	8 (4.4)	66 (34.6)	17 (9.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Completed high school	57 (23.7)	66 (29.2)	31 (6.9)	60 (36.8)	37 (20.4)	84 (44.0)	39 (22.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vocational school	19 (7.9)	10 (4.4)	110 (24.4)	3 (1.8)	16 (8.8)	0 (0.0)	11 (6.3)	23 (12.9)	243 (73.4)	0 (0.0)	0 (0.0)
Some college	56 (23.2)	38 (16.8)	56 (12.4)	41 (25.2)	53 (29.3)	21 (11.0)	31 (17.8)	94 (52.8)	0 (0.0)	0 (0.0)	0 (0.0)
College graduate	21 (8.7)	9 (4.0)	100 (22.2)	14 (8.6)	28 (15.5)	12 (6.3)	38 (21.8)	32 (18.0)	65 (19.6)	0 (0.0)	0 (0.0)
Graduate studies	73 (30.3)	4 (1.8)	75 (16.7)	39 (23.9)	39 (21.5)	8 (4.2)	38 (21.8)	29 (16.3)	23 (6.9)	155 (100.0)	81 (100.0)
Unknown	0 (0.0)	0 (0.0)	73 (16.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Smoking status, n (%)											
Never	241 (100.0)	17 (7.5)	50 (11.1)	30 (17.5)	41 (22.5)	33 (17.3)	38 (21.8)	33 (18.2)	43 (12.5)	18 (11.6)	25 (30.9)
Former	0 (0.0)	44 (19.5)	290 (64.4)	70 (40.9)	106 (58.2)	70 (36.6)	90 (51.7)	67 (37.0)	126 (36.5)	95 (61.3)	48 (59.3)
Current	0 (0.0)	165 (73.0)	110 (24.4)	71 (41.5)	35 (19.2)	88 (46.1)	46 (26.4)	81 (44.8)	176 (51.0)	42 (27.1)	8 (9.9)
BMI[2] (kg/m^2, median (5th-95th percentile))	25.7 (19.9, 38.0)	26.9 (20.0, 41.4)	26.7 (21.1, 35.7)	23.6 (19.5, 31.6)	26.6 (20.8, 33.2)	25.4 (20.1, 33.0)	25.2 (20.2, 33.2)	24.3 (19.8, 31.8)	24.2 (19.7, 33.8)	26.6 (21.7, 33.4)	25.1 (21.7, 31.9)
MV use, all participants, n (%)											
Never	0 (0.0)	0 (0.0)	325 (72.2)	84 (49.1)	0 (0.0)	0 (0.0)	77 (44.3)	0 (0.0)	0 (0.0)	0 (0.0)	41 (50.6)
Ever	0 (0.0)	0 (0.0)	57 (12.7)	86 (50.3)	0 (0.0)	0 (0.0)	97 (55.7)	0 (0.0)	0 (0.0)	0 (0.0)	29 (35.8)
No current	131 (54.4)	161 (71.2)	0 (0.0)	0 (0.0)	76 (41.8)	160 (83.8)	0 (0.0)	114 (63.0)	196 (56.8)	76 (49.0)	0 (0.0)
Current	110 (45.6)	64 (28.3)	0 (0.0)	0 (0.0)	95 (52.2)	31 (16.2)	0 (0.0)	60 (33.1)	133 (38.6)	78 (50.3)	0 (0.0)
Missing	0 (0.0)	1 (0.4)	68 (15.1)	1 (0.6)	11 (6.0)	0 (0.0)	0 (0.0)	7 (3.9)	16 (4.6)	1 (0.6)	11 (13.6)
MV use in those with available data, n (%)											
No (Never+No current)	131 (54.4)	161 (71.6)	325 (85.1)	84 (49.4)	76 (44.4)	160 (83.8)	77 (44.3)	114 (65.5)	196 (59.6)	76 (49.4)	41 (58.6)
Yes (Ever+Current)	110 (45.6)	64 (28.4)	57 (14.9)	86 (50.6)	95 (55.6)	31 (16.2)	97 (55.7)	60 (34.5)	133 (40.4)	78 (50.6)	29 (41.4)

SUPPLEMENTAL TABLE 1 (CONTINUED)

Region	Nordic				Asia				Australia	
Cohort[1]	MDCS	NSHDS	ATBC	HUNT	SCS[3]	SCHS	SMHS	SWHS	MCCS[4]	*P*
Numbers, n	198	244	200	193	513	379	421	416	353	
Sex (female), n(%)	109 (55.1)	121 (49.6)	0 (0.0)	130 (67.4)	0 (0.0)	99 (26.1)	0 (0.0)	416 (100.0)	141 (39.9)	<0.001
Age (years, mean (5th-95th percentile))	61.7 (49.1, 71.3)	59.8 (40.2, 60.3)	60.0 (51.0, 67.0)	59.1 (38.8, 76.6)	58.0 (47.6, 64.0)	68.0 (57.0, 78.0)	66.0 (45.0, 73.0)	62.0 (43.0, 69.0)	61.0 (45.0, 67.4)	<0.001
Education, n (%)										
Less than high school	91 (46.4)	110 (45.6)	63 (31.5)	101 (53.7)	366 (71.3)	370 (97.6)	15 (3.6)	92 (22.1)	232 (65.7)	<0.001
Completed high school	49 (25.0)	33 (13.7)	1 (0.5)	60 (31.9)	78 (15.2)	6 (1.6)	49 (11.9)	94 (22.6)	37 (10.5)	
Vocational school	14 (7.1)	72 (29.9)	81 (40.5)	0 (0.0)	17 (3.3)	3 (0.8)	154 (37.4)	103 (24.8)	33 (9.3)	
Some college	42 (21.4)	26 (10.8)	43 (21.5)	9 (4.8)	0 (0.0)	0 (0.0)	111 (26.9)	85 (20.4)	9 (2.5)	
College graduate	0 (0.0)	0 (0.0)	12 (6.0)	10 (5.3)	52 (10.1)	0 (0.0)	38 (9.2)	23 (5.5)	42 (11.9)	
Graduate studies	0 (0.0)	0 (0.0)	0 (0.0)	8 (4.3)	0 (0.0)	0 (0.0)	45 (10.9)	19 (4.6)	0 (0.0)	
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoking status, n (%)										
Never	50 (25.3)	30 (12.3)	0 (0.0)	27 (14.0)	51 (9.9)	80 (21.1)	49 (11.6)	381 (91.6)	49 (13.9)	<0.001
Former	71 (35.9)	76 (31.1)	0 (0.0)	43 (22.3)	11 (2.1)	89 (23.5)	71 (16.9)	5 (1.2)	145 (41.1)	
Current	77 (38.9)	138 (56.6)	200 (100.0)	123 (63.7)	451 (87.9)	210 (55.4)	301 (71.5)	30 (7.2)	159 (45.0)	
BMI[2] (kg/m^2, median (5th-95th percentile))	24.8 (20.1, 30.8)	26.0 (20.5, 33.9)	26.2 (20.9, 32.1)	26.3 (20.3, 34.5)	21.6 (17.9, 27.6)	22.8 (18.2, 27.1)	23.7 (18.7, 29.0)	24.4 (19.5, 30.2)	27.5 (21.2, 35.6)	<0.001
MV use, all participants, n (%)										
Never	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001
Ever	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
No current	0 (0.0)	113 (46.3)	0 (0.0)	37 (19.2)	0 (0.0)	0 (0.0)	386 (91.7)	393 (94.5)	306 (86.7)	
Current	0 (0.0)	27 (11.1)	0 (0.0)	28 (14.5)	0 (0.0)	0 (0.0)	35 (8.3)	23 (5.5)	47 (13.3)	
Missing	198 (100.0)	104 (42.6)	200 (100.0)	128 (66.3)	513 (100.0)	379 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
MV use in those with available data, n (%)										
No (Never+No current)	n.a.	113 (80.7)	n.a.	37 (56.9)	n.a.	n.a.	386 (91.7)	393 (94.5)	306 (86.7)	
Yes (Ever+Current)	n.a.	27 (19.3)	n.a.	28 (43.1)	n.a.	n.a.	35 (8.3)	23 (5.5)	47 (13.3)	

[1]ATBC, The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CLUE, The Campaign Against Cancer and Stroke (CLUE I) and the Campaign Against Cancer and Heart Disease (CLUE II); CPS-II, The American Cancer Society Cancer Prevention Study-II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; HUNT, The Nord-Trøndelag Health Study; MCCS, The Melbourne Collaborative Cohort Study; MDCS, The Malmö Diet and Cancer Study; MEC, The Multiethnic Cohort; NHS, The Nurses' Health Study; NSHDS, The Northern Sweden Health and Disease Study Cohort; NYUWHS, The New York University Women's Health Study; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCCS, The Southern Community Cohort Study; SCHS, The Singapore Chinese Health Study; SCS, The Shanghai Cohort Study; SMHS, The Shanghai Men's Health Study; SWHS, The Shanghai Women's Health Study; WHI, The Women's Health Initiative; WHS, Women's Health Study.

[2]BMI was calculated as weight/height2.

[3]SCS consisted of ethnic Chinese only.

[4]MCCS consisted of 75% Australian born, and 25% direct immigrants from Italy and Greece.

SUPPLEMENTAL TABLE 2
Crude geometric mean (5,95 percentiles) biomarker concentrations for the total population and by geographic region[1]

Region	Total	US	Nordic	Asia	Australia
Number, n	5314	2397	835	1729	353
Vitamin scores					
TVS		0.45 (-1.24, 2.19)	-0.12 (-1.17, 1.14)	-0.56 (-1.76, 0.78)	-0.01 (-1.03, 0.99)
BVS		0.43 (-1.18, 2.30)	-0.41 (-1.57, 0.95)	-0.34 (-1.54, 1.01)	-0.27 (-1.35, 0.94)
FVS		0.33 (-1.36, 1.79)	0.26 (-0.87, 1.37)	-0.64 (-2.03, 0.81)	0.30 (-0.81, 1.39)
Vitamins					
B2 (nmol/L)	20.3 (5.8, 89.6)	23.9 (7.2, 116.7)	16.1 (5.3, 47.5)	17.8 (4.7, 74.1)	22.2 (9.3, 62.8)
PLP (nmol/L)	41.3 (13.8, 195.5)	54.7 (16.4, 270.9)	32.5 (13.1, 101.1)	32.4 (12.2, 117.8)	34.4 (14.3, 110.4)
PL (nmol/L)	17.4 (5.2, 112.7)	22.3 (4.5, 184.5)	15.1 (6.0, 52.3)	13.1 (5.3, 47.3)	17.8 (9.2, 56.0)
PA (nmol/L)	25.2 (6.7, 187.4)	41.2 (10.2, 323.8)	22.6 (10.9, 80.8)	13.7 (4.5, 62.6)	22.3 (11.0, 72.0)
Folate (nmol/L)	20.0 (6.3, 90.3)	32.1 (8.1, 114.8)	10.6 (4.8, 28.7)	15.3 (6.7, 40.5)	13.7 (5.5, 35.2)
B12 (pmol/L)	428 (232, 768)	442 (230, 810)	465 (263, 790)	398 (227, 681)	401 (242, 659)
Vit A (µmol/L)	2.11 (1.32, 3.15)	2.23 (1.44, 3.30)	2.32 (1.61, 3.23)	1.84 (1.17, 2.82)	2.29 (1.60, 3.24)
Vit D (nmol/L)	54.5 (25.5, 101.5)	60.3 (25.9, 110.7)	58.1 (30.2, 100.9)	45.5 (23.1, 84.1)	56.8 (31.5, 97.4)
aTOC (µmol/L)	30.6 (17.4, 58.4)	34.5 (19.4, 67.0)	31.9 (21.1, 50.2)	24.7 (15.8, 41.3)	34.4 (23.3, 55.2)
gTOC (µmol/L)	3.66 (1.04, 9.95)	4.12 (0.94, 10.80)	3.02 (1.22, 7.30)	3.81 (1.11, 9.87)	2.10 (0.90, 5.05)
One-carbon metabolites					
tMet (µmol/L)	26.8 (18.6, 40.4)	26.6 (18.8, 39.7)	26.1 (18.5, 38.1)	27.4 (18.0, 42.1)	26.8 (19.6, 36.8)
tHcy (µmol/L)	11.8 (6.8, 21.9)	11.0 (6.4, 19.8)	11.6 (6.9, 20.9)	12.8 (7.4, 26.4)	13.1 (8.0, 22.0)
Cysta (µmol/L)	0.205 (0.097, 0.472)	0.178 (0.087, 0.400)	0.188 (0.097, 0.441)	0.262 (0.132, 0.567)	0.195 (0.111, 0.414)
tCys (µmol/L)	296 (232, 377)	305 (238, 386)	297 (230, 392)	281 (226, 348)	304 (253, 368)
Serine (µmol/L)	120.4 (76.6, 197.1)	108.0 (70.5, 163.5)	130.8 (84.2, 202.1)	135.3 (88.3, 217.7)	117.6 (86.8, 158.1)
Glycine (µmol/L)	268 (170, 441)	265 (168, 440)	266 (165, 439)	275 (174, 448)	250 (178, 381)

Choline (µmol/L)	12.9 (8.1, 22.5)	12.6 (7.8, 19.5)	12.3 (7.4, 21.7)	14.3 (8.7, 28.4)	14.7 (9.6, 21.9)
Betaine (µmol/L)	39.9 (20.6, 69.9)	35.9 (17.7, 62.4)	33.3 (20.4, 53.8)	51.2 (33.1, 78.4)	37.4 (22.1, 59.3)
DMG (µmol/L)	4.11 (2.15, 7.88)	3.47 (1.87, 6.64)	4.17 (2.45, 7.40)	5.17 (3.19, 9.19)	4.02 (2.41, 7.10)
Tryptophan + metabolites					
Trp (µmol/L)	65.9 (46.2, 90.7)	63.7 (43.7, 90.5)	67.8 (49.2, 92.3)	67.5 (49.3, 90.4)	68.3 (52.3, 89.7)
Kyn (µmol/L)	1.52 (1.05, 2.22)	1.53 (1.02, 2.34)	1.52 (1.06, 2.18)	1.50 (1.09, 2.14)	1.53 (1.13, 2.17)
KA (nmol/L)	44.4 (22.0, 91.0)	44.1 (21.2, 92.5)	38.5 (20.7, 69.9)	47.6 (23.7, 97.6)	46.2 (26.1, 81.5)
AA (nmol/L)	14.4 (8.0, 29.1)	14.7 (8.1, 28.9)	14.5 (8.4, 29.1)	13.9 (7.6, 28.8)	15.2 (8.4, 30.6)
HK (nmol/L)	37.1 (20.3, 70.5)	35.0 (18.9, 65.2)	37.6 (21.6, 63.9)	40.1 (21.9, 81.1)	36.7 (22.3, 65.8)
XA (nmol/L)	12.1 (4.6, 29.1)	11.5 (4.2, 29.4)	11.8 (5.1, 26.5)	13.0 (5.4, 29.9)	12.2 (4.9, 26.5)
HAA (nmol/L)	31.9 (16.1, 59.6)	30.2 (14.3, 61.5)	33.5 (18.9, 58.7)	33.8 (18.1, 59.8)	31.3 (17.4, 53.2)
QA (nmol/L)	362 (208, 685)	378 (207, 741)	336 (197, 605)	356 (216, 605)	356 (222, 598)
Other					
CRP (µg/L)	1.76 (0.24, 13.17)	2.25 (0.30 ,14.75)	1.57 (0.22 ,11.09)	1.28 (0.20, 10.52)	2.14 (0.37, 11.78)
KTR (nmol/µmol)	23.1 (16.4, 34.5)	24.0 (16.6, 37.0)	22.4 (16.5, 32.2)	22.2 (16.0, 32.1)	22.4 (16.0, 31.6)
PAr (PA/(PLP+PL))	0.48 (0.16, 1.02)	0.58 (0.22, 1.20)	0.50 (0.23, 0.94)	0.33 (0.11, 0.69)	0.47 (0.22, 0.82)
Creat (µmol/L)	74.7 (52.3, 105.3)	74.8 (51.3, 109.0)	73.6 (54.9, 97.5)	75.4 (52.3, 105.0)	73.2 (52.6, 103.0)
HK/XA	3.66 (1.39, 7.72)	3.69 (1.27, 8.25)	3.66 (1.58, 7.08)	3.69 (1.52, 7.16)	3.36 (1.46, 6.69)
Cotinine (nmol/L)	44 (0, 1868)	18 (0, 1963)	153 (0, 1693)	81 (1, 1780)	47 (0, 2070)
Arginine (µmol/L)	71.8 (28.2, 145.0)	59.3 (19.5, 125.0)	96.5 (51.2, 172.0)	82.3 (44.1, 144.6)	66.5 (37.9, 107.4)
ADMA (µmol/L)	0.545 (0.411, 0.714)	0.529 (0.397, 0.701)	0.549 (0.424, 0.703)	0.564 (0.435, 0.739)	0.546 (0.430, 0.684)
SDMA (µmol/L)	0.590 (0.408, 0.865)	0.574 (0.392, 0.833)	0.566 (0.411, 0.785)	0.628 (0.434, 0.933)	0.588 (0.448, 0.786)
hArg (µmol/L)	1.95 (0.98, 3.70)	1.76 (0.91, 3.39)	1.78 (0.95, 3.26)	2.33 (1.30, 4.06)	2.01 (1.11, 3.50)
MMA (µmol/L)	0.181 (0.105, 0.385)	0.177 (0.109, 0.330)	0.164 (0.100, 0.306)	0.199 (0.106, 0.500)	0.173 (0.108, 0.317)

Online Supplemental Material

[1]AA, anthranilic acid; ADMA, asymmetric dimethylarginine; α-tocopherol, αTOC; B2, riboflavin; B12, vitamin B12; BVS, B-Vitamin Score; CRP, C-reactive protein; Cysta, cystathionine; DMG, dimethylglycine; FVS, Fat-soluble Vitamin Score; γTOC, γ-tocopherol; HAA, 3-hydroxyanthranilic acid; hArg, homoarginine; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine/tryptophan ratio; Kyn, kynurenine; MMA, methylmalonic acid; PA, 4-pyridoxic acid; PAr, PLP/(PL+PA); PL, pyridoxal; PLP, pyridoxal 5'-phosphate; QA, quinolinic acid; SDMA, symmetric dimethylarginine, tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; Trp, tryptophan; TVS, Total Vitamin Score; Vit A, all-trans retinol; Vit D, 25-OH vitamin D2 + season adjusted 25-OH vitamin D3; XA, xanthurenic acid.

SUPPLEMENTAL TABLE 3
Crude geometric mean (5,95 percentiles) biomarker concentrations for US cohorts[1,2]

Cohort	WHI	SCCS	PLCO	NYUWHS	CPS-II	CLUE	MEC	WHS	NHS	HPFS	PHS
Number, n	241	226	450	171	182	191	174	181	345	155	81
Vitamin scores											
TVS	0.42 (-1.09, 1.63)	-0.56 (-1.79, 1.06)	0.59 (-1.21, 2.17)	0.71 (-0.90, 2.75)	1.36 (-0.42, 3.06)	0.17 (-1.11, 1.63)	1.13 (-0.25, 2.29)	0.11 (-1.28, 1.59)	0.38 (-1.06, 1.94)	0.37 (-1.01, 1.82)	0.35 (-0.88, 1.53)
BVS	0.52 (-0.88, 2.01)	-0.13 (-1.33, 1.33)	0.63 (-1.06, 2.42)	0.73 (-0.97, 3.07)	1.10 (-0.64, 3.00)	0.19 (-1.20, 1.94)	1.05 (-0.42, 2.55)	-0.07 (-1.42, 1.44)	0.26 (-1.32, 2.10)	0.14 (-1.39, 2.02)	0.14 (-1.10, 1.43)
FVS	0.16 (-1.36, 1.43)	-0.89 (-2.47, 0.61)	0.34 (-1.30, 1.84)	0.46 (-0.81, 1.78)	1.23 (-0.34, 2.51)	0.10 (-1.08, 1.27)	0.86 (-0.28, 1.80)	0.30 (-1.01, 1.51)	0.40 (-0.95, 1.57)	0.53 (-0.64, 1.57)	0.50 (-0.70, 1.53)
Vitamins											
B2 (nmol/L)	23.4 (9.8, 74.6)	15.3 (4.6, 85.8)	29.5 (9.4, 131.3)	33.3 (9.4, 242.6)	34.4 (8.1, 184.3)	25.6 (11.8, 74.2)	25.1 (10.5, 68.2)	13.3 (4.8, 57.9)	27.2 (10.7, 138.1)	18.8 (6.1, 116.5)	16.6 (6.4, 76.0)
PLP (nmol/L)	58.7 (15.3, 252.3)	40.4 (16.7, 116.2)	48.3 (14.2, 250.8)	63.3 (14.9, 397.5)	101.4 (29.1, 516.4)	44.2 (14.7, 186.7)	80.0 (21.8, 312.2)	52.5 (19.0, 185.7)	43.5 (15.2, 189.3)	65.4 (21.7, 313.3)	58.4 (24.8, 136.3)
PL (nmol/L)	26.6 (8.0, 150.7)	5.6 (2.2, 22.3)	35.1 (10.6, 316.4)	38.6 (8.1, 1429.8)	26.5 (5.8, 366.9)	27.8 (10.5, 122.1)	42.0 (12.7, 184.6)	10.2 (4.3, 30.4)	24.4 (8.2, 189.4)	14.6 (4.3, 136.7)	13.2 (5.7, 55.2)
PA (nmol/L)	40.3 (11.6, 189.2)	16.1 (5.7, 98.2)	50.1 (13.6, 432.0)	51.7 (10.7, 1182.0)	93.4 (17.8, 975.9)	32.2 (10.2, 222.4)	58.2 (16.3, 269.1)	30.3 (12.4, 112.2)	37.5 (13.0, 278.1)	45.8 (18.2, 336.2)	39.9 (18.2, 161.5)
Folate (nmol/L)	49.4 (19.1, 114.1)	19.0 (7.8, 52.6)	51.5 (15.3, 137.0)	27.9 (7.8, 124.5)	35.5 (13.0, 100.0)	21.8(6.4, 89.8)	66.6 (26.9, 138.8)	18.7 (6.2, 55.2)	26.4 (7.3, 99.4)	21.4 (7.1, 61.3)	26.3 (8.4, 60.3)
B12 (pmol/L)	389 (172, 822)	440 (257, 710)	423 (205, 805)	525 (304, 944)	551 (296, 1764)	437(232, 743)	502 (246, 1094)	445 (254, 741)	422 (233, 735)	400 (229, 678)	406 (219, 697)
Vit A (µmol/L)	2.02 (1.54, 2.69)	1.93 (1.12, 3.30)	2.09 (1.27, 3.33)	2.30 (1.65, 3.28)	2.69 (1.96, 3.88)	2.18 (1.49, 3.13)	2.35 (1.79, 3.15)	2.29 (1.54, 3.26)	2.31 (1.65, 3.27)	2.37 (1.68, 3.10)	2.51 (1.80, 3.33)
Vit D (nmol/L)	55.5 (25.7, 100.9)	35.1 (17.1, 71.1)	60.9 (29.7, 110.9)	61.2 (30.1, 102.9)	78.9 (39.9, 135.6)	61.0 (30.7, 94.4)	76.5 (49.9, 112.7)	61.6 (29.7, 106.2)	63.5 (30.5, 112.2)	66.6 (37.8, 110.7)	64.2 (33.4, 104.2)
aTOC (µmol/L)	36.4 (19.9, 67.0)	23.4 (14.5, 37.8)	37.8 (20.5, 74.7)	36.7 (23.5, 77.3)	46.3 (26.1, 89.3)	28.8 (18.4, 49.1)	41.5 (24.5, 67.1)	32.0 (20.9, 48.8)	33.5 (21.8, 52.1)	34.8 (22.9, 62.6)	32.1 (20.8, 65.1)
gTOC (µmol/L)	2.48 (0.61, 8.13)	6.45 (2.80, 13.46)	3.60 (0.81, 10.92)	3.99 (1.08, 9.76)	3.48 (1.02, 10.57)	5.81 (2.31, 10.96)	2.38 (0.72, 7.41)	5.90 (2.57, 11.27)	5.15 (1.59, 10.00)	4.65 (1.27, 10.32)	3.62 (1.17, 8.01)
One-carbon metabolites											
tMet (µmol/L)	22.7 (18.2, 28.1)	24.9 (15.6, 42.5)	27.0 (18.4, 41.1)	27.0 (19.3, 45.7)	28.2 (18.6, 50.2)	29.0 (20.2, 46.9)	28.9 (21.8, 36.7)	25.7 (19.2, 34.6)	27.1 (20.6, 36.2)	27.1 (19.3, 37.5)	25.9 (19.0, 33.3)
tHcy (µmol/L)	7.3 (5.0, 11.6)	14.4 (8.4, 24.7)	9.8 (6.2, 15.3)	9.8 (6.3, 15.9)	13.1 (8.3, 19.5)	11.4 (7.6, 18.9)	10.4 (7.0, 17.0)	12.1 (7.9, 22.5)	12.1 (8.0, 19.6)	13.7 (9.0, 20.9)	11.8 (8.5, 17.8)
Cysta (µmol/L)	0.120 (0.066, 0.227)	0.189 (0.083, 0.462)	0.196 (0.094, 0.440)	0.177 (0.091, 0.365)	0.212 (0.102, 0.494)	0.216 (0.106, 0.479)	0.184 (0.104, 0.336)	0.151 (0.084, 0.310)	0.170 (0.092, 0.352)	0.188 (0.104, 0.365)	0.178 (0.114, 0.324)
tCys (µmol/L)	273 (226, 332)	283 (216, 382)	307 (237, 386)	290 (241, 348)	341 (270, 424)	310 (258, 381)	329 (282, 402)	303 (236, 396)	318 (257, 386)	308 (245, 368)	294 (249, 358)
Serine (µmol/L)	81.6 (58.7, 116.9)	104.0 (74.4, 149.3)	121.7 (90.2, 164.1)	147.1 (113.4,	95.5 (62.9, 134.6)	111.2 (75.9, 179.9)	99.3 (76.0, 133.6)	107.7 (75.6, 159.6)	118.8 (87.2, 165.4)	98.0 (70.6, 135.9)	88.0 (62.6, 118.6)

				186.6)							
Glycine (µmol/L)	215 (133, 391)	248 (163, 395)	279 (196, 399)	322 (226, 525)	242 (160, 386)	279 (182, 454)	253 (176, 401)	286 (172, 508)	300 (195, 520)	236 (167, 339)	231 (163, 322)
Choline (µmol/L)	8.3 (6.0, 11.5)	14.4 (10.3, 20.7)	12.3 (8.3, 18.0)	12.5 (8.6, 17.8)	14.9 (10.4, 21.2)	14.3 (10.0, 20.3)	11.5 (8.7, 15.6)	11.8 (8.6, 17.6)	15.1 (10.7, 22.5)	13.1 (9.1, 18.8)	11.8 (8.9, 16.9)
Betaine (µmol/L)	24.2 (12.7, 42.9)	44.3 (28.7, 73.5)	37.6 (18.8, 61.9)	37.6 (24.4, 59.7)	40.5 (18.9, 73.7)	40.6 (24.9, 64.7)	40.2 (25.0, 64.6)	31.4 (16.5, 54.6)	30.8 (17.4, 49.9)	40.6 (26.8, 61.3)	39.7 (25.9, 60.5)
DMG (µmol/L)	2.45 (1.44, 4.48)	4.43 (2.68, 9.12)	3.56 (1.92, 6.07)	3.41 (1.96, 7.00)	4.30 (2.45, 7.55)	4.10 (2.50, 6.80)	3.60 (1.92, 6.28)	3.17 (1.99, 5.27)	2.91 (1.75, 5.12)	3.88 (2.35, 6.42)	3.76 (2.42, 6.94)
Tryptophan + metabolites											
Trp (µmol/L)	50.6 (36.4, 63.7)	54.3 (37.7, 75.9)	64.2 (43.1, 91.3)	65.9 (46.3, 90.5)	71.8 (48.3, 103.2)	66.8 (44.6, 93.6)	67.1 (49.9, 88.3)	68.1 (51.8, 87.2)	68.4 (48.3, 91.2)	67.0 (47.7, 88.1)	63.5 (49.2, 80.4)
Kyn (µmol/L)	1.29 (0.92, 1.85)	1.25 (0.88, 1.92)	1.66 (1.06, 2.60)	1.50 (1.08, 2.15)	1.79 (1.28, 2.46)	1.56 (1.08, 2.21)	1.66 (1.19, 2.35)	1.51 (1.08, 2.09)	1.53 (1.08, 2.20)	1.64 (1.15, 2.39)	1.59 (1.15, 2.04)
KA (nmol/L)	31.9 (18.2, 58.7)	35.5 (13.9, 78.4)	47.9 (23.7, 102.9)	41.6 (22.5, 80.5)	55.1 (25.0, 117.2)	43.4 (22.2, 85.0)	54.3 (29.6, 105.2)	41.6 (21.0, 83.3)	42.8 (22.5, 78.6)	53.0 (28.2, 95.4)	57.1 (33.3, 98.4)
AA (nmol/L)	13.9 (7.6, 28.2)	14.7 (7.5, 42.2)	14.7 (7.8, 26.5)	12.8 (7.5, 23.3)	15.9 (9.7, 27.1)	13.0 (7.7, 23.9)	16.9 (10.5, 32.9)	13.0 (7.9, 23.0)	15.6 (8.9, 30.3)	15.2 (8.6, 27.1)	18.4 (10.3, 30.9)
HK (nmol/L)	30.3 (18.9, 54.5)	29.8 (14.5, 77.9)	39.8 (22.6, 71.9)	36.6 (23.7, 65.7)	34.6 (17.9, 63.0)	37.9 (21.4, 63.9)	39.1 (25.7, 64.5)	32.8 (16.4, 62.9)	36.5 (21.6, 66.9)	30.4 (16.1, 53.3)	30.8 (15.8, 48.7)
XA (nmol/L)	7.2 (2.4, 14.7)	8.6 (2.6, 24.1)	12.1 (4.5, 31.4)	11.7 (5.4, 31.8)	15.8 (6.5, 34.1)	12.3 (4.8, 29.4)	11.7 (4.2, 25.0)	12.8 (4.9, 36.0)	11.6 (4.7, 28.0)	14.9 (7.2, 29.1)	13.4 (6.4, 26.6)
HAA (nmol/L)	26.1 (15.7, 47.2)	19.3 (9.2, 44.3)	35.2 (18.8, 71.6)	33.6 (18.6, 58.7)	42.1 (23.2, 82.4)	35.8 (20.0, 66.0)	35.9 (20.8, 58.8)	24.4 (12.5, 50.5)	30.3 (16.4, 54.3)	25.2 (14.3, 45.6)	27.7 (15.0, 43.9)
QA (nmol/L)	324 (198, 628)	342 (172, 844)	434 (229, 809)	336 (210, 612)	458 (280, 778)	364 (204, 708)	399 (241, 743)	370 (219, 641)	357 (208, 710)	372 (224, 677)	401 (256, 690)
Other											
CRP (µg/L)	2.23 (0.30, 13.18)	2.67 (0.30, 16.09)	2.08 (0.24, 14.37)	1.49 (0.15, 10.97)	3.03 (0.46, 17.05)	2.39 (0.36, 15.19)	1.54 (0.23, 13.03)	2.46 (0.32, 13.13)	2.72 (0.39, 15.89)	2.75 (0.48, 15.36)	1.26 (0.28 ,5.77)
KTR (nmol/µmol)	25.5 (18.1, 39.6)	23.1 (15.6, 40.0)	25.9 (18.5, 40.0)	22.7 (16.2, 32.9)	24.9 (16.5, 38.4)	23.4 (16.9, 36.2)	24.7 (17.9, 35.8)	22.1 (16.1, 30.4)	22.3 (15.7, 35.4)	24.4 (16.8, 35.3)	25.0 (17.0, 35.7)
PAr (PA/(PLP+PL))	0.51 (0.24, 1.00)	0.49 (0.14, 0.97)	0.65 (0.28, 1.25)	0.54 (0.24, 1.02)	0.81 (0.27, 1.83)	0.49 (0.21, 0.89)	0.52 (0.23, 1.05)	0.53 (0.23, 0.99)	0.61 (0.26, 1.21)	0.60 (0.28, 1.09)	0.61 (0.28, 1.39)
Creat (µmol/L)	59.0 (44.1, 79.2)	84.8 (59.5, 136.5)	76.1 (54.5, 102.0)	64.8 (48.4, 84.3)	82.6 (58.6, 120.0)	80.0 (57.6, 115.4)	83.6 (55.6, 123.0)	69.7 (53.0, 92.9)	69.6 (51.4, 93.5)	87.9 (67.0, 119.3)	84.5 (68.1, 107.0)
HK/XA	5.13 (1.94, 12.85)	4.64 (1.40, 10.97)	3.76 (1.32, 8.06)	3.49 (1.40, 6.55)	2.48 (0.99, 4.74)	3.59 (1.51, 7.01)	3.87 (1.75, 9.07)	3.08 (1.11, 8.27)	3.87 (1.36, 8.54)	2.28 (0.96, 3.87)	2.58 (1.09, 4.46)
Cotinine (nmol/L)	0 (0, 2)	344 (2, 2629)	9 (0, 1413)	33 (0, 1982)	5 (0, 1623)	60 (0, 2140)	3 (0, 1198)	32 (0, 1244)	77 (0, 2219)	12 (0, 1875)	3 (0, 1000)
Arginine (µmol/L)	57.7 (37.6, 85.3)	31.9 (10.5, 89.5)	98.5 (64.8, 139.0)	109.1 (79.6, 147.5)	51.2 (24.1, 101.9)	84.7 (55.7, 122.0)	67.7 (37.7, 108.4)	44.2 (14.9, 83.5)	47.8 (21.8, 90.1)	30.9 (10.9, 70.7)	45.1 (20.3, 76.4)
ADMA (µmol/L)	0.456 (0.339, 0.617)	0.523 (0.410, 0.718)	0.505 (0.380, 0.655)	0.549 (0.422, 0.672)	0.549 (0.438, 0.713)	0.549 (0.428, 0.687)	0.563 (0.434, 0.727)	0.559 (0.436, 0.713)	0.560 (0.442, 0.735)	0.534 (0.399, 0.713)	0.521 (0.403, 0.691)
SDMA (µmol/L)	0.476 (0.346, 0.737)	0.582 (0.401, 0.895)	0.595 (0.420, 0.830)	0.578 (0.424, 0.779)	0.621 (0.465, 0.836)	0.585 (0.436, 0.804)	0.633 (0.403, 1.053)	0.540 (0.397, 0.747)	0.624 (0.451, 0.883)	0.480 (0.346, 0.673)	0.557 (0.424, 0.739)

hArg (µmol/L)	1.53 (0.78, 3.08)	2.01 (0.97, 3.83)	1.68 (0.88, 3.10)	1.68 (0.95, 3.60)	1.97 (1.00, 3.52)	1.83 (1.04, 3.40)	1.93 (0.96, 3.92)	1.79 (0.93, 3.70)	1.66 (0.92, 3.26)	1.85 (1.15, 2.89)	1.81 (1.00, 2.87)
MMA (µmol/L)	0.172 (0.111, 0.309)	0.172 (0.109, 0.315)	0.180 (0.110, 0.365)	0.163 (0.101, 0.283)	0.211 (0.131, 0.435)	0.198 (0.116, 0.350)	0.176 (0.100, 0.387)	0.168 (0.114, 0.275)	0.166 (0.103, 0.289)	0.174 (0.111, 0.305)	0.171 (0.110, 0.356)

[1]CLUE, The Campaign Against Cancer and Stroke (CLUE I) and the Campaign Against Cancer and Heart Disease (CLUE II); CPS-II, The American Cancer Society Cancer Prevention Study-II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; MEC, The Multiethnic Cohort; NHS, The Nurses' Health Study; NYUWHS, The New York University Women's Health Study; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCCS, The Southern Community Cohort Study; WHI, The Women's Health Initiative; WHS, Women's Health Study.

[2]AA, anthranilic acid; ADMA, asymmetric dimethylarginine; α-tocopherol, αTOC; B2, riboflavin; B12, vitamin B12; BVS, B-Vitamin Score; CRP, C-reactive protein; Cysta, cystathionine; DMG, dimethylglycine; FVS, Fat-soluble Vitamin Score; γTOC, γ-tocopherol; HAA, 3-hydroxyanthranilic acid; hArg, homoarginine; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine/tryptophan ratio; Kyn, kynurenine; MMA, methylmalonic acid; PA, 4-pyridoxic acid; PAr, PLP/(PL+PA); PL, pyridoxal; PLP, pyridoxal 5'-phosphate; QA, quinolinic acid; SDMA, symmetric dimethylarginine, tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; Trp, tryptophan; TVS, Total Vitamin Score; Vit A, all-trans retinol; Vit D, 25-OH vitamin D2 + season adjusted 25-OH vitamin D3; XA, xanthurenic acid.

SUPPLEMENTAL TABLE 4
Crude geometric mean (5,95 percentiles) biomarker concentrations for Nordic, Asian and Australian cohorts[1,2]

Region	Nordic				Asian				Australia
Cohort	MDCS	NSHDS	ATBC	HUNT	SCS	SCHS	SMHS	SWHS	MCCS
Number, n	198	244	200	193	513	379	421	416	353
Vitamin scores									
TVS	-0.04 (-1.06, 1.10)	-0.30 (-1.20, 0.92)	0.05 (-1.26, 1.34)	-0.15 (-1.17, 0.86)	-0.71 (-1.73, 0.33)	0.04 (-1.20, 1.23)	-0.77 (-2.00, 0.52)	-0.69 (-1.78, 0.61)	-0.01 (-1.03, 0.99)
BVS	-0.39 (-1.54, 0.95)	-0.59 (-1.71, 0.87)	-0.19 (-1.30, 1.04)	-0.44 (-1.63, 0.73)	-0.41 (-1.47, 0.75)	-0.11 (-1.55, 1.34)	-0.58 (-1.75, 0.91)	-0.24 (-1.24, 1.02)	-0.27 (-1.35, 0.94)
FVS	0.38 (-0.58, 1.29)	0.13 (-0.88, 1.04)	0.33 (-1.12, 1.94)	0.24 (-0.68, 1.19)	-0.85 (-2.02, 0.32)	0.19 (-0.79, 1.17)	-0.77 (-2.11, 0.57)	-1.02 (-2.26, 0.40)	0.30 (-0.81, 1.39)
Vitamins									
B2 (nmol/L)	19.7 (8.9, 60.2)	10.4 (3.9, 32.8)	18.6 (9.0, 44.9)	19.3 (8.8, 49.4)	23.1 (9.6, 75.0)	23.5 (7.5, 96.1)	12.7 (3.4, 71.9)	14.2 (4.3, 63.6)	22.2 (9.3, 62.8)
PLP (nmol/L)	37.2 (16.1, 132.0)	32.9 (14.2, 78.7)	32.2 (12.3, 104.4)	28.2 (12.1, 72.9)	20.6 (9.9, 48.6)	37.4 (12.4, 205.5)	37.4 (16.6, 106.6)	43.3 (19.7, 125.4)	34.4 (14.3, 110.4)
PL (nmol/L)	23.3 (10.8, 77.6)	8.8 (4.6, 17.9)	16.1 (8.2, 47.3)	17.9 (9.1, 48.2)	15.3 (6.9, 50.8)	20.9 (7.8, 142.3)	9.0 (4.0, 27.5)	10.3 (5.2, 29.7)	17.8 (9.2, 56.0)
PA (nmol/L)	27.7 (11.9, 124.3)	19.9 (10.8, 40.9)	22.6 (11.4, 62.7)	21.5 (10.2, 69.2)	8.2 (2.7, 29.2)	23.1 (8.8, 185.5)	14.3 (5.9, 62.0)	14.4 (6.2, 63.4)	22.3 (11.0, 72.0)
Folate (nmol/L)	12.3 (5.6, 44.0)	9.1 (4.3, 38.7)	12.2 (6.6, 25.8)	9.4 (4.7, 20.8)	15.7 (7.1, 37.1)	15.8 (7.2, 45.3)	13.4 (5.8, 39.2)	16.5 (7.6, 45.3)	13.7 (5.5, 35.2)
B12 (pmol/L)	372 (207, 674)	507 (287, 931)	518 (325, 823)	468 (272, 761)	403 (231, 681)	415 (210, 730)	359 (221, 585)	420 (271, 667)	401 (242, 659)
Vit A (µmol/L)	2.30 (1.66, 3.09)	2.14 (1.48, 2.85)	2.52 (1.78, 3.72)	2.38 (1.80, 3.12)	1.82 (1.23, 2.59)	2.12 (1.45, 3.12)	1.89 (1.21, 2.88)	1.60 (1.04, 2.39)	2.29 (1.60, 3.24)
Vit D (nmol/L)	63.8 (38.9, 96.3)	56.6 (32.9, 89.2)	51.0 (25.0, 104.8)	62.4 (35.3, 104.2)	41.6 (21.9, 71.8)	64.8 (44.1, 95.4)	42.9 (22.9, 75.6)	39.0 (21.7, 67.7)	56.8 (31.5, 97.4)
aTOC (µmol/L)	33.1 (23.5, 49.4)	32.5 (23.3, 45.8)	33.6 (20.6, 59.1)	28.5 (18.8, 43.0)	22.7 (16.2, 33.6)	31.0 (22.0, 45.3)	22.5 (14.6, 39.3)	24.6 (15.6, 41.5)	34.4 (23.3, 55.2)
gTOC (µmol/L)	2.72 (1.21, 5.72)	2.56 (1.30, 5.41)	2.54 (1.00, 5.90)	4.94 (2.36, 9.79)	3.81 (2.01, 7.04)	1.65 (0.78, 3.74)	4.99 (2.33, 9.69)	6.21 (2.71, 12.27)	2.10 (0.90, 5.05)
One-carbon metabolites									
tMet (µmol/L)	25.8 (17.8, 39.8)	24.5 (18.7, 31.9)	27.8 (20.8, 36.5)	26.7 (17.5, 41.5)	30.1 (20.7, 44.9)	27.8 (18.8, 40.9)	26.2 (17.7, 39.9)	25.3 (16.9, 40.2)	26.8 (19.6, 36.8)
tHcy (µmol/L)	11.3 (7.0, 20.9)	9.8 (6.1, 16.2)	13.8 (8.4, 21.8)	12.4 (7.7, 22.5)	13.9 (8.5, 32.6)	14.9 (9.1, 25.7)	13.7 (8.4, 32.9)	9.4 (6.2, 15.2)	13.1 (8.0, 22.0)
Cysta (µmol/L)	0.214 (0.118, 0.430)	0.154 (0.086, 0.364)	0.173 (0.103, 0.316)	0.231 (0.108, 0.573)	0.283 (0.154, 0.567)	0.277 (0.148, 0.591)	0.269 (0.142, 0.587)	0.219 (0.112, 0.485)	0.195 (0.111, 0.414)
tCys (µmol/L)	286 (226, 368)	275 (226, 327)	338 (259, 439)	296 (241, 362)	275 (222, 345)	304 (250, 367)	273 (219, 332)	279 (226, 330)	304 (253, 368)
Serine (µmol/L)	110.8 (76.9, 158.4)	104.6 (79.2, 145.2)	150.6 (112.7, 195.0)	177.7 (140.7, 224.2)	184.8 (141.1, 248.6)	125.8 (93.0, 167.2)	112.8 (77.2, 157.7)	118.4 (84.2, 169.6)	117.6 (86.8, 158.1)

Glycine (µmol/L)	238 (160, 423)	223 (153, 385)	283 (212, 395)	347 (247, 521)	359 (273, 504)	237 (172, 342)	247 (173, 362)	253 (159, 433)	250 (178, 381)
Choline (µmol/L)	11.7 (8.1, 17.3)	9.1 (6.6, 12.7)	12.9 (8.9, 19.0)	17.9 (12.1, 28.3)	20.9 (13.3, 40.3)	13.0 (9.7, 17.3)	12.3 (8.7, 17.6)	11.3 (7.9, 15.9)	14.7 (9.6, 21.9)
Betaine (µmol/L)	32.0 (20.4, 55.3)	29.7 (18.7, 46.1)	39.4 (26.0, 61.0)	33.7 (20.7, 53.0)	58.0 (39.4, 86.9)	45.0 (29.1, 66.7)	51.8 (34.2, 76.1)	48.7 (32.5, 77.1)	37.4 (22.1, 59.3)
DMG (µmol/L)	3.91 (2.30, 7.22)	3.79 (2.34, 6.70)	4.96 (3.13, 7.76)	4.19 (2.54, 7.65)	5.76 (3.61, 10.01)	4.87 (2.96, 8.77)	5.31 (3.42, 9.01)	4.68 (2.85, 8.09)	4.02 (2.41, 7.10)
Tryptophan + metabolites									
Trp (µmol/L)	63.9 (46.0, 86.6)	61.6 (48.3, 77.0)	76.0 (57.6, 97.0)	72.1 (53.3, 95.4)	75.0 (55.9, 98.8)	66.5 (48.4, 86.8)	63.9 (47.9, 83.0)	63.7 (48.5, 83.9)	68.3 (52.3, 89.7)
Kyn (µmol/L)	1.42 (0.98, 2.15)	1.42 (1.01, 1.93)	1.64 (1.20, 2.23)	1.62 (1.18, 2.25)	1.54 (1.15, 2.16)	1.62 (1.17, 2.32)	1.45 (1.09, 2.01)	1.40 (1.02, 1.94)	1.53 (1.13, 2.17)
KA (nmol/L)	36.2 (21.2, 67.8)	37.4 (19.7, 70.3)	41.3 (24.2, 69.3)	39.4 (20.2, 70.9)	47.2 (23.4, 94.2)	49.3 (27.2, 111.3)	44.9 (23.2, 84.6)	49.3 (22.3, 100.7)	46.2 (26.1, 81.5)
AA (nmol/L)	14.8 (8.7, 29.1)	12.5 (7.6, 20.8)	13.7 (8.8, 23.2)	18.3 (10.2, 47.1)	16.2 (8.2, 50.3)	14.4 (7.7, 26.0)	12.8 (7.0, 21.9)	12.2 (7.8, 20.1)	15.2 (8.4, 30.6)
HK (nmol/L)	33.1 (19.8, 63.1)	36.7 (22.5, 62.7)	41.6 (26.2, 64.7)	39.8 (23.5, 67.1)	47.1 (24.5, 100.5)	41.0 (26.0, 77.4)	35.4 (20.1, 66.1)	37.2 (21.0, 68.3)	36.7 (22.3, 65.8)
XA (nmol/L)	12.7 (5.7, 28.7)	10.6 (4.4, 23.6)	10.5 (5.3, 23.5)	14.4 (6.7, 27.3)	14.8 (5.9, 33.1)	13.1 (6.0, 28.7)	12.3 (5.2, 28.9)	11.8 (4.9, 26.5)	12.2 (4.9, 26.5)
HAA (nmol/L)	36.5 (20.1, 63.0)	30.3 (18.6, 52.4)	35.3 (21.7, 58.7)	32.8 (17.2, 57.0)	32.9 (17.3, 64.2)	35.6 (20.2, 57.5)	32.8 (17.8, 56.2)	34.2 (19.0, 59.8)	31.3 (17.4, 53.2)
QA (nmol/L)	332 (198, 608)	315 (195, 559)	368 (235, 591)	334 (191, 723)	332 (203, 543)	377 (227, 689)	354 (208, 638)	373 (241, 598)	356 (222, 598)
Other									
CRP (µg/L)	1.36 (0.28, 8.06)	1.52 (0.19, 11.08)	2.09 (0.31, 15.13)	1.39 (0.18, 9.22)	0.83 (0.12, 8.96)	1.42 (0.26, 9.03)	1.63 (0.28, 13.38)	1.53 (0.23, 11.74)	2.14 (0.37, 11.78)
KTR (nmol/µmol)	22.3 (15.8, 33.8)	23.0 (17.2, 33.1)	21.6 (16.4, 27.5)	22.4 (16.5, 32.4)	20.6 (15.3, 28.8)	24.3 (17.6, 35.8)	22.7 (16.6, 32.4)	21.9 (16.2, 30.5)	22.4 (16.0, 31.6)
PAr (PA/(PLP+PL))	0.49 (0.21, 0.92)	0.51 (0.25, 0.92)	0.49 (0.26, 0.85)	0.51 (0.24, 1.05)	0.28 (0.07, 0.59)	0.43 (0.18, 0.84)	0.35 (0.13, 0.66)	0.30 (0.14, 0.56)	0.47 (0.22, 0.82)
Creat (µmol/L)	73.3 (53.9, 98.0)	70.7 (54.0, 91.5)	80.1 (63.3, 104.0)	71.4 (54.6, 94.9)	84.9 (64.0, 109.0)	74.7 (50.0, 115.2)	80.7 (64.4, 102.0)	61.5 (48.7, 76.2)	73.2 (52.6, 103.0)
HK/XA	2.93 (1.35, 5.79)	4.02 (1.86, 7.75)	4.39 (1.90, 7.84)	3.19 (1.47, 6.04)	3.87 (1.42, 7.93)	3.59 (1.70, 7.44)	3.71 (1.49, 6.14)	3.56 (1.57, 6.62)	3.36 (1.46, 6.69)
Cotinine (nmol/L)	28 (0, 2066)	135 (1, 1645)	782 (218, 1577)	187 (2, 1634)	390 (5, 2082)	61 (0, 1863)	187 (2, 1680)	6 (1, 551)	47 (0, 2070)
Arginine (µmol/L)	78.3 (50.8, 114.4)	66.6 (42.7, 95.9)	133.8 (90.6, 193.1)	136.4 (103.0, 181.2)	107.3 (62.0, 169.1)	70.3 (43.1, 108.1)	71.0 (35.9, 118.0)	79.3 (49.1, 125.2)	66.5 (37.9, 107.4)
ADMA (µmol/L)	0.540 (0.408, 0.725)	0.521 (0.417, 0.625)	0.589 (0.466, 0.756)	0.554 (0.436, 0.706)	0.584 (0.440, 0.785)	0.587 (0.475, 0.739)	0.535 (0.415, 0.688)	0.550 (0.437, 0.692)	0.546 (0.430, 0.684)
SDMA (µmol/L)	0.545 (0.387, 0.753)	0.532 (0.427, 0.692)	0.644 (0.478, 0.901)	0.555 (0.412, 0.756)	0.706 (0.516, 0.994)	0.646 (0.455, 0.981)	0.613 (0.458, 0.862)	0.542 (0.398, 0.736)	0.588 (0.448, 0.786)
hArg (µmol/L)	1.70 (1.03, 2.87)	1.62 (0.84, 2.95)	2.11 (1.18, 3.66)	1.77 (0.91, 3.23)	2.27 (1.32, 3.95)	2.47 (1.35, 4.20)	2.39 (1.35, 4.30)	2.22 (1.15, 3.87)	2.01 (1.11, 3.50)
MMA (µmol/L)	0.189	0.140	0.157	0.183	0.192	0.243	0.197	0.175	0.173

(0.111, 0.412)	(0.095, 0.216)	(0.101, 0.275)	(0.111, 0.310)	(0.110, 0.404)	(0.115, 0.701)	(0.102, 0.485)	(0.099, 0.359)	(0.108, 0.317)

[1]ATBC, The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; HUNT, The Nord-Trøndelag Health Study; MCCS, The Melbourne Collaborative Cohort Study; MDCS, The Malmö Diet and Cancer Study; NSHDS, The Northern Sweden Health and Disease Study Cohort; SCHS, The Singapore Chinese Health Study; SCS, The Shanghai Cohort Study; SMHS, The Shanghai Men's Health Study; SWHS, The Shanghai Women's Health Study.

[2]AA, anthranilic acid; ADMA, asymmetric dimethylarginine; α-tocopherol, αTOC; B2, riboflavin; B12, vitamin B12; BVS, B-Vitamin Score; CRP, C-reactive protein; Cysta, cystathionine; DMG, dimethylglycine; FVS, Fat-soluble Vitamin Score; γTOC, γ-tocopherol; HAA, 3-hydroxyanthranilic acid; hArg, homoarginine; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine/tryptophan ratio; Kyn, kynurenine; MMA, methylmalonic acid; PA, 4-pyridoxic acid; PAr, PLP/(PL+PA); PL, pyridoxal; PLP, pyridoxal 5'-phosphate; QA, quinolinic acid; SDMA, symmetric dimethylarginine, tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; Trp, tryptophan; TVS, Total Vitamin Score; Vit A, all-trans retinol; Vit D, 25-OH vitamin D2 + season adjusted 25-OH vitamin D3; XA, xanthurenic acid.

SUPPLEMENTAL TABLE 5
Geometric mean biomarker concentrations (95% CI) for US cohorts from generalized linear models[1,2,3]

Cohort	WHI	SCCS	PLCO	NYUWHS	CPS-II	CLUE	MEC	WHS	NHS	HPFS	PHS
Number, n	241	226	450	171	182	191	174	181	345	155	81
Vitamin scores											
TVS	0.10	-0.34	0.43	0.73	1.14	0.24	0.95	0.10	0.38	0.25	0.21
	(-0.03 ,0.22)	(-0.45 ,-0.23)	(0.35 ,0.51)	(0.59 ,0.86)	(1.02 ,1.27)	(0.11 ,0.36)	(0.83 ,1.08)	(-0.02 ,0.23)	(0.28 ,0.48)	(0.11 ,0.40)	(0.03 ,0.40)
BVS	0.17	0.02	0.55	0.71	0.93	0.22	0.91	-0.11	0.21	0.09	0.02
	(0.04 ,0.29)	(-0.09 ,0.14)	(0.46 ,0.63)	(0.57 ,0.85)	(0.80 ,1.06)	(0.10 ,0.35)	(0.78 ,1.04)	(-0.25 ,0.02)	(0.11 ,0.32)	(-0.06 ,0.24)	(-0.17 ,0.21)
FVS	0.03	-0.81	0.24	0.50	1.11	0.06	0.72	0.32	0.42	0.44	0.36
	(-0.08, 0.15)	(-0.91, -0.70)	(0.16, 0.32)	(0.38, 0.63)	(0.99, 1.23)	(-0.06, 0.18)	(0.60, 0.85)	(0.20, 0.44)	(0.32, 0.51)	(0.30, 0.58)	(0.19, 0.54)
Vitamins											
B2 (nmol/L)	19.2	17.0	26.9	35.3	29.9	26.3	21.8	13.7	27.6	16.4	14.5
	(17.2, 21.4)	(15.4, 18.8)	(25.0, 29.0)	(31.4, 39.7)	(26.8, 33.4)	(23.6, 29.3)	(19.5, 24.4)	(12.2, 15.3)	(25.3, 30.1)	(14.4, 18.7)	(12.3, 17.1)
PLP (nmol/L)	48.3	43.7	46.4	61.8	94.5	44.5	76.8	51.0	43.2	63.7	54.3
	(43.6, 53.6)	(39.7, 48.1)	(43.3, 49.7)	(55.2, 69.2)	(84.9, 105.1)	(40.0, 49.4)	(68.9, 85.7)	(45.8, 56.9)	(39.7, 46.9)	(56.2, 72.2)	(46.4, 63.6)
PL (nmol/L)	21.0	6.3	32.8	38.8	23.2	29.3	37.7	10.0	23.9	14.2	12.1
	(18.4, 23.9)	(5.6, 7.1)	(30.1, 35.8)	(33.7, 44.7)	(20.3, 26.5)	(25.7, 33.4)	(32.9, 43.2)	(8.7, 11.5)	(21.5, 26.5)	(12.2, 16.7)	(9.9, 14.7)
PA (nmol/L)	30.7	19.1	44.2	55.2	76.2	33.5	47.2	31.2	38.0	43.4	33.8
	(26.9, 34.9)	(17.0, 21.5)	(40.5, 48.1)	(48.0, 63.5)	(66.8, 87.0)	(29.4, 38.2)	(41.2, 54.0)	(27.2, 35.7)	(34.3, 42.2)	(37.2, 50.7)	(27.8, 41.1)
Folate (nmol/L)	38.2	21.6	48.4	27.1	31.2	22.8	59.9	18.1	25.2	21.6	24.5
	(34.8, 41.8)	(19.9, 23.5)	(45.6, 51.5)	(24.5, 29.9)	(28.4, 34.2)	(20.8, 25.0)	(54.5, 65.9)	(16.4, 19.9)	(23.4, 27.1)	(19.4, 24.1)	(21.3, 28.1)
B12 (pmol/L)	360	451	424	513	542	440	499	434	411	409	411
	(339, 381)	(427, 475)	(408, 441)	(482, 546)	(511, 576)	(415, 467)	(469, 530)	(409, 461)	(393, 431)	(382, 439)	(376, 449)
Vit A (µmol/L)	2.06	1.94	2.04	2.38	2.66	2.17	2.29	2.36	2.38	2.27	2.41
	(1.99, 2.14)	(1.88, 2.01)	(1.99, 2.08)	(2.29, 2.47)	(2.56, 2.76)	(2.10, 2.25)	(2.21, 2.38)	(2.27, 2.45)	(2.32, 2.45)	(2.18, 2.37)	(2.28, 2.54)
Vit D (nmol/L)	53.7	36.0	59.0	62.4	76.5	59.7	74.0	62.3	64.8	64.9	61.3
	(50.9, 56.7)	(34.3, 37.9)	(56.9, 61.2)	(58.8, 66.1)	(72.4, 80.8)	(56.5, 63.0)	(70.0, 78.4)	(58.9, 65.9)	(62.0, 67.6)	(60.8, 69.2)	(56.5, 66.5)
aTOC (µmol/L)	33.1	24.6	37.0	36.0	43.8	28.9	39.5	31.1	32.3	35.1	31.9
	(31.6, 34.6)	(23.6, 25.6)	(36.0, 38.2)	(34.3, 37.8)	(41.9, 45.9)	(27.6, 30.2)	(37.7, 41.4)	(29.7, 32.6)	(31.2, 33.5)	(33.3, 37.0)	(29.8, 34.1)
gTOC (µmol/L)	2.64	6.28	3.60	4.07	3.56	5.87	2.40	6.06	5.31	4.67	3.56
	(2.43, 2.88)	(5.81, 6.78)	(3.40, 3.81)	(3.72, 4.46)	(3.27, 3.88)	(5.39, 6.39)	(2.20, 2.63)	(5.55, 6.62)	(4.96, 5.68)	(4.22, 5.17)	(3.14, 4.05)
One-carbon metabolites											
tMet (µmol/L)	24.8	25.7	27.7	29.2	30.1	29.9	29.9	27.7	29.3	26.7	26.2
	(24.0, 25.6)	(24.9, 26.5)	(27.1, 28.3)	(28.2, 30.2)	(29.1, 31.1)	(29.0, 30.9)	(28.9, 31.0)	(26.8, 28.7)	(28.5, 30.1)	(25.7, 27.8)	(24.9, 27.5)
tHcy (µmol/L)	8.1	14.4	9.3	11.2	12.8	11.3	9.6	13.5	13.3	12.6	10.8
	(7.8, 8.5)	(13.8, 15.0)	(9.1, 9.6)	(10.6, 11.8)	(12.2, 13.4)	(10.8, 11.9)	(9.2, 10.1)	(12.8, 14.2)	(12.8, 13.8)	(11.9, 13.3)	(10.0, 11.5)
Cysta (µmol/L)	0.124	0.198	0.181	0.202	0.196	0.217	0.162	0.168	0.184	0.170	0.157
	(0.116. 0.132)	(0.187. 0.210)	(0.174. 0.190)	(0.188. 0.216)	(0.183. 0.210)	(0.203. 0.232)	(0.152. 0.174)	(0.157. 0.179)	(0.175. 0.194)	(0.158. 0.184)	(0.143. 0.174)
tCys (µmol/L)	262	295	295	305	323	315	307	314	327	297	277
	(257, 267)	(290, 300)	(291, 299)	(299, 311)	(317, 330)	(309, 321)	(301, 313)	(308, 320)	(322, 332)	(290, 304)	(269, 285)
Serine (µmol/L)	82	103	124	144	98	107	102	106	117	100	91
	(80, 84)	(100, 105)	(122, 127)	(139, 148)	(95, 101)	(104, 110)	(99, 106)	(102, 109)	(115, 120)	(96, 103)	(87, 95)

Glycine (µmol/L)	207 (199, 215)	248 (240, 257)	286 (279, 294)	309 (297, 322)	243 (234, 252)	274 (264, 284)	257 (247, 267)	273 (262, 283)	286 (278, 295)	247 (236, 259)	245 (232, 259)
Choline (µmol/L)	8.6 (8.3, 8.9)	14.6 (14.2, 15.1)	11.9 (11.6, 12.1)	13.2 (12.7, 13.7)	14.5 (14.0, 15.0)	13.9 (13.4, 14.4)	11.0 (10.6, 11.4)	12.4 (11.9, 12.8)	15.7 (15.2, 16.1)	12.4 (11.9, 12.9)	11.2 (10.6, 11.8)
Betaine (µmol/L)	26.6 (25.5, 27.7)	44.2 (42.5, 46.0)	35.9 (34.9, 36.9)	44.3 (42.4, 46.4)	40.3 (38.6, 42.1)	41.2 (39.4, 42.9)	38.0 (36.4, 39.7)	36.7 (35.1, 38.4)	35.4 (34.3, 36.7)	35.5 (33.7, 37.3)	34.6 (32.5, 36.9)
DMG (µmol/L)	2.66 (2.54, 2.79)	4.45 (4.25, 4.65)	3.41 (3.30, 3.52)	3.94 (3.74, 4.16)	4.24 (4.04, 4.46)	4.22 (4.02, 4.43)	3.35 (3.19, 3.53)	3.65 (3.47, 3.84)	3.29 (3.17, 3.42)	3.46 (3.26, 3.67)	3.35 (3.11, 3.61)
Tryptophan + metabolites											
Trp (µmol/L)	53.9 (52.5, 55.4)	53.2 (51.9, 54.6)	63.9 (62.7, 65.1)	68.6 (66.5, 70.6)	73.6 (71.5, 75.7)	65.9 (64.1, 67.7)	67.8 (65.9, 69.8)	71.1 (69.1, 73.1)	71.8 (70.2, 73.4)	64.3 (62.2, 66.4)	61.4 (58.9, 64.0)
Kyn (µmol/L)	1.30 (1.26, 1.34)	1.30 (1.27, 1.34)	1.57 (1.53, 1.60)	1.60 (1.55, 1.66)	1.68 (1.63, 1.73)	1.57 (1.52, 1.62)	1.52 (1.48, 1.57)	1.60 (1.55, 1.65)	1.60 (1.56, 1.64)	1.52 (1.47, 1.58)	1.44 (1.38, 1.51)
KA (nmol/L)	32.5 (30.5, 34.5)	37.3 (35.2, 39.4)	43.9 (42.2, 45.8)	46.5 (43.5, 49.7)	51.2 (48.1, 54.6)	43.6 (41.0, 46.4)	48.7 (45.7, 52.0)	45.9 (43.1, 49.0)	46.8 (44.5, 49.1)	46.9 (43.5, 50.5)	49.1 (44.8, 54.0)
AA (nmol/L)	13.0 (12.2, 13.8)	16.0 (15.1, 16.9)	13.5 (12.9, 14.0)	13.9 (13.0, 14.8)	14.4 (13.5, 15.3)	12.7 (12.0, 13.5)	14.9 (14.0, 15.9)	13.8 (13.0, 14.7)	16.5 (15.7, 17.3)	14.0 (13.0, 15.0)	16.0 (14.6, 17.5)
HK (nmol/L)	29.6 (28.1, 31.3)	30.9 (29.4, 32.5)	38.0 (36.7, 39.4)	37.3 (35.2, 39.5)	32.6 (30.9, 34.5)	38.2 (36.2, 40.4)	36.4 (34.4, 38.5)	33.1 (31.3, 35.0)	36.7 (35.2, 38.4)	29.3 (27.4, 31.2)	29.2 (26.9, 31.7)
XA (nmol/L)	7.9 (7.3, 8.5)	8.5 (7.9, 9.1)	11.7 (11.1, 12.3)	12.3 (11.2, 13.4)	15.9 (14.6, 17.3)	12.0 (11.0, 13.0)	11.6 (10.7, 12.7)	13.6 (12.4, 14.8)	12.4 (11.6, 13.2)	13.8 (12.5, 15.2)	12.4 (11.0, 14.1)
HAA (nmol/L)	27.6 (26.1, 29.2)	19.2 (18.2, 20.2)	33.8 (32.6, 35.1)	35.5 (33.4, 37.7)	42.0 (39.7, 44.5)	35.7 (33.7, 37.8)	35.2 (33.2, 37.4)	26.0 (24.5, 27.6)	32.8 (31.3, 34.3)	23.2 (21.6, 24.8)	25.3 (23.3, 27.6)
QA (nmol/L)	293 (279, 308)	383 (366, 401)	387 (374, 401)	363 (344, 384)	397 (377, 417)	370 (351, 389)	337 (320, 355)	391 (371, 412)	372 (357, 387)	340 (320, 361)	342 (317, 369)
Other											
CRP (µg/L)	2.05 (1.73, 2.43)	2.97 (2.54, 3.47)	1.98 (1.77, 2.22)	1.48 (1.23, 1.79)	2.64 (2.22, 3.15)	2.54 (2.14, 3.02)	1.32 (1.10, 1.58)	2.35 (1.97, 2.82)	2.46 (2.14, 2.82)	2.90 (2.36, 3.56)	1.31 (1.01, 1.70)
KTR (nmol/µmol)	24.2 (23.4, 25.0)	24.5 (23.8, 25.2)	24.5 (24.0, 25.1)	23.4 (22.5, 24.2)	22.9 (22.1, 23.6)	23.8 (23.0, 24.6)	22.5 (21.7, 23.3)	22.5 (21.7, 23.3)	22.3 (21.8, 22.9)	23.7 (22.8, 24.7)	23.5 (22.3, 24.7)
PAr (PA/(PLP+PL))	0.435 (0.405, 0.467)	0.370 (0.347, 0.396)	0.536 (0.511, 0.563)	0.517 (0.478, 0.559)	0.608 (0.564, 0.655)	0.443 (0.412, 0.476)	0.405 (0.375, 0.436)	0.504 (0.467, 0.544)	0.546 (0.516, 0.579)	0.527 (0.484, 0.575)	0.499 (0.447, 0.557)
Creat (µmol/L)	64.0 (62.3, 65.8)	85.1 (83.0, 87.3)	71.9 (70.6, 73.2)	73.5 (71.3, 75.7)	81.1 (78.9, 83.4)	80.0 (77.8, 82.2)	78.7 (76.5, 81.0)	78.4 (76.2, 80.6)	78.3 (76.6, 80.0)	78.3 (75.7, 80.9)	74.5 (71.5, 77.7)
HK/XA	3.76 (3.49, 4.06)	3.65 (3.41, 3.91)	3.26 (3.10, 3.42)	3.04 (2.80, 3.30)	2.05 (1.90, 2.21)	3.20 (2.96, 3.45)	3.13 (2.90, 3.39)	2.44 (2.26, 2.64)	2.97 (2.79, 3.15)	2.12 (1.94, 2.32)	2.35 (2.10, 2.64)
Cotinine (nmol/L)	20 (16, 24)	83 (69, 100)	30 (26, 34)	48 (38, 59)	26 (21, 32)	60 (49, 73)	12 (10, 15)	40 (32, 49)	70 (60, 83)	28 (22, 35)	20 (15, 27)
Arginine (µmol/L)	58.4 (55.4, 61.5)	32.0 (30.5, 33.5)	98.0 (94.7, 101.5)	113.1 (106.9, 119.8)	51.1 (48.5, 53.9)	83.9 (79.6, 88.4)	66.6 (63.0, 70.4)	45.5 (43.0, 48.0)	49.0 (47.0, 51.1)	30.3 (28.4, 32.2)	44.2 (40.9, 47.9)
ADMA (µmol/L)	0.454 (0.443, 0.464)	0.533 (0.522, 0.544)	0.497 (0.489, 0.504)	0.565 (0.551, 0.579)	0.536 (0.524, 0.549)	0.550 (0.537, 0.563)	0.543 (0.530, 0.556)	0.570 (0.557, 0.584)	0.566 (0.556, 0.577)	0.523 (0.509, 0.538)	0.509 (0.491, 0.527)
SDMA (µmol/L)	0.468 (0.454,	0.606 (0.589,	0.566 (0.555,	0.630 (0.610,	0.585 (0.567,	0.589 (0.571,	0.581 (0.562,	0.576 (0.558,	0.657 (0.641,	0.453 (0.436,	0.512 (0.489,

	0.482)	0.624)	0.578)	0.652)	0.604)	0.607)	0.599)	0.595)	0.673)	0.470)	0.537)
hArg (μmol/L)	1.63 (1.54, 1.71)	1.98 (1.88, 2.07)	1.64 (1.59, 1.70)	1.76 (1.66, 1.87)	2.02 (1.92, 2.14)	1.80 (1.70, 1.90)	1.96 (1.86, 2.07)	1.91 (1.81, 2.02)	1.81 (1.73, 1.88)	1.72 (1.61, 1.83)	1.67 (1.54, 1.81)
MMA (μmol/L)	0.168 (0.159, 0.178)	0.182 (0.172, 0.192)	0.169 (0.163, 0.176)	0.175 (0.165, 0.187)	0.194 (0.183, 0.206)	0.201 (0.189, 0.213)	0.158 (0.148, 0.168)	0.176 (0.166, 0.187)	0.172 (0.164, 0.180)	0.167 (0.156, 0.180)	0.157 (0.143, 0.171)

[1]CLUE, The Campaign Against Cancer and Stroke (CLUE I) and the Campaign Against Cancer and Heart Disease (CLUE II); CPS-II, The American Cancer Society Cancer Prevention Study-II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; MEC, The Multiethnic Cohort; NHS, The Nurses' Health Study; NYUWHS, The New York University Women's Health Study; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCCS, The Southern Community Cohort Study; WHI, The Women's Health Initiative; WHS, Women's Health Study.

[2]AA, anthranilic acid; ADMA, asymmetric dimethylarginine; α-tocopherol, αTOC; B2, riboflavin; B12, vitamin B12; BVS, B-Vitamin Score; CRP, C-reactive protein; Cysta, cystathionine; DMG, dimethylglycine; FVS, Fat-soluble Vitamin Score; γTOC, γ-tocopherol; HAA, 3-hydroxyanthranilic acid; hArg, homoarginine; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine/tryptophan ratio; Kyn, kynurenine; MMA, methylmalonic acid; PA, 4-pyridoxic acid; PAr, PLP/(PL+PA); PL, pyridoxal; PLP, pyridoxal 5'-phosphate; QA, quinolinic acid; SDMA, symmetric dimethylarginine, tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; Trp, tryptophan; TVS, Total Vitamin Score; Vit A, all-trans retinol; Vit D, 25-OH vitamin D2 + season adjusted 25-OH vitamin D3; XA, xanthurenic acid.

[3]General linear models, adjusted for age, sex, smoking (former vs. never, current vs. never).

SUPPLEMENTAL TABLE 6
Geometric mean biomarker concentrations (95% CI) for Nordic, Asian, Australian cohorts from generalized linear models[1,2,3]

Region	Nordic				Asian				Australia
Cohort	MDCS	NSHDS	ATBC	HUNT	SCS	SCHS	SMHS	SWHS	MCCS
Number, n	198	244	200	193	513	379	421	416	353
Vitamin scores									
TVS	-0.12 (-0.24 ,0.00)	-0.31 (-0.42 ,-0.20)	0.20 (0.08 ,0.33)	-0.07 (-0.19 ,0.05)	-0.43 (-0.52 ,-0.34)	0.01 (-0.08 ,0.09)	-0.61 (-0.69 ,-0.52)	-0.84 (-0.93 ,-0.74)	-0.05 (-0.14 ,0.03)
BVS	-0.42 (-0.55 ,-0.30)	-0.51 (-0.62 ,-0.40)	0.07 (-0.06 ,0.19)	-0.39 (-0.52 ,-0.27)	-0.16 (-0.25 ,-0.07)	-0.11 (-0.20 ,-0.02)	-0.43 (-0.52 ,-0.34)	-0.49 (-0.59 ,-0.39)	-0.23 (-0.32 ,-0.13)
FVS	0.39 (0.27, 0.50)	0.19 (0.09, 0.30)	0.42 (0.30, 0.53)	0.28 (0.17, 0.39)	-0.73 (-0.81, -0.65)	0.14 (0.06, 0.23)	-0.74 (-0.82, -0.66)	-1.06 (-1.15, -0.96)	0.31 (0.22, 0.39)
Vitamins									
B2 (nmol/L)	19.9 (17.9, 22.1)	11.2 (10.2, 12.3)	21.4 (19.2, 23.9)	20.5 (18.4, 22.8)	27.4 (25.3, 29.6)	22.6 (20.9, 24.4)	13.6 (12.6, 14.7)	12.8 (11.8, 14.0)	22.9 (21.2, 24.8)
PLP (nmol/L)	35.9 (32.4, 39.7)	33.8 (30.9, 37.1)	38.3 (34.5, 42.5)	29.2 (26.4, 32.3)	23.4 (21.7, 25.2)	38.8 (36.0, 41.8)	41.0 (38.1, 44.1)	36.4 (33.5, 39.6)	34.5 (32.0, 37.2)
PL (nmol/L)	22.5 (19.9, 25.6)	9.5 (8.4, 10.6)	19.3 (17.0, 22.0)	18.6 (16.4, 21.2)	18.3 (16.7, 20.1)	20.8 (19.0, 22.9)	9.9 (9.1, 10.9)	8.8 (7.9, 9.8)	18.2 (16.5, 20.0)
PA (nmol/L)	27.3 (24.1, 31.0)	22.6 (20.2, 25.3)	27.2 (23.9, 31.0)	23.1 (20.4, 26.2)	10.0 (9.1, 11.0)	21.6 (19.7, 23.7)	15.4 (14.1, 16.9)	12.9 (11.7, 14.3)	23.4 (21.4, 25.7)
Folate (nmol/L)	12.0 (11.0, 13.2)	9.7 (9.0, 10.6)	14.9 (13.6, 16.3)	9.8 (8.9, 10.7)	19.1 (17.8, 20.4)	15.8 (14.8, 16.9)	15.0 (14.1, 16.0)	13.8 (12.9, 14.9)	14.2 (13.3, 15.2)
B12 (pmol/L)	370 (350, 391)	510 (485, 537)	542 (512, 575)	465 (439, 492)	422 (405, 440)	418 (401, 435)	372 (357, 388)	394 (376, 413)	406 (390, 424)
Vit A (µmol/L)	2.31 (2.24, 2.39)	2.17 (2.10, 2.23)	2.49 (2.41, 2.58)	2.43 (2.34, 2.51)	1.81 (1.77, 1.86)	2.08 (2.03, 2.13)	1.86 (1.81, 1.90)	1.66 (1.61, 1.70)	2.28 (2.22, 2.34)
Vit D (nmol/L)	64.1 (60.9, 67.6)	57.6 (54.9, 60.4)	52.8 (50.0, 55.7)	63.6 (60.4, 67.1)	42.9 (41.3, 44.7)	64.4 (62.0, 67.0)	43.3 (41.7, 45.0)	38.5 (36.8, 40.1)	56.8 (54.7, 59.1)
aTOC (µmol/L)	33.1 (31.7, 34.5)	33.6 (32.3, 34.9)	36.1 (34.6, 37.8)	28.5 (27.3, 29.7)	24.8 (24.0, 25.6)	30.8 (29.8, 31.8)	23.7 (22.9, 24.4)	23.3 (22.5, 24.1)	34.9 (33.8, 36.1)
gTOC (µmol/L)	2.72 (2.51, 2.96)	2.54 (2.35, 2.73)	2.44 (2.24, 2.66)	4.99 (4.60, 5.42)	3.64 (3.43, 3.87)	1.64 (1.55, 1.75)	4.83 (4.55, 5.13)	6.50 (6.08, 6.95)	2.08 (1.95, 2.21)
One-carbon metabolites									
tMet (µmol/L)	27.7 (26.9, 28.6)	25.2 (24.5, 26.0)	27.8 (27.0, 28.8)	29.4 (28.5, 30.4)	31.8 (31.1, 32.6)	28.9 (28.2, 29.6)	26.7 (26.1, 27.3)	27.9 (27.2, 28.6)	27.4 (26.8, 28.1)
tHcy (µmol/L)	11.7 (11.2, 12.2)	10.3 (9.9, 10.7)	12.3 (11.8, 12.9)	12.9 (12.3, 13.5)	12.7 (12.3, 13.2)	13.7 (13.2, 14.1)	12.4 (12.0, 12.9)	11.0 (10.6, 11.4)	13.2 (12.8, 13.7)
Cysta (µmol/L)	0.221 (0.208. 0.236)	0.166 (0.157. 0.176)	0.164 (0.154. 0.175)	0.244 (0.229. 0.260)	0.278 (0.265. 0.291)	0.251 (0.240. 0.263)	0.250 (0.239. 0.262)	0.250 (0.237. 0.263)	0.200 (0.191. 0.210)
tCys (µmol/L)	290 (284, 295)	287 (282, 292)	343 (337, 350)	305 (299, 310)	280 (277, 284)	292 (288, 296)	271 (267, 274)	283 (279, 287)	309 (305, 314)
Serine (µmol/L)	112 (109, 115)	103 (101, 106)	150 (146, 154)	175 (171, 180)	183 (180, 187)	128 (125, 131)	113 (111, 116)	116 (114, 119)	117 (114, 119)

Glycine (µmol/L)	239 (231, 248)	223 (215, 230)	289 (278, 300)	339 (327, 351)	369 (360, 379)	240 (233, 246)	254 (248, 261)	243 (236, 251)	253 (246, 260)
Choline (µmol/L)	11.7 (11.3, 12.1)	9.3 (9.0, 9.6)	12.5 (12.1, 13.0)	18.3 (17.7, 18.9)	20.4 (19.9, 20.9)	12.6 (12.2, 12.9)	11.8 (11.5, 12.1)	12.1 (11.7, 12.4)	14.6 (14.3, 15.0)
Betaine (µmol/L)	33.1 (31.8, 34.5)	30.7 (29.6, 31.9)	35.2 (33.8, 36.7)	36.0 (34.5, 37.5)	52.2 (50.7, 53.9)	41.7 (40.5, 43.0)	45.9 (44.5, 47.3)	55.3 (53.4, 57.1)	37.1 (36.0, 38.3)
DMG (µmol/L)	4.02 (3.84, 4.22)	3.96 (3.79, 4.13)	4.45 (4.24, 4.67)	4.42 (4.21, 4.63)	5.28 (5.09, 5.46)	4.48 (4.33, 4.64)	4.74 (4.58, 4.90)	5.35 (5.15, 5.56)	4.04 (3.90, 4.18)
Tryptophan + metabolites									
Trp (µmol/L)	64.7 (63.0, 66.4)	61.0 (59.5, 62.4)	72.6 (70.6, 74.6)	73.3 (71.4, 75.3)	71.1 (69.7, 72.5)	66.0 (64.7, 67.3)	61.4 (60.2, 62.6)	66.7 (65.3, 68.2)	67.4 (66.1, 68.8)
Kyn (µmol/L)	1.44 (1.40, 1.48)	1.48 (1.44, 1.52)	1.64 (1.59, 1.69)	1.68 (1.64, 1.74)	1.56 (1.52, 1.59)	1.54 (1.51, 1.58)	1.41 (1.38, 1.44)	1.49 (1.45, 1.52)	1.54 (1.51, 1.57)
KA (nmol/L)	37.0 (34.8, 39.3)	39.6 (37.5, 41.8)	40.8 (38.4, 43.4)	42.0 (39.6, 44.6)	47.0 (44.9, 49.1)	46.1 (44.1, 48.2)	42.5 (40.7, 44.4)	53.5 (51.0, 56.2)	46.3 (44.2, 48.4)
AA (nmol/L)	14.9 (14.1, 15.8)	13.3 (12.6, 14.1)	14.5 (13.7, 15.4)	19.2 (18.1, 20.4)	17.8 (17.0, 18.6)	13.6 (13.0, 14.2)	12.7 (12.2, 13.3)	12.4 (11.8, 13.0)	15.4 (14.7, 16.1)
HK (nmol/L)	33.2 (31.5, 35.0)	38.0 (36.2, 39.8)	43.0 (40.7, 45.3)	40.6 (38.5, 42.8)	47.9 (46.1, 49.8)	39.8 (38.3, 41.4)	35.5 (34.2, 36.9)	38.1 (36.5, 39.7)	37.1 (35.7, 38.6)
XA (nmol/L)	12.8 (11.9, 13.9)	10.4 (9.7, 11.2)	10.2 (9.4, 11.0)	14.9 (13.8, 16.2)	13.7 (12.9, 14.5)	13.0 (12.3, 13.8)	11.7 (11.0, 12.4)	12.5 (11.8, 13.4)	11.9 (11.2, 12.6)
HAA (nmol/L)	37.1 (35.2, 39.2)	30.4 (28.9, 31.9)	34.4 (32.6, 36.4)	34.3 (32.5, 36.2)	31.5 (30.2, 32.8)	35.1 (33.7, 36.5)	31.4 (30.1, 32.6)	35.9 (34.4, 37.6)	30.8 (29.6, 32.1)
QA (nmol/L)	334 (318, 351)	345 (330, 361)	402 (383, 423)	356 (339, 374)	367 (354, 381)	352 (339, 364)	359 (346, 372)	379 (364, 394)	365 (352, 379)
Other									
CRP (µg/L)	1.38 (1.17, 1.63)	1.66 (1.43, 1.93)	2.15 (1.81, 2.55)	1.34 (1.14, 1.59)	0.93 (0.82, 1.05)	1.29 (1.14, 1.46)	1.68 (1.49, 1.90)	1.64 (1.43, 1.88)	2.28 (2.02, 2.59)
KTR (nmol/µmol)	22.3 (21.6, 23.0)	24.3 (23.6, 25.0)	22.6 (21.8, 23.3)	23.0 (22.3, 23.7)	21.9 (21.4, 22.4)	23.4 (22.8, 23.9)	23.0 (22.4, 23.5)	22.3 (21.7, 22.9)	22.8 (22.3, 23.4)
PAr (PA/(PLP+PL))	0.458 (0.427, 0.491)	0.512 (0.481, 0.546)	0.462 (0.430, 0.497)	0.477 (0.445, 0.512)	0.231 (0.219, 0.243)	0.346 (0.328, 0.364)	0.296 (0.281, 0.312)	0.283 (0.267, 0.300)	0.437 (0.415, 0.461)
Creat (µmol/L)	74.8 (72.8, 76.8)	72.8 (71.1, 74.5)	74.4 (72.4, 76.4)	75.6 (73.6, 77.6)	78.6 (77.1, 80.2)	70.4 (69.1, 71.8)	73.7 (72.3, 75.2)	68.8 (67.4, 70.3)	73.0 (71.5, 74.4)
HK/XA	2.59 (2.41, 2.78)	3.64 (3.41, 3.89)	4.23 (3.92, 4.56)	2.72 (2.53, 2.93)	3.50 (3.32, 3.70)	3.05 (2.89, 3.22)	3.04 (2.88, 3.21)	3.03 (2.86, 3.22)	3.12 (2.95, 3.29)
Cotinine (nmol/L)	42 (35, 51)	77 (65, 92)	48 (39, 59)	84 (69, 103)	42 (36, 48)	41 (36, 48)	49 (42, 56)	69 (58, 81)	47 (41, 54)
Arginine (µmol/L)	79.4 (75.5, 83.5)	67.7 (64.7, 70.9)	129.9 (123.3, 136.9)	138.0 (131.2, 145.2)	105.1 (101.2, 109.1)	68.7 (66.1, 71.3)	69.4 (66.9, 72.0)	81.5 (78.2, 85.0)	66.9 (64.5, 69.5)
ADMA (µmol/L)	0.546 (0.534, 0.558)	0.532 (0.521, 0.543)	0.585 (0.571, 0.598)	0.559 (0.546, 0.572)	0.587 (0.578, 0.597)	0.572 (0.563, 0.581)	0.529 (0.520, 0.538)	0.563 (0.553, 0.574)	0.552 (0.543, 0.561)
SDMA (µmol/L)	0.555 (0.539, 0.572)	0.560 (0.545, 0.575)	0.636 (0.617, 0.656)	0.575 (0.558, 0.592)	0.713 (0.697, 0.729)	0.608 (0.595, 0.622)	0.592 (0.579, 0.605)	0.569 (0.556, 0.583)	0.599 (0.586, 0.613)
hArg (µmol/L)	1.69 (1.61, 1.78)	1.60 (1.52, 1.67)	2.06 (1.95, 2.17)	1.84 (1.75, 1.94)	2.17 (2.09, 2.25)	2.50 (2.40, 2.59)	2.29 (2.20, 2.38)	2.27 (2.17, 2.36)	1.97 (1.89, 2.04)
MMA (µmol/L)	0.191	0.149	0.158	0.189	0.200	0.229	0.193	0.185	0.176

	(0.181, 0.202)	(0.142, 0.157)	(0.149, 0.168)	(0.179, 0.200)	(0.192, 0.209)	(0.219, 0.238)	(0.185, 0.201)	(0.177, 0.194)	(0.169, 0.184)

[1]MDCS, The Malmö Diet and Cancer Study; NSHDS, The Northern Sweden Health and Disease Study Cohort; ATBC, The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; HUNT, The Nord-Trøndelag Health Study; SMHS, The Shanghai Men’s Health Study; SWHS, The Shanghai Women’s Health Study; SCHS, The Singapore Chinese Health Study; SCS, The Shanghai Cohort Study; MCCS, The Melbourne Collaborative Cohort Study.

[2]AA, anthranilic acid; ADMA, asymmetric dimethylarginine; α-tocopherol, αTOC; B2, riboflavin; B12, vitamin B12; BVS, B-Vitamin Score; CRP, C-reactive protein; Cysta, cystathionine; DMG, dimethylglycine; FVS, Fat-soluble Vitamin Score; γTOC, γ-tocopherol; HAA, 3-hydroxyanthranilic acid; hArg, homoarginine; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine/tryptophan ratio; Kyn, kynurenine; MMA, methylmalonic acid; PA, 4-pyridoxic acid; PAr, PLP/(PL+PA); PL, pyridoxal; PLP, pyridoxal 5'-phosphate; QA, quinolinic acid; SDMA, symmetric dimethylarginine, tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; Trp, tryptophan; TVS, Total Vitamin Score; Vit A, all-trans retinol; Vit D, 25-OH vitamin D2 + season adjusted 25-OH vitamin D3; XA, xanthurenic acid.

[3]General linear models, adjusted for age, sex, smoking (former vs. never, current vs. never).

SUPPLEMENTAL TABLE 7
Prevalence of vitamin deficiency by region[1,2]

	Deficient, % of population						
	Total	US, all	US, no MV[3] use	US, MV[3] use	Nordic	Asia	Australia
B2	3.3	1.4	1.8	0.8	4.2	6.3	0.3
PLP	16.1	9.4	12.7	3.3	20.7	23.5	15.0
Folate	1.9	0.5	0.6	0.4	6.0	1.5	3.7
B12	0.8	0.8	0.9	0.7	0.6	0.8	1.1
Vit A	0.2	0.2	0.2	0.1	0.0	0.3	0.0
Vit D	9.1	7.3	9.5	3.3	4.8	14.7	4.5
αTOC	0.3	0.1	0.2	0.0	0.1	0.5	0.3

[1]αTOC, α-tocopherol; B2, riboflavin; B12, vitamin B12; PLP, Pyridoxal 5'-phosphate, Vit A, all-trans retinol; Vit D, season adjusted 25-OH vitamin D3+25-OH vitamin D2.

[2]Cutoff concentrations for deficiency: αTOC, 12 µmol/L; B2, 5 nmol/L; B12, 150 pM; folate, 5 nmol/L; PLP, 20 nmol/L; vitamin A, 0.7 µmol/L; 25-OH vitamin D, 30 nmol/L.

[3]MV, Multivitamin supplement.

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SUPPLEMENTAL TABLE 8

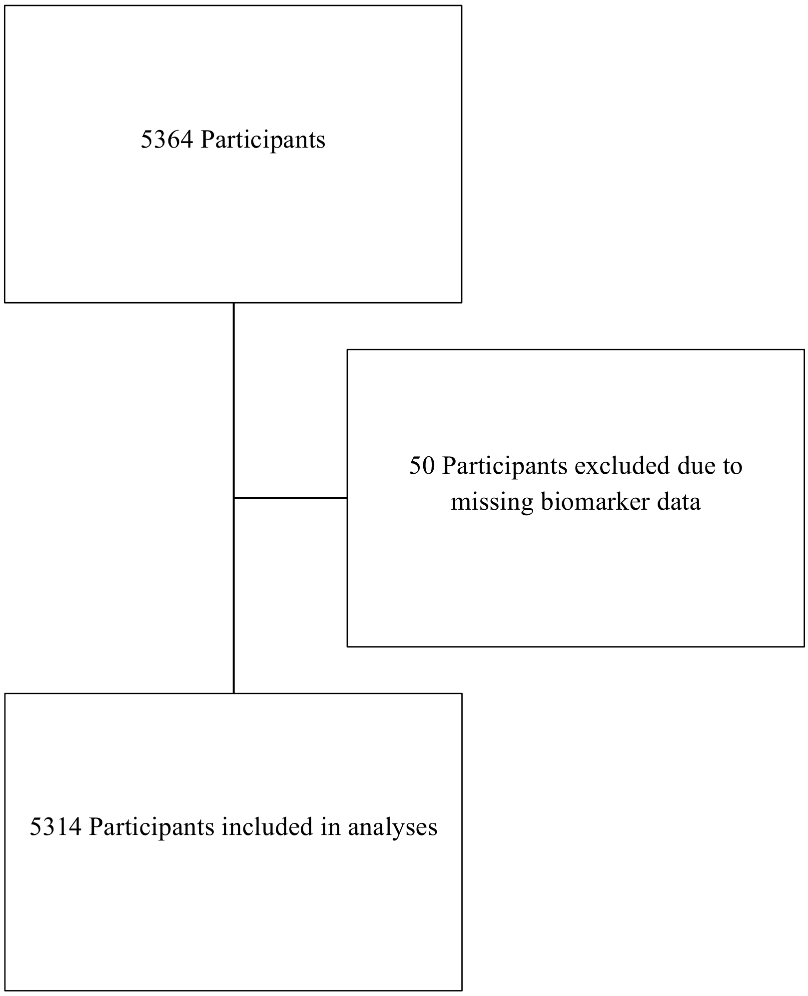
Between-batch CV of the measured biomarkers in the quality control plasma[1]

Vitamins		CV (%)
	B2	7.1
	PLP	5.2
	PL	12.3
	PA	8.6
	Folate	9.5
	B12	6.8
	Vit A	6.6
	25-OH Vit D2	n.a.[2]
	25-OH Vit D3	7.4
	αTOC	6.2
	γTOC	8.0
One-carbon metabolites		
	Met	2.0
	MetSO	28.7
	tHcy	3.4
	Cysta	3.8
	tCys	2.9
	Serine	2.5
	Glycine	2.5
	Choline	9.0
	Betaine	4.3
	DMG	8.3
Tryptophan + metabolites		
	Trp	2.9
	Kyn	2.5
	KA	5.5
	AA	14.1
	HK	8.1
	XA	14.6
	HAA	5.8
	QA	5.4
Other		
	CRP	7.8
	Creat	3.9
	Cotinine	5.7
	Arginine	4.3
	ADMA	10.5
	SDMA	10.6
	hArg	10.2
	MMA	3.3

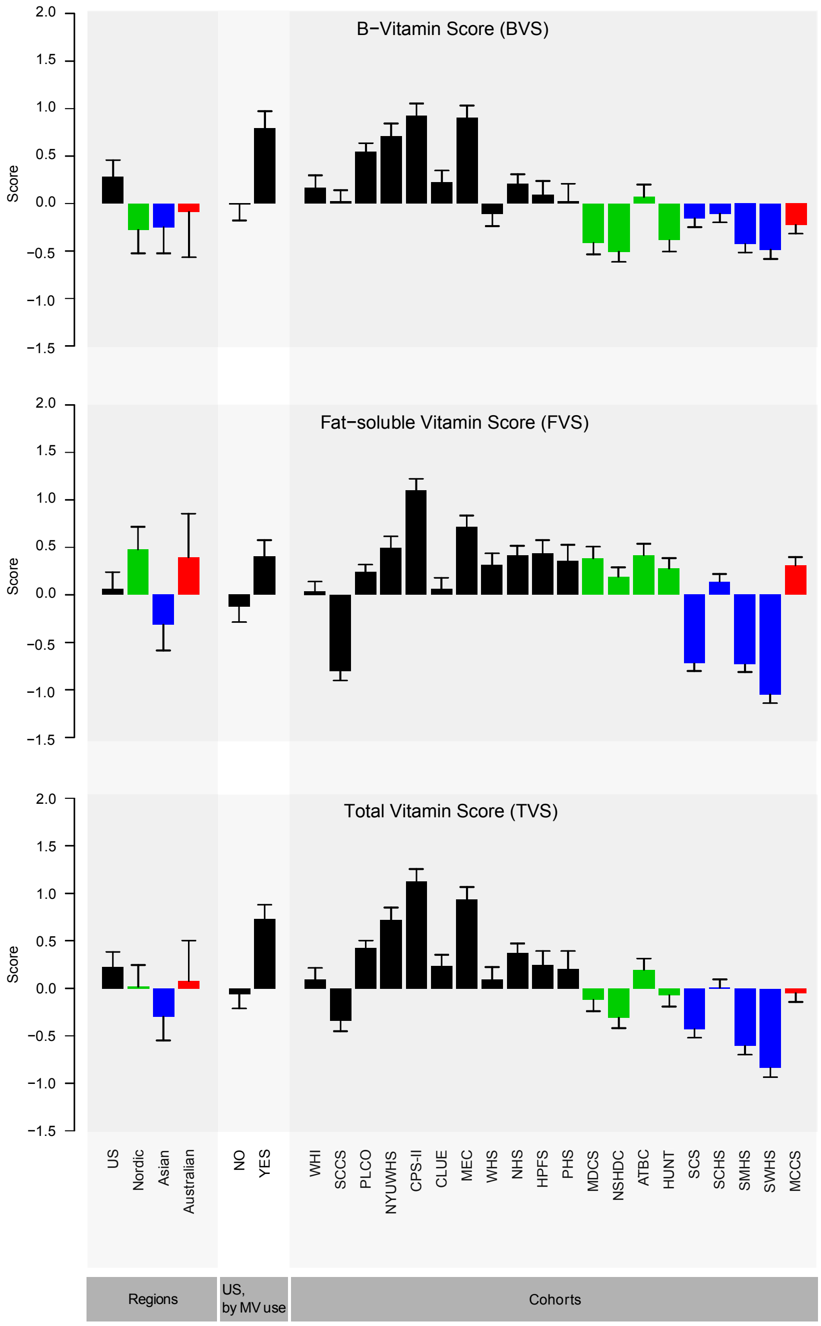
[1]AA, anthranilic acid; ADMA, asymmetric dimethylarginine; α-tocopherol, αTOC; B2, riboflavin; B12, vitamin B12; CRP, C-reactive protein; Cysta, cystathionine; DMG, dimethylglycine; γTOC, γ-tocopherol; HAA, 3-hydroxyanthranilic acid; hArg, homoarginine; HK, 3-hydroxykynurenine; KA, kynurenic acid; Kyn, kynurenine; MMA, methylmalonic acid; PA, 4-pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate; QA, quinolinic acid; SDMA, symmetric dimethylarginine, tCys, total cysteine; tHcy, total homocysteine; tMet, total methionine; Trp, tryptophan; Vit A, all-trans retinol; XA, xanthurenic acid.

[2]In the QC plasma, 25-OH Vitamin D2 was below the LOD of the assay.

Online Supplemental Material

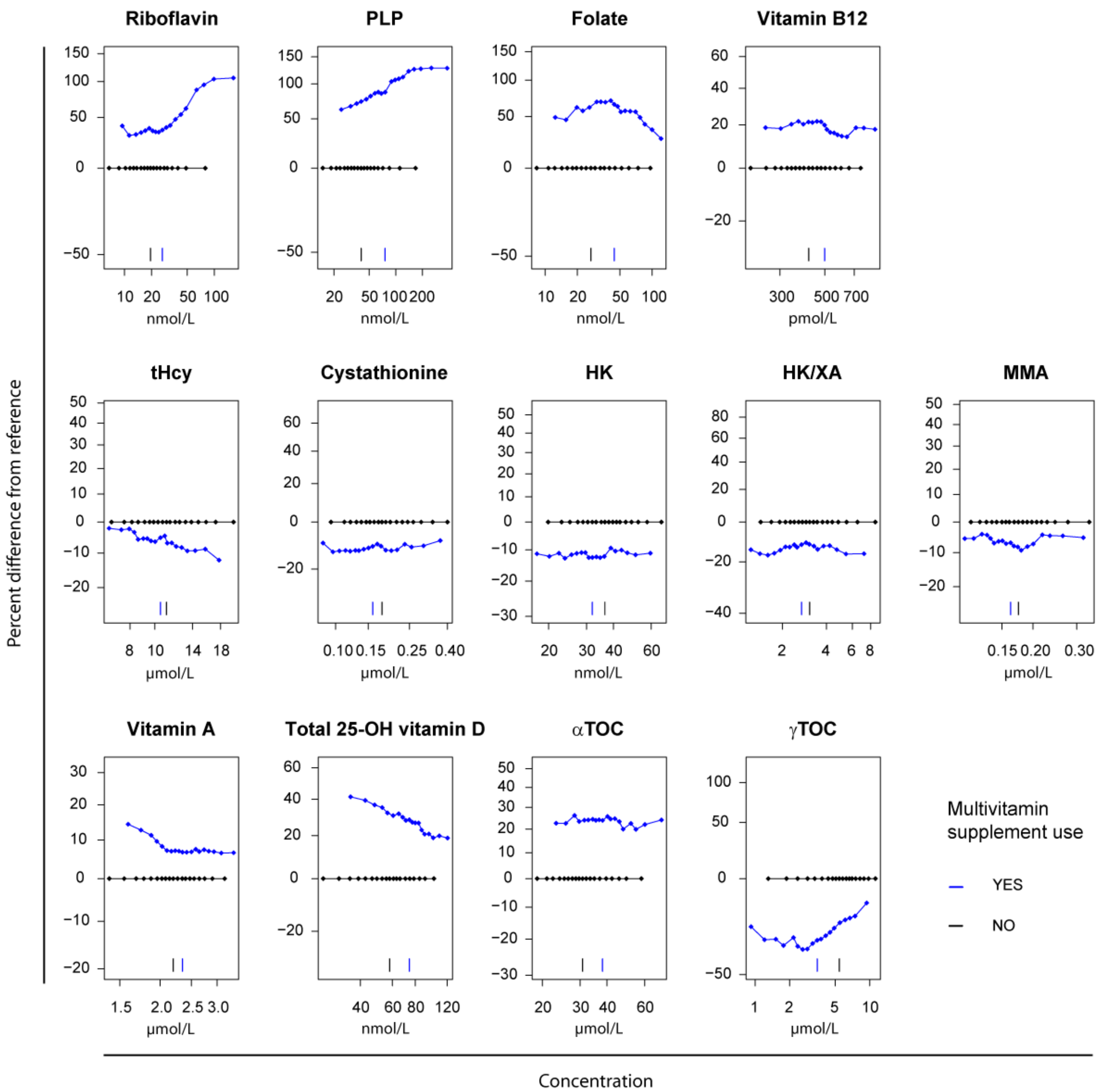


Supplemental Figure 1. Flow chart of participants



Supplemental Figure 2. Geometric means of the three vitamin scores. Black, US; green, Nordic; blue, Asia; red, Australia. Error bars indicate 95% CI. The B-Vitamin Score (BVS) was obtained from riboflavin, PLP, folate and vitamin B12, the Fat-soluble Vitamin Score (FVS) from vitamin A, total 25-OH vitamin D and aTOC, and the Total Vitamin Score (TVS) combined all seven vitamins, as described in the text. Geometric means (95% CI) by region were estimated using mixed models adjusted for age, gender and smoking (former vs. never, current vs. never) with cohort as random effect. Geometric means (95% CI) by cohort were estimated by using generalized linear models adjusted for age, gender and smoking (former vs. never, current vs. never). MV, Multivitamin supplement.

Cohort abbreviations: ATBC, The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CLUE, The Campaign Against Cancer and Stroke (CLUE I) and the Campaign Against Cancer and Heart Disease (CLUE II); CPS-II, The American Cancer Society Cancer Prevention Study-II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; HUNT, The Nord-Trøndelag Health Study; MCCS, The Melbourne Collaborative Cohort Study; MDCS, The Malmö Diet and Cancer Study; MEC, The Multiethnic Cohort; NHS, The Nurses' Health Study; NSHDS, The Northern Sweden Health and Disease Study Cohort; NYUWHS, The New York University Women's Health Study; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCCS, The Southern Community Cohort Study; SCHS, The Singapore Chinese Health Study; SCS, The Shanghai Cohort Study; SMHS, The Shanghai Men's Health Study; SWHS, The Shanghai Women's Health Study; WHI, The Women's Health Initiative; WHS, Women's Health Study.



Supplemental Figure 3. Distribution of vitamins and vitamin markers in USA by multivitamin supplement use from quantile regression (adjusted for age (years) at blood sampling, sex, smoking (former vs. never, current vs. never) and cohort) shown by (5,10,15,20,25,30,35, 40,45,50,55,60,65,70,75,80,85,90,95) percentiles. The y-axis in each panel is scaled to show three standard deviations of the distributions for the biomarker in that panel. The vertical line in each panel indicates the 50th quantile for each group. αTOC, α-tocopherol; γTOC, γ-tocopherol HK, 3-hydroxykynurenine; HK/XA, HK/xanthurenic acid; MMA, methylmalonic acid; PLP, pyridoxal 5'-phosphate; tHcy, total homocysteine; Total 25-OH vitamin D, 25-OH vitamin D2 + season-adjusted 25-OH vitamin D3.