A Minimum of Six Days of Diet Recording Is Needed to Assess Usual Vitamin K Intake among Older Adults¹,²

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Abstract
There is a growing interest in the role of vitamin K in health, especially in aging populations. Knowledge of inter- and intra-individual variability of dietary vitamin K intake could be useful to accurately assess usual intake and rank participants in epidemiological studies. Our objectives were to: 1) estimate the variance components of vitamin K intake; 2) investigate whether day of the week, season, and energy intake are factors related to intra-individual variance; and 3) calculate the requisite number of days to achieve desired degrees of accuracy for estimating individual vitamin K intake, ranking individuals and estimating regression coefficient. Vitamin K intake was assessed in 939 older adults (67–84 y) enrolled in the Québec Longitudinal Study on Nutrition and Successful Aging study using 2 sets of 3 nonconsecutive multiple-pass 24-h dietary recalls (24HR) collected 6 mo apart. Each set included 2 weekdays and one weekend day. Intra- to inter-individual variance ratios for vitamin K intake were 3.2 (95% CI = 2.6–3.9) overall, 2.6 (95% CI = 2.1–3.5) for men, and 3.7 (95% CI = 2.9–5.0) for women. Day of the week (weekdays) and season (May to October) were positively and significantly associated with vitamin K intake but explained a negligible part of intra-individual variation (<1%). Adjusting for energy intake explained <7% of variance and did not affect the variance ratio. Six to 13 24HR are required to properly rank individuals according to their usual vitamin K intake and limit attenuation of the regression coefficient. These results should be considered in studies planning to assess vitamin K intakes in older adults. J. Nutr. 141: 341–346, 2011.

Introduction
There is a growing number of epidemiological studies focusing on possible relationships between vitamin K intake and health, notably cardiovascular diseases (1–8), bone health (9–14), metabolic disorders (15,16), and cancer (17,18). In such investigations, one major issue is the need to accurately rank individuals according to their usual nutrient intake to limit the attenuation of regression coefficients and the probability of false negative results. However, individual vitamin K intake is characterized by high day-to-day variability compared with between-individual variance (19,20). This requires a greater number of days of diet recording to estimate usual intake and correctly rank individuals. Admittedly, daily diet recording method is time-consuming and labor intensive to process. Thus, it is important to determine the number of days required to be cost-effective while limiting ranking error.

Variance components for vitamin K intake were examined in only 2 studies (19,20). One study reported a within- to between-variance ratio in postmenopausal women of 2.6 and recommended a minimum of 5 nonconsecutive days of diet recording/person to adequately rank individuals. Although calculations were based on a relatively large sample (n = 362), the use of a 3-consecutive-day food record (FR)¹⁰ might have led to an underestimation of the variance ratio (21). Furthermore, these results cannot be generalized to men, because variance ratios could differ between sexes (22–24). In the other study, the small sample size (n = 34) allowed for only a rough estimate of variance components (20).

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10 Abbreviations used: CNF, Canadian Nutrient File; FR, food record; 24HR, 24-h dietary recall; NuAge, Québec Longitudinal Study on Nutrition and Successful Aging.
Day of the week and season could contribute to daily variation in diet (25). Variation in energy intake could also explain some part of the variance in micronutrient intake. Whether ranking of individuals is preserved despite these factors has not been determined thus far for vitamin K. Higher consumption of vitamin K has been reported on weekdays, but the extent to which the day of the week accounted for intra-individual variance was not indicated (19). Three studies examined seasonal variation in vitamin K intake and, surprisingly, did not find a significant effect (19,26,27).

Poor vitamin K intake has been mostly associated with adverse age-related outcomes. Knowledge of variance components and factors associated with day-to-day variability could thus be useful for studies planning to assess vitamin K intake in older adults. Using data from the Québec Longitudinal Study on Nutrition and Successful Aging study (NuAge) cohort, our purposes were to: 1) estimate the variance components of vitamin K intake; 2) investigate whether day of the week, season, and energy intake are factors related to intra-individual variance; and 3) calculate the requisite number of days to achieve a desired degree of accuracy for estimating individual vitamin K intake, ranking individuals, and estimating the regression coefficient.

Methods

Data source. Data were obtained from the NuAge study (28). The NuAge study was designed to determine whether optimal dietary intakes throughout senior years are associated with successful aging. Details of this study can be found elsewhere (28). Briefly, NuAge is a 3.5-year longitudinal observational study of 1793 men and women aged 67 through senior years are associated with successful aging. Using data from the NuAge study (28), our purposes were to: 1) estimate the variance components of vitamin K intake; 2) investigate whether day of the week, season, and energy intake are factors related to intra-individual variance; and 3) calculate the requisite number of days to achieve a desired degree of accuracy for estimating individual vitamin K intake, ranking individuals, and estimating the regression coefficient.

Statistical analyses. Distribution of phylloquinone intake was positively skewed. Thus, ln-transformed data were used in all models and equations. Where phylloquinone intake was 0 μg/d, a value of 1 μg/d was imputed. Geometric mean phylloquinone intakes were calculated. The data set had a 2-level hierarchical structure in which each 24HR (level 1; n = 5634) was nested in individuals (level 2; n = 939). Letting $Y_{ij}$ be phylloquinone intake for the $i^{th}$ subject at the $j^{th}$ 24HR, the variance component model was written as follows:

$$Y_{ij} = \gamma_{00} + \mu_{0i} + e_{ij}$$

where $\gamma_{00}$ is the overall mean of phylloquinone intake, $\mu_{0i}$ the error term at level 2, and $e_{ij}$ the error term at level 1, whereas $\sigma_{\mu}^2$ and $\sigma_{e}^2$ represent inter- and intra-individual variances, respectively. Calculations were first made for all participants taking into account their standardized weight in the population based on sex, age group (67–72, 73–77, and 78–84 y), and area of residence (Sherbrooke region, Montréal, and Laval) as estimated by the Institut de la Statistique du Québec based on the 1996 Canadian census. The variances were also estimated separately for men and women.

The effects of the 3 level 1 predictors, day of the week (coded 0 for weekdays and 1 for weekend days), season (coded 0 for November to April and 1 for May to October), and energy intake (expressed as MJ) were first analyzed by fitting a series of univariate models. Each univariate model was defined by:

$$Y_{ij} = \gamma_{00} + \gamma_{10}X_{i1} + \mu_{0i} + e_{ij}$$

where $X_{i1}$ is the value for the $p^{th}$ level 1 predictor ($p = 1, 2$, or $3$), $\gamma_{00}$ is the regression coefficient across individuals (fixed component) for the $p^{th}$ predictor, and $\mu_{0i}$ the error term for the regression coefficient at level 2 (random component) for the $p^{th}$ predictor. For ease of interpretation, energy intake was centered at its grand mean. For each predictor, 2 hypotheses were tested: the fixed effect is null ($H_0: \gamma_{p0} = 0$) and the effect is constant across individuals ($H_2: \sigma_{\mu p} = 0$). Acceptance of both hypotheses implies that the level 1 predictor will not be included in the subsequent unstructured multivariate model including up to 3 level 1 predictors ($p = 3$). The multivariate model was defined as follows:

$$Y_{ij} = \gamma_{00} + \sum (\gamma_{ip}X_{ij} + \mu_{0i}) + \mu_{ij} + e_{ij}$$

Parameter estimation in the multilevel models was done using iterative generalized least squares provided by MLwiN 2.20 software (34). In all
models, the significance of individual fixed effects was evaluated using a bilateral $t$ test. Random effects were tested using likelihood ratio (univariate models) and Wald tests (multivariate model) with halved $P$-value. Residual analysis was performed. To verify if the sample used in the present study was representative of the NuAge cohort, independent $t$ tests and $\chi^2$ tests were used. $P < 0.05$ was considered significant.

The number of 24HR ($n$) needed to estimate “true” mean vitamin K intake for an individual within a certain confidence limit ($D$) was calculated using (25):

$$D = \frac{Z \cdot CV}{\sqrt{n}},$$

where $D$ is one-half the length of the interval (as a percentage of “true” mean), $Z_n$ is the normal deviate at a certain CI ($Z_{0.05} = 1.96$ for 95% CI), and $CV$ is the coefficient of intra-individual variation calculated as $\sigma_x / \mu_x$. Similarly, the number of 24HR needed to ensure the desired correlation ($r$) between estimated and “true” dietary intake (unobservable) was determined using the following equation (24,35,36):

$$r = \left[ 1 + \frac{\sigma_x^2}{\sigma_y^2} \right]^{-1/2},$$

where $\sigma_x^2 / \sigma_y^2$ is the variance ratio as determined by the variance component model. The correlation coefficient $r$ is thus a measure of confidence of ranking or classification of individuals into fractions (e.g. fourths). Finally, the attenuation of the true regression coefficient was estimated as follows (22,24):

$$a = \left( 1 - \left[ 1 + \frac{\sigma_x^2}{\sigma_y^2} \right]^{-1} \right) \times 100,$$

where $a$ is the proportion (%) of attenuation of regression coefficient.

### Results

The study sample was mainly Caucasian, with fewer men than women (Table 1). Mean BMI was in the normal range for older adults. Overall, there was no difference between NuAge participants included in the vitamin K intake analyses and those who did not.

#### Variance components of vitamin K intake in older adults

Variance components for all individuals and by sex are presented in Table 2. The geometric mean vitamin K intake across individuals was 70.0 $\mu$g/d (geometric mean). A large proportion of the variation in vitamin K intake resulted from day-to-day variability, with the variance ratios estimated at 3.2 (95% CI = 2.6–3.9) for the total weighted sample, 2.6 (95% CI = 2.1–3.5) for men, and 3.7 (95% CI = 2.9–5.0) for women.

#### Factors explaining intra-individual variance

Multilevel analyses revealed a significant and independent effect of day of the week, season, and energy intake on individual dietary vitamin K intake (Tables 3 and 4). Weekdays were associated with greater consumption of vitamin K ($\gamma_{01}$) and this effect was constant across individuals ($\sigma_{\mu2}^2$; Table 4). Also, vitamin K intake was higher for 24HR collected from May to October than those collected from November to April ($\gamma_{02}$; Table 4). The seasonal effect varied significantly across individuals ($\sigma_{\mu2}^2$) and declined as individual vitamin K intakes increased ($\sigma_{\mu2}^2$; Table 4). Interestingly, regression coefficients of season and day of the week remained significant and quite similar in the multivariable model, which indicated that their effects were mostly independent of energy intake (Table 4). Although significant, day of the week and season contributed to only a very small proportion of the intra-individual variance in vitamin K intake (<1%). Further analysis showed no interaction between day of the week and season (data not shown).

Energy intake was significantly and positively associated with vitamin K intake, although the impact was quite small ($\gamma_{03}$; Table 4). Adjusting for energy intake decreased both inter- and intra-individual variability, but the variance ratio remained similar (3.3). As with season, the effect of energy intake varied across individuals ($\sigma_{\mu3}^2$) and declined as individual vitamin K intake increased ($\sigma_{\mu3}^2$; Table 4). Adjusting for age and sex did not affect the regression coefficients of any of these factors (data not shown).

#### Number of days of diet recording needed to assess phyloquinone intake

Relationships between number of 24HR and accuracy of individual estimate of vitamin K intake,

### TABLE 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vitamin K intake analyses</th>
<th>Excluded</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>939</td>
<td>854</td>
<td>0.59</td>
</tr>
<tr>
<td>Women, %</td>
<td>53</td>
<td>52</td>
<td>0.14</td>
</tr>
<tr>
<td>Age, y</td>
<td>74.3 ± 4.1</td>
<td>74.6 ± 4.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Caucasian, %</td>
<td>99</td>
<td>98</td>
<td>0.17</td>
</tr>
<tr>
<td>Education, y</td>
<td>11.8 ± 4.4</td>
<td>11.5 ± 4.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Living alone, %</td>
<td>32</td>
<td>34</td>
<td>0.21</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>28.0 ± 4.6</td>
<td>27.6 ± 4.3</td>
<td>0.21</td>
</tr>
</tbody>
</table>

1 Values are means ± SD.
2 Refers to participants with missing 24HR or incomplete dietary coding.
3 Data were analyzed by $\chi^2$ or $t$ tests as appropriate.

### TABLE 2

<table>
<thead>
<tr>
<th>Parameter estimates of the variance component models for vitamin K intake in healthy community-dwelling older adults$_{1,2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total$^3$</td>
</tr>
<tr>
<td>$n = 939$</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Fixed effect</td>
</tr>
<tr>
<td>Overall mean vitamin K intake, $\gamma_{00}$</td>
</tr>
<tr>
<td>Random effects</td>
</tr>
<tr>
<td>Inter-individual variance, $\sigma_{\mu0}^2$</td>
</tr>
<tr>
<td>Intra-individual variance, $\sigma_{\mu0}^2$</td>
</tr>
<tr>
<td>Deviance</td>
</tr>
</tbody>
</table>

1 Vitamin K intake data ($\mu$g/d) were ln-transformed prior to analyses.
2 Values are parameter estimates (SE).
3 Data were weighted according to sex, age group, and area of residence to ensure representativeness of the elderly population.

### TABLE 3

<table>
<thead>
<tr>
<th>Level 1 predictors</th>
<th>Fixed effect</th>
<th>Random effects$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t$ ratio</td>
<td>$p$</td>
</tr>
<tr>
<td>Day of the week</td>
<td>-4.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(weekend vs. weekdays)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season (May to October vs. November to April)</td>
<td>3.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Energy intake, MJ/d</td>
<td>17.20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Vitamin K intake data ($\mu$g/d) were assessed using six 24HR by individual. Data were ln-transformed prior to analyses.
2 Likelihood ratio tests with 2 of df were used to assess the significance of the random components (variance and covariance with the intercept).

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Variance components of vitamin K intake. In this study, ranking, and estimation of the regression coefficient are illustrated (Fig. 1). Because adjustments for day of the week, season, and energy intake did not change the variance ratio, calculations were conducted with the weighted pooled intra-individual variance and weighted inter-individual variance (Table 2). The 6 nonconsecutive 24HR used in the present study estimated individual usual vitamin K intake within a range of ±14% of the “true” mean. The correlation coefficient between estimated and “true” vitamin K intake was $r = 0.81$, i.e. $65–70\%$ of individuals would be correctly classified into the extreme fractions of fourths and fifths. Thus, the regression coefficient with vitamin K intake as an independent variable would be attenuated by 34%.

Calculations indicated that 13 24HR are needed to accurately estimate individual usual vitamin K intake ($±10\%$) and ensure a correlation of $r = 0.90$ and an attenuation factor < 20% (Fig. 1). In comparison, that level of accuracy in ranking can be reached with 5 24HR when assessing energy intake (data not shown). With $<13$ d, the attenuation factor of the regression coefficient grows exponentially as the number of days decreases (Fig. 1). Alternatively, additional days of diet recording continues to improve the ranking but at an increasingly limited rate (Fig. 1). In a logistic regression model, attenuation is also a function of the “true” relative risk (RR). For example, if the true RR is between 1.5 and 3.0, the estimated RR would be attenuated by 27–38% with $r = 0.80$ and 14–20% with $r = 0.90$ (21). See Hartman et al. (21) for RR attenuation factors as a function of the ranking accuracy ($r$) at different levels of “true” RR.

### Discussion

**Variance components of vitamin K intake.** In this study, hierarchical modeling was used to extend our current understanding of variance partitioning of vitamin K intake in older adults. In agreement with previous studies (19,20), our results showed high day-to-day variability compared with inter-individual variance. In a group of 362 postmenopausal women, Booth et al. (19) reported a variance ratio of 2.6, a value less than ours, especially when compared with that calculated in women. This discrepancy could be due to the use of consecutive days, which could lead to an underestimation of day-to-day variance (21). The younger sample (41–71 vs. 67–84 y) could also contribute to the difference, although no evidence supports that variance ratios in nutrient intakes vary with age in adults (24,36).

Variance ratios for phyloquinone were also reported in a study where dietary intake of fat-soluble vitamins was measured in 34 healthy adults (20). Although the small sample allowed for only a crude estimate, the variance ratio for vitamin K was by far the highest of all vitamins. Similarly, the variance ratio found in our study was larger than those calculated among older adults for most other nutrients (24,37). This high intra-individual variance most likely results from the large amounts of vitamin K found in a few episodically consumed foods such as green leafy vegetables. Also, the relatively homogenous sample of generally healthy elderly Caucasians might account in part for the low variance. In agreement with previous studies (19,20), our results showed high day-to-day variability compared with inter-individual variance in older adults (23,24,36) as well as in adults in general (22,38). An overall lower energy intake and a smaller inter-individual variance

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**Table 4** Parameter estimates from fitting linear-multilevel model testing day of the week, season, and energy intake as intra-individual predictors of vitamin K intake in older adults ($n = 939$)

<table>
<thead>
<tr>
<th>Model fixed effects</th>
<th>Parameter estimate (SE)</th>
<th>t test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept, $\gamma_0$</td>
<td>4.243 (0.021)</td>
<td>202.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Day of the week, $\gamma_1$</td>
<td>$-0.109$ (0.021)</td>
<td>$-5.19$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Season, $\gamma_2$</td>
<td>0.091 (0.022)</td>
<td>4.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Energy intake, $\gamma_3$</td>
<td>0.006 (0.005)</td>
<td>17.20</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**Figure 1** Confidence limit of estimated usual vitamin K intake of individuals (A); correlation coefficient between the estimated and the true vitamin K intake (B); and attenuation of regression coefficient with vitamin K intake as independent variable (C) as a function of the number of nonconsecutive multiple-pass 24HR.
could explain the consistently higher ratio observed in women (36). This result should be considered in studies including women exclusively.

**Nature of the day-to-day variability.** Consistent with our results, Booth et al. (19) reported that vitamin K intake was significantly higher on weekdays. In our study, the effect of day of the week did not vary across individuals, even after adjustment for energy intake. Such a fixed day of the week pattern likely reflects a behavioral response to our weekdays/weekend cycle. In previous studies, no seasonal variation in vitamin K intakes was found, although this was examined in northern Europe and northeastern USA (19,26,27). This could be explained by limitations in study designs. In Price et al. (26), the sample was small and analyzed by sex (34 women; 31 men), which could have led to lack of statistical power. In the other 2 studies, dietary intake was assessed by 1 FR of 4 or 7 consecutive days/individual. Because participants and seasons were not crossed, the seasonal effect was probably dampened. In the present study, the powerful design revealed the seasonal effect. Seasonal variation could be attributable to an increased availability and lower prices of fresh vegetables during the spring and summer months, notably green leafy vegetables. The fact that the seasonal effect was stronger in those with low vitamin K intakes could indicate that they were more likely to change their diet composition according to seasonal availability of foods than those with high intake. Factors such as low income could explain this result.

Although the day of the week and season had a significant and independent effect on vitamin K intake, they explained a negligible portion of intra-individual variance, which is consistent with numerous studies on macro- and other micronutrients (21,39–42). Thus, our results strongly suggest that day-to-day variation in vitamin K intake is almost entirely a random fluctuation around the individual’s “true” mean. Ranking of individuals would most likely be preserved regardless of for which day or season vitamin K intake is estimated (21). Also, effects of season and day of the week remained similar after adjustment for energy intake, suggesting that they related to variation in diet composition. Thus, when describing absolute intake, and food sources of phylloquinone, accounting for these factors could still be valuable. Adjusting for total energy intake slightly decreased both intra- and inter-individual variances. A weak effect of energy intake was expected, because food sources of phylloquinone are not energy dense. Although it did not improve the variance ratio, adjustment for energy intake could be done to account for confounding and individual differences in energy requirements.

**Implications for designing research.** Booth et al. (19) estimated that 5–10 independent days are required to assess vitamin K intake. Although their recommendations are lower than ours at the same level of accuracy ($r = 0.80–0.90$), they remain comparable (5–10 vs. 6–13 nonconsecutive days). Our results are also in line with those of Hartman et al. (21), who concluded that 7–14 nonconsecutive days are generally adequate ($r = 0.90$) for most nutrients. In nutritional epidemiology, the expected effect size is usually small and thus could easily be dampened by measurement error due to insufficient days of diet recording. To our knowledge, 2 epidemiologic studies have used 24HR or FR to assess phylloquinone intake (13,16). In Rejnmark et al. (13), phylloquinone intake was assessed in a large cohort of perimenopausal women using a FR (4–7 d). In that latter study, no association was found with bone health, a result inconsistent with previous reports using FFQ (9–11,14). In light of our findings, the lack of association reported by Rejnmark et al. (13) could be a false negative due to insufficient days of measurements. It is noteworthy that Pan et al. (16), using only one 24HR, also found no association between phylloquinone intake and metabolic syndrome in younger adults.

Collecting the “ideal” 13 24HR per individual would not be realistic in most studies. It would be more practical and cost-effective to use a FFQ validated for phylloquinone (9,43). Assessing phylloquinone intake by 24HR still remains valuable for validating FFQ, measuring absolute intake, and comparing with DRI, and examining food sources. In those cases, Figure 1 can prove useful to determine the number of days needed. Because calculations were made on a pooled intra-individual variance, individuals with the highest intra-individual variation, notably women, would be the ones most likely to be misclassified. Hence, investigators should be advised to examine data from men and women separately.

**Strengths and limitations of the study.** Our results are based on a powerful methodological and analytical design. The multiple-pass method was used to improve consistency across interviewers and decrease memory bias. Incidentally, vitamin K intake (as geometric mean) reported was in accordance with previous work using daily records in similar populations (27,43). The NuAge cohort excluded frail or institutionalized elders. Although this limits generalization, this cohort has the advantage of including the oldest old (>75 y). A nonrandom sample of the NuAge cohort was used in the present study. Nevertheless, our sample remained large and did not differ from those who were excluded. Vitamin K was assessed as phylloquinone, the predominant K vitamer. We acknowledge that menaquinones can also contribute to total vitamin K intake. However, menaquinones intakes could not be assessed due to the lack of data in the CNF.

In view of the growing interest in the emerging roles of vitamin K in human physiology, the present study provides important information as to the best methodological practices for assessing vitamin K intake in older adults. Based on a comprehensive picture of the variance components, we highlighted that vitamin K intake was characterized by high within- to between-individual variance ratio. Accordingly, we recommend a minimum of 6 nonconsecutive days of diet recording to properly rank individuals and lessen the probability of false negative results in epidemiologic studies. In addition, we found that day of the week and season, while significant, explained a negligible portion of the intra-individual variance. Vitamin K intake could thus be assessed regardless of which day or season the diet recording occurs.

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Literature Cited


