Circulating Phylloquinone Concentrations of Adults in the United States Differ According to Race and Ethnicity

M. Kyla Shea, Sarah L. Booth, Jennifer A. Nettleton, Gregory L. Burke, Haiying Chen, and Stephen B. Kritchevsky

Abstract

Differences in micronutrient status are reported to contribute to racial and ethnic differences in chronic diseases. Diseases related to vitamin K are reported to differ by race and ethnicity, but it is unclear if circulating vitamin K concentrations similarly differ. We examined racial and ethnic differences in serum phylloquinone (K1) in the Multiethnic Study of Atherosclerosis (MESA) (mean ± SD age = 62 ± 10 y; 52% female; 262 white, 180 African American, 169 Hispanic, 93 Chinese American). Overall, 25% had serum K1 <0.1 nmol/L (the lower limit of detection). The prevalence of low serum K1 was 4% in Chinese Americans compared with 24% of whites, 29% of African Americans, and 33% of Hispanics. Compared with whites, Chinese Americans were significantly less likely to have serum K1 <0.1 nmol/L [OR (95% CI): 0.23 (0.09–0.53), adjusted for serum TG, K1 intake, age, sex, BMI, smoking, total cholesterol, site, season, and lipid-lowering medication use]. African Americans and Hispanics had similar odds to whites for having serum K1 <0.1 nmol/L [OR (95% CI): 1.30 (0.79–2.15) and 1.19 (0.66–2.15), respectively; fully adjusted]. In participants with detectable concentrations (n = 523), (natural log) serum K1 was higher in the Chinese Americans compared with whites, African Americans, and Hispanics (geometric mean ± SEM = 2.2 ± 0.1 nmol/L vs. 1.2 ± 0.1 nmol/L, 1.5 ± 0.1 nmol/L, and 1.1 ± 0.1 nmol/L, respectively, adjusted for serum TG, K1 intake, and additional covariates; all P < 0.001). These findings suggest circulating K1 differs by race and ethnicity in U.S. adults, especially among those of Chinese American descent, which merits consideration in the design and interpretation of future population-based and clinical studies of vitamin K and related diseases. J. Nutr. 142: 1060–1066, 2012.

Introduction

Accumulating evidence indicates that low vitamin K status may be a risk factor for chronic diseases, such as cardiovascular disease (CVD), bone loss, and osteoarthritis, which are reported to vary by race and ethnicity (1–5). Racial and ethnic differences in nutrient status are reported to account for some of the racial and ethnic variation in other health outcomes (6–8), so it is possible that racial and ethnic differences in vitamin K status may partially account for the observed racial and ethnic differences in health outcomes related to vitamin K.

The primary form of vitamin K in the Western diet is phylloquinone (K1), which is found in green leafy vegetables and vegetable oils, and dietary K1 intake was reported to differ among racial and ethnic groups in NHANES (1999–2004) (9). Two small studies with modest numbers of participants suggested that biomarkers of vitamin K status differed among ethnicities (10,11); however, these studies collected samples from individuals of different ethnic backgrounds residing in different countries. The majority of population-based studies of vitamin K status have been limited to Caucasians, a group in whom the prevalence of low vitamin K status is reported to be 25–33% (12–16). We used data from the Multiethnic Study of Atherosclerosis (MESA), a large, population-based cohort comprised of white, African American, Chinese American, and Hispanic adults living in the US to determine if concentrations of serum K1 [the predominate form of vitamin K in circulation (17)] differed by race and ethnicity. Understanding the vitamin K...
status of racial and ethnic groups in the population is important for the design and interpretation of targeted interventions focusing on health outcomes related to vitamin K.

**Methods**

The MESA study is a large, ongoing, observational study examining the prevalence and determinants of subclinical CVD in a multi-ethnic cohort that began in 2000–2002. The MESA cohort (n = 6814) was recruited from 6 U.S. communities, including: Forsyth County, NC; northern Manhattan and the Bronx, NY; Baltimore County, MD; St. Paul, MN; Chicago and Maywood, IL; and Los Angeles County, CA. The cohort is 38% non-Hispanic white, 28% African American, 22% Hispanic, and 12% Chinese American, all of whom were free of clinically diagnosed CVD at baseline. The study design and methods of MESA have been described in detail (18). MESA was approved by the Institutional Review Boards at all 6 study sites (Wake Forest University, Columbia University, John Hopkins University, University of Minnesota, Northwestern University, University of California at Los Angeles) and this substudy was additionally approved by the Institutional Review Boards at Wake Forest University and Tufts University. All participants gave written informed consent.

A randomly chosen subgroup of 780 MESA participants who were not taking warfarin (a vitamin K antagonist) had serum K1 measured from samples obtained at the baseline visit. These participants did not differ from the 6034 participants who did not have serum K1 measured by taking the natural-log (ln). Because 25% of participants had serum K1 <0.1 nmol/L [the LLD for this assay (19)], the ln-transformed distributions were bimodal (Fig. 1). Therefore, our analytical approach was 2-fold (1): logistic regression was used to determine the OR (95% CI) for having serum K1 <0.1 nmol/L (below the LLD) in African Americans, Hispanics, and Chinese Americans compared with whites. Whites were chosen as the reference group, because they provided the largest sample size and therefore the mean outcome measures for that group were estimated with greater precision (2); we determined the OR (95% CI) for having serum K1 lower than the median among participants with detectable K1 (1.4 nmol/L) in African Americans, Hispanics, and Chinese Americans compared with whites. Racial and ethnic differences in continuous measures of serum K1 were also examined using general linear models. Analysis of serum K1 was adjusted for age, sex, BMI, smoking status, total cholesterol, education, season, study site, and K1 intake. Because serum K1 was natural-log transformed, geometric means were calculated for each racial and ethnic group by back-transforming the unadjusted and adjusted means. SE of the geometric means were estimated using the delta method (SE geometric mean = square root [(geometric mean) 2 x (SEM of ln-transformed outcome)]). The correlation between (ln) serum K1 and (ln) K1 intake was calculated using Spearman correlation coefficients adjusted for TG and energy intake. Because McKeown et al. (27) reported the linear relationship between circulating K1 and K1 intake reached a plateau at intakes of 200 μg/d, we subsequently determined the correlations among participants with detectable serum K1 who reported intakes of ≥200 μg/d. Stepwise logistic and linear models were used to determine the percent variability of circulating K1 that is attributable to race and ethnicity, with a P < 0.15 specified for model inclusion. The following variables, chosen based on biological plausibility and a similar analysis of circulating K1 in the Framingham Offspring Study (28), were entered: TG, race (Chinese American, Hispanic, African American), total cholesterol, BMI, age, sex, education, lipid-lowering medication use (yes/no), current smoker (yes/no), study site, and season. All analyses were carried out using SAS v 9.1 and were considered significant when P < 0.05.

**Results**

The overall age of participants was 62 ± 10 y (mean ± SD). The mean BMI among Chinese Americans was in the normal range by U.S. standards, whereas the mean BMI among whites, African Americans, and Hispanics would be considered overweight. Hispanics had the highest mean TG and total cholesterol concentrations compared with the other 3 racial and ethnic groups. Education differed significantly according to race and ethnicity, with over one-half of white participants reporting graduating from college, which was higher than the other groups (Table 1).

The prevalence of serum K1 <0.1 nmol/L was 24% in whites, 29% in African Americans, 33% in Hispanics, and 4% in Chinese American (Fig. 1). In fully adjusted analysis, Chinese American had 0.23-fold lower odds of having serum K1 <0.1 nmol/L than whites, whereas the odds for African Americans and Hispanics did not differ from whites (Table 2). Among participants with detectable serum K1, Chinese American and African Americans were significantly less likely than whites to have serum K1 below the median level (1.4 nmol/L) in adjusted models (Table 2).
Energy-adjusted K1 intakes were significantly lower among Hispanics compared with the 3 other racial and ethnic groups (Table 1) and these differences persisted following adjustment for education, age, sex, BMI, current smoker, season, and study site. Among participants with detectable serum K1, Chinese Americans had higher concentrations compared with whites, African Americans, and Hispanics. Serum K1 remained significantly higher among Chinese Americans compared with whites, African Americans, and Hispanics after adjustment for TG (Table 1). The racial and ethnic differences were similar after further adjustment for K1 intake, education, age, sex, BMI, cholesterol, lipid-lowering medication use, current smoking, season, and study site.

The energy-adjusted K1 intake of participants with serum K1, \(0.1 \text{ nmol/L}\) was 80 \(\pm\) 4 mg/d (geometric mean \(\pm\) SEM), less than among participants with detectable serum K1, 102 \(\pm\) 3 \(\mu\)g/d (\(P < 0.01\)). Overall among participants with detectable serum K1, (ln) serum K1 positively correlated with (ln) K1 intake (\(r = 0.20;\ P < 0.001\) adjusted for TG and energy intake). However, the correlation between serum K1 and K1 intake was not significant among African Americans (\(r = 0.04;\ P = 0.64\)), Hispanics (\(r = 0.14;\ P = 0.13\)), and Chinese Americans (\(r = 0.01;\ P = 0.90\)) but was significant in whites (\(r = 0.21;\ P = 0.003\)). When participants who reported dietary K1 intake \(>200 \text{ mg/d}\) were excluded (\(n = 104\)), the correlations between (ln) serum K1 and (ln) K1 intake were not appreciably changed (white: \(n = 158,\ r = 0.23,\ P = 0.004\); African American: \(n = 92,\ r = 0.03,\ P = 0.78\); Hispanic: \(n = 96,\ r = 0.18,\ P = 0.07\); Chinese American: \(n = 73,\ r = 0.14,\ P = 0.23\)).

Using stepwise linear regression of participants with detectable serum K1, Chinese Americans and African American race and ethnicity and TG were significant predictors of serum K1 (Table 3), which along with K1 intake and BMI explained 21\% of the variability in serum K1 in this group. When the outcome of having serum K1 less than the LLD (considered dichotomously) was similarly analyzed, only 10\% of the variability in the outcome was explained by the included predictors. In this analysis, Chinese American ethnicity, TG, K1 intake, total cholesterol, lipid-lowering medication use, season, and study site were identified as significant predictors of serum K1.

**Discussion**

The overall distributions of serum K1 were right-skewed and did not normalize following a log transformation, unlike other nutrient biomarkers, which are normalized by such transformation (29,30). Upon inspection, the basis appeared to be the strong bimodal distribution of serum K1 and the large proportion of persons (25\%) with concentrations \(\leq 0.1 \text{ nmol/L}\) (the LLD for this assay), which has been used to measure circulating K1 in other population-based studies (13,21). Although participants with \(\leq 0.1 \text{ nmol/L}\) circulating K1 reported consuming slightly less dietary K1 (80 vs. 103 \(\mu\)g/d), it is unlikely that this observed difference in intake fully accounted for having circulating K1 \(\leq 0.1 \text{ nmol/L}\) for 2 reasons. Outcomes of dietary depletion-repletion studies found step-wise repletion using up to 450 \(\mu\)g/d K1 did not increase circulating K1 concentrations to within the normal range for adults (31). Although participants with \(\leq 0.1 \text{ nmol/L}\) circulating K1 reported consuming slightly less dietary K1 (80 vs. 103 \(\mu\)g/d), it is unlikely that this observed difference in intake fully accounted for having circulating K1 \(\leq 0.1 \text{ nmol/L}\) for 2 reasons. Outcomes of dietary depletion-repletion studies found step-wise repletion using up to 450 \(\mu\)g/d K1 did not increase circulating K1 concentrations to within the normal range for adults (31). Although participants with \(\leq 0.1 \text{ nmol/L}\) circulating K1 reported consuming slightly less dietary K1 (80 vs. 103 \(\mu\)g/d), it is unlikely that this observed difference in intake fully accounted for having circulating K1 \(\leq 0.1 \text{ nmol/L}\) for 2 reasons. Outcomes of dietary depletion-repletion studies found step-wise repletion using up to 450 \(\mu\)g/d K1 did not increase circulating K1 concentrations to within the normal range for adults (31). Although participants with \(\leq 0.1 \text{ nmol/L}\) circulating K1 reported consuming slightly less dietary K1 (80 vs. 103 \(\mu\)g/d), it is unlikely that this observed difference in intake fully accounted for having circulating K1 \(\leq 0.1 \text{ nmol/L}\) for 2 reasons. Outcomes of dietary depletion-repletion studies found step-wise repletion using up to 450 \(\mu\)g/d K1 did not increase circulating K1 concentrations to within the normal range for adults (31). Although participants with \(\leq 0.1 \text{ nmol/L}\) circulating K1 reported consuming slightly less dietary K1 (80 vs. 103 \(\mu\)g/d), it is unlikely that this observed difference in intake fully accounted for having circulating K1 \(\leq 0.1 \text{ nmol/L}\) for 2 reasons. Outcomes of dietary depletion-repletion studies found step-wise repletion using up to 450 \(\mu\)g/d K1 did not increase circulating K1 concentrations to within the normal range for adults (31).

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Chinese American participants were less likely to have serum K1 concentrations lower than the LLD. Similar results were seen among participants with detectable serum K1; Chinese American participants were significantly less likely to have concentrations less than the median (1.4 nmol/L) compared with the other racial and ethnic groups. Likewise, Chinese American participants had higher serum K1 when analyzed as a continuous outcome in those with detectable concentrations, and African Americans had higher concentrations than whites or Hispanics. The higher circulating K1 concentration we observed in Chinese Americans is consistent with 2 previous small studies of vitamin K status, especially in cohorts that include racially homogeneous (28). Race and ethnicity (Chinese American and African American) accounted 3–9% of the variability, whereas site no longer met the } \text{value}\text{ of } \approx 0.05\text{ (Tukey’s Honestly Significant Difference test). K1, phylloquinone.} \text{P value based on chi-square analysis.} \text{P value is for overall F-statistic, based on unadjusted ANOVA.} \text{The outcome was transformed using natural log; means presented are geometric means ± SEM.} \text{To convert mmol/L to mg/dL, multiply by 88.57.} \text{To convert mmol/L to mg/dL, multiply by 38.67.} \text{P value is for overall F-statistic for race and ethnicity based on ANCOVA using natural log-transformed outcome, adjusted for kcal/d; means presented are geometric, least-square means ± SEM.} \text{Further adjusted for education, age, sex, BMI, current smoker, season, and study site.} \text{Analysis limited to participants with detectable serum K1 (≥0.1 nmol/L, n = 523).} \text{P value is for overall F-statistic for race and ethnicity based on ANCOVA using natural log-transformed outcome, adjusted for TG; means presented are geometric, least-square means ± SEM.} \text{Further adjusted for K1 intake, education, age, sex, BMI, total cholesterol, lipid-lowering medication use, current smoker, season, and study site.}
validated for macronutrient intake (22) but not specifically for vitamin K intake. It may lack the sensitivity to quantify K1 intake precisely enough to correlate with serum measures. The correlations between serum measures and intakes differ by race and ethnicity because of dietary pattern differences. Even though the FFQ was modified to include foods more commonly consumed by the racial and ethnic groups in MESA (23,24), it is plausible the FFQ did not sufficiently query specific foods that contribute to vitamin K status in racial and ethnic groups. Serum K1 reflects intakes over the previous 12 h, whereas the FFQ

### TABLE 2

<table>
<thead>
<tr>
<th>(ln) Serum K1&lt;0.1 nmol/L (yes/no)</th>
<th>Parameter estimate</th>
<th>Variability, %</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>523</td>
<td>704</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race (compared with whites)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese American</td>
<td>0.37</td>
<td>&lt;0.001</td>
<td>9.4</td>
<td>0.23 (0.09–0.53)</td>
</tr>
<tr>
<td>Hispanic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>0.19</td>
<td>&lt;0.001</td>
<td>2.7</td>
<td>—</td>
</tr>
<tr>
<td>(ln) serum TG, mmol/L</td>
<td>0.29</td>
<td>&lt;0.001</td>
<td>7.4</td>
<td>0.51 (0.34–0.74)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>−0.01</td>
<td>0.01</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>(ln) K1 intake, μg/d</td>
<td>0.04</td>
<td>0.11</td>
<td>0.4</td>
<td>0.74 (0.59–0.94)</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid-lowering medication use</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Season (compared with January–March)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April–June</td>
<td>0.58</td>
<td>0.37–0.93</td>
<td>0.02</td>
<td></td>
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<tr>
<td>July–September</td>
<td>0.49</td>
<td>0.29–0.83</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>October–December</td>
<td>0.58</td>
<td>0.35–0.98</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Site (compared with St. Paul, MN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Los Angeles, CA</td>
<td>0.53</td>
<td>0.29–0.97</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Forsyth County, NC</td>
<td>0.44</td>
<td>0.24–0.81</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>New York, NY</td>
<td>0.79</td>
<td>0.44–1.43</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Chicago, IL</td>
<td>0.36</td>
<td>0.19–0.69</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Baltimore County, MD</td>
<td>0.35</td>
<td>0.18–0.67</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Total variability explained by model, %</td>
<td>20.9</td>
<td>10.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. K1, phyloquinone; LLD, lower limit of detection; MESA, Multiethnic Study of Atherosclerosis.
2. Limited to participants with detectable serum K1 and based on stepwise linear regression, the following exposures were entered: serum TG, race and ethnicity (Chinese American, Hispanic, African American), total cholesterol, BMI, age, sex, education (category), lipid-lowering medication use, current smoker, season, and study site; entry P < 0.15. Dash, did not meet P < 0.15 entry criteria.
3. Based on stepwise logistic regression, the following exposures were entered: TG, race and ethnicity (Chinese American, Hispanic, African American), total cholesterol, BMI, age, sex, education (category), lipid-lowering medication use, current smoker, season, and study site; entry P < 0.15.
4. LLD for serum K1 using HPLC assay is 0.1 nmol/L (19).
examining whether or not different forms of vitamin K vary by status. As novel roles for K1 and menaquinones are emerging, circulating K1 is considered a global indicator of vitamin K status in the US, K1 is the primary dietary form of vitamin K and additional vitamin K measures in MESA would have provided do not appear to have an important role in the typical U.S. diet found in smaller amounts in meats and dairy-based foods (38).

Menadione (vitamin K3) and menaquinone (vitamin K2). Menaquinones 4–10 which are (10,11). Furthermore, other forms of vitamin K exist, such as menaquinone (vitamin K2). Menaquinones 4–10 [which are found in smaller amounts in meats and dairy-based foods (38)] do not appear to have an important role in the typical U.S. diet (17) but may be present in different amounts in diets consumed by certain racial and ethnic groups. Although inclusion of additional vitamin K measures in MESA would have provided further insight into racial and ethnic differences in vitamin K status in the US, K1 is the primary dietary form of vitamin K and circulating K1 is considered a global indicator of vitamin K status. As novel roles for K1 and menaquinones are emerging, examining whether or not different forms of vitamin K vary by race and ethnicity merits future study. The correlation between circulating K1 and K1 intake among whites in MESA was modest but similar to what has been reported using a single blood draw and adjusted for TG in other Caucasian adults (28,35), suggesting it may be a suitable for ranking white adults according to K1 intake. The FFQ appeared to be less accurate in estimating K1 across other racial and ethnic groups. However, our primary aim was not to describe racial and ethnic differences in K1 intake and we acknowledge that vitamin K intake may have accounted for more of the variability in serum K1 if a more precise dietary measure was available. Dietary assessment tools that estimate vitamin K intake across different ethnic groups need to be developed and validated. Compared with other population-based studies of nutrient biomarkers (39–43), our sample size was modest. There were fewer Chinese American participants compared with the other groups and a disproportionate number of participants excluded due to incomplete data were African American.

This is the first report to our knowledge that the circulating K1 concentrations differ by race and ethnicity among adults living in the United States, especially among persons of Chinese American descent. Racial and ethnic differences in serum K1 persisted after adjustment for K1 intake, suggesting the differences we observed were not entirely dependent on dietary intakes. As novel roles for vitamin K in health and disease are emerging (12), the racial and ethnic differences in serum K1 we found may have implications for the design and interpretation of intervention studies focused on health-outcomes related to vitamin K. The extent to which the differences in circulating K1 influence racial and ethnic differences in vitamin K-related health outcomes merits future investigation.

Acknowledgments
M.K.S. designed the study, performed the statistical analyses, and drafted the manuscript; S.L.B., J.A.N., G.L.B., H.C., and S.B.K. contributed to the design of the analyses, the interpretation of the data, and writing of the manuscript; and SLB was also responsible for laboratory analyses. All authors read and approved the final manuscript.

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8. Alvarez JA, Ashraf AP, Hunter GR, Gower BA. Serum 25-hydroxyvitamin D and parathyroid hormone are independent determinants of whole-body


