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Protein–calorie malnutrition does not predict subtle vitamin K depletion in hospitalized patients

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Objective: Recent studies suggest that subtle vitamin K depletion has far-reaching consequences. As this entity is not associated with prothrombin time elevation, it is important to determine whether alternate methods can help identify it. We investigated subtle vitamin K depletion in a hospital setting and determined whether protein–calorie malnutrition predicts its presence.
Design, setting, subjects: Using a high-pressure liquid chromatography (HPLC) assay of plasma phylloquinone and a food frequency questionnaire for phylloquinone intake, we examined the phylloquinone status of 27 hospitalized patients with normal coagulation parameters, no liver disease, and no recent warfarin use. We assessed protein–calorie nutritional status with Reilly's criteria and anthropometrics.
Results: 51% of patients (95% Cl = 31% to 70%) had evidence of subtle vitamin K depletion as defined by a subnormal plasma phylloquinone concentration. Patients whose phylloquinone intake was less than the Recommended Daily Allowance (RDA) over the preceding year had lower plasma phylloquinone concentrations when compared to other patients: median (range) 0.106 nmol/l (0.022–0.461) versus 0.301 nmol/l (0.067–3.928), respectively ($P = 0.023$). Plasma phylloquinone concentrations were no different, however, between well-nourished and malnourished patients: median (range) 0.245 nmol/l (0.022–0.522) versus 0.188 nmol/l (0.067–3.928), respectively ($P = 0.782$).
Conclusions: Subtle vitamin K depletion is common among hospitalized patients and protein–calorie malnutrition does not predict its presence.
Descriptors: phylloquinone; protein–calorie malnutrition; vitamin K

Introduction

The consequences of subtle vitamin K depletion are not yet fully realized. A co-factor for the post-translational conversion of glutamic acid residues of specific proteins into γ-carboxyglutamic acid residues, vitamin K is best known for its role as a co-factor in hemostasis. Recent evidence suggests, however, that this vitamin is a carboxylation cofactor in a variety of other tissues as well, including bone, cartilage, kidney, placenta, lung, and testicular tissue (Binkley & Suttie, 1995; Shearer *et al*, 1996). Although the clinical consequences of this vitamin's pervasive activity remain unknown, some have suggested that the optimal vitamin concentration in extra-hepatic tissues may be higher than that necessary for the hepatic synthesis of procoagulants (Anonymous, 1982) and that subtle depletion may result in such effects as compromised skeletal integrity (Binkley & Suttie, 1995). Thus, screening for vitamin K depletion requires more sensitive criteria than prolongation of the prothrombin time, as the undercarboxylation of vitamin K-dependent proteins precedes clotting factor depression in the vitamin K-depleted state (Anonymous, 1982).

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Two previous studies have already determined that subtle vitamin K depletion is prevalent among certain patient populations, and one of these studies has underscored the difficulty in diagnosing it. Using a radioimmunoassay for undercarboxylated prothrombin, Krasinski *et al* (1985) observed sutble vitamin K depletion in 31% of their patients with chronic gastrointestinal disorders. Similar preliminary data have been reported by Duquette and Ferland (1994). Krasinski's paper also stated that there were no signficant differences in protein–calorie nutritional status between vitamin K-replete patients and those with subtle vitamin K depletion. However, the criteria these researchers used for the assessment of protein–calorie malnutrition were inadequate. These investigators measured only serum albumin, serum total protein, and fecal fat. It is well recognized that serum albumin and protein are crude and nonspecific indicators of protein–calorie nutritional status among inpatients (Jeejeebhoy *et al*, 1990) and that fecal fat is a measure of malabsorption, not nutritional status. To test the hypothesis that subtle vitamin K depletion is not associated with protein–calorie malnutrition, one must utilize a set of parameters that accurately reflect general nutritional status.

In view of the potential ramifications of subtle vitamin K depletion and the difficulty in identifying it, we studied the vitamin K status and the protein–calorie nutritional status of a group of hospitalized patients. The purpose of this study was to determine whether protein–calorie nutritional status predicts the presence of subtle vitamin K depletion.

Methods

Over a one-year period, patients from the Adult Gastroenterology Service and the General Internal Medicine inpatient services at New England Medical Center were recruited. This study was approved by the Human Investigation Review Committee at New England Medical Center, and patients were enrolled only after the proper written consent had been obtained.

Patients were excluded from participation if they had one of the following: abnormal prothrombin time, abnormal partial thromboplastin time, ALT or AST equal to or greater than three times normal, a history of cirrhosis, recent or ongoing warfarin use, or a creatinine of ≥ 2.5 g/dl. Blood samples, both for determining study eligibility and for data collection, were drawn after admission to the hospital.

Patients who were enrolled into the study were assessed for plasma phylloquinone concentration, reported habitual intake of phylloquinone, and protein–calorie nutritional status within the first 72 h of hospital admission. Blood for phylloquinone was drawn after subjects had fasted for at least 8 h. Blood tube samples for phylloquinone were wrapped in aluminum foil to avoid light exposure and were transported to the laboratory immediately on ice. Samples were stored at $-70°C$ until the time of analysis. Plasma phylloquinone was determined by reversed-phase HPLC with the use of post-column solid-phase chemical reduction of phylloquinone to its hydroquinone followed by fluorometric detection, as described previously (Haroon *et al*, 1986). Values for phylloquinone were compared to normal values from age- and sex-matched controls, as determined by an earlier study from our laboratory (Sadowski *et al*, 1989). In this earlier study, control samples had been obtained, handled, and assayed in the same manner as described above. Because the principal dietary source of vitamin K is phylloquinone, we considered plasma phylloquinone concentration an appropriate static measure of vitamin K nutritional status. (Sokoll & Sadowski, 1996).

The usual phylloquinone dietary intake over the preceding year was estimated with a semi-quantitative food frequency questionnaire specific for phylloquinone-rich foods. (Table 1) Patients were asked how often and what size of portion of each food item they usually consumed by one of the authors (C.L.). The phylloquinone content of these foods has been previously determined by HPLC as published elsewhere (Booth *et al*, 1995). We defined the normal dietary intake of phylloquinone as 60 μg/day, or slightly below the RDA of 65–80 μg/day (National Research Council, 1989) and base this definition on our own earlier data which show that the average dietary intake of vitamin K in the American population is slightly below the RDA. (Booth *et al*, 1996).

Nutritional assessment with anthropometric measurements was performed by one of us (C.L.). Protein–calorie malnutrition was determined by the likelihood-of-malnutrition criteria established by Reilley *et al* (1988), with minor modifications. Patients were deemed to have a high likelihood of malnutrition if they had any one of the following criteria: serum albumin < 3.5 g/dl, absolute lymphocyte count $< 1.5 \times 10^9$ cells/l, body weight of less than 80% of ideal, an unintentional weight loss of 4.5 kg (10 pounds) or more over the preceding 3 months, or an unintentional loss of 15% or more of body weight over the preceding three months. Reilly's criteria have been validated in a prior study (Reilly *et al*, 1988) that demonstrated that patients who met these criteria for malnutrition were significantly more likely to suffer morbidity and mortality during their hospitalization ($P < 0.001$) and to incur greater healthcare costs ($P < 0.0001$), both of which are accepted outcomes of protein–calorie malnutrition in the hospitalized patient. Finally, upper-arm circumference and triceps skinfold thickness were measured and assessed, as described by Frisancho (1981).

Table 1 Phylloquinone-rich foods included in food frequency questionnaire

Vegetable	*Oil*
broccoli	oils—generic
brussel sprouts	soybean oil
cabbage	canola oil
cauliflower	other vegetable oil
kale	margarine
lettuce	*Miscellaneous*
spinach	yogurt
turnips	tofu
Legumes	
chick peas	
green beans	
lentils	
soy beans	

Daily chart reviews began immediately after patients were enrolled into the study. Antibiotic use, including type and duration, were recorded.

Statistical analyses were performed with the computer software package SYSTAT (version 5.2.1 for MacIntosh, SPSS Inc., Chicago, IL, USA). Results are expressed as mean values with standard deviations or as median values with ranges. Confidence intervals (95%) were determined for the proportion of patients with subtle vitamin K depletion. The Mann–Whitney *U*-test was used to compare plasma phylloquinone between (1) patients with a high likelihood of protein–calorie malnutrition versus those with apparently normal protein–calorie nutritional status and (2) patients who reported phylloquinone intake below 60 μg/day *vs* those who reported consuming more. A subset analysis using Pearson's correlation coefficient was used to determine whether plasma phylloquinone correlated with specific malnutrition indicators such as albumin, total lymphocyte count, and the absolute difference between subjects' individual anthropometric measurements and those of sex- and age-matched median population control values, obtained from standard tables for triceps skinfold thickness and upper-arm muscle circumference (Frisancho, 1981). A *P*-value of < 0.05 was considered statistically significant in all analyses.

Results

Twenty-seven patients were enrolled into the study. Of these, 22 were inpatients on the Adult Gastrointestinal Service and the remainder were from the General Internal Medicine Service. The admitting diagnosis or symptom of patients enrolled into the study is listed in Table 2. The sex ratio of subjects was 59% female, 41% male. The subjects' mean age ± s.d. was 46 years ± 16. All 27 subjects completed all aspects of the study except for one subject who was unable to complete the semi-quantitative food frequency questionnaire.

Table 2 Admitting diagnosis or symptom of patients enrolled into the study ($n=27$)

Gastrointestinal bleeding	8
Pyelonephritis	1
Rheumatalogical disease	2
Diarrhoea	1
Inflammatory bowel disease exacerbation	5
Diabetic ketoacidosis	1
Small-bowel obstruction	1
Fever of unknown origin	1
Cardiac complaint	2
Irritable bowel syndrome	2
Meningitis	1
Bronchitis	1
Graves' disease	1

Five subjects were receiving antibiotics at the time of the study and two others had received them in the preceding 2 months. None of the five patients who were receiving antibiotics at the time of recruitment had received them for longer than 3 days. None of the antibiotics administered belonged to the group of drugs with the methyltetrazole-thiol side-chain, which has been associated with prolongation of the prothrombin time (Lipsky 1994) and with interference with menaquinone production by endogeneous gut bacteria (Suttie, 1995).

Although all subjects had a normal prothrombin time, we still found a significant prevalance of subtle vitamin K depletion: 51% of patients (95% CI = 31%–70%) had low plasma phylloquinone concentrations, defined as values less than two standard deviations below previously referenced age- and sex-matched control means from our laboratory (Sokoll & Sadowski, 1996) (Figure 1). Moreover, patients whose habitual phylloquinone intake was $<60\,\mu g/day$ ($n=8$) had lower plasma phylloquinone concentrations compared to those who reported higher intakes ($n=18$): median (range) 0.106 nmol/l (0.022–0.461) *vs* 0.301 nmol/l (0.067–3.928), respectively, ($P=0.023$) (Figure 2).

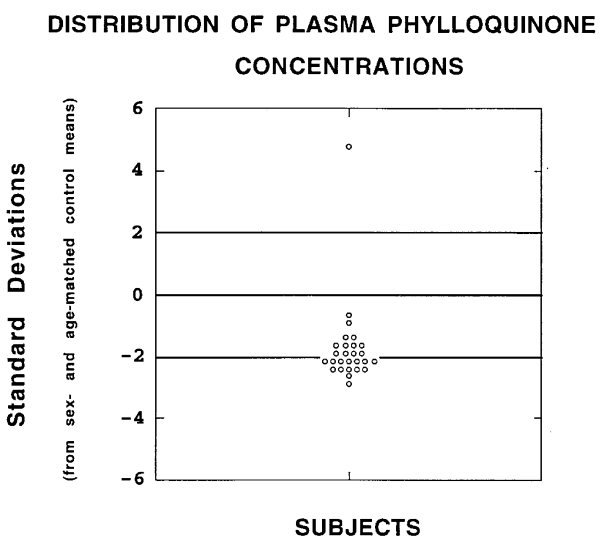

Figure 1 The distribution of plasma phylloquinone levels in 27 hospitalized patients with respect to standard deviations from the mean from age- and sex-matched control values. For the few patients who did not fit into one of the age groups studied in our earlier data, the mean age and standard deviations from the mean of the group as a whole were used for plotting.

Comparision of plasma phylloquinone concentrations between well-nourished patients ($n=7$) and those with a high likelihood of malnutrition ($n=20$) revealed no difference: 0.245 nmol/l (0.022–0.522) *vs* 0.188 nmol/l (0.067–3.928), respectively ($P=0.782$) (Figure 3). In a subset analysis of the nutritional assessment data, no correlation was found between plasma phylloquinone and serum albumin ($r=-0.242$; $P=0.244$) or between plasma phylloquinone and total lymphocyte count ($r=-0.495$; $P=0.806$). Neither did anthropometric data correlate with plasma phylloquinone. The absolute difference between subjects' individual anthropometric measurements and those of earlier-reported, median, age- and sex-matched, population-based normal values (Friscancho, 1981) was correlated with subjects' plasma phylloquinone concentrations and showed no relationship: comparisons for triceps skinfold thickness were $r=-0.164$; $P=0.434$; and for upper-arm circumference $r=0.177$, $P=0.409$.

Discussion

Our data suggest that an assessment of vitamin K status should arise independently of an assessment of protein–calorie nutritional status. The presence of protein–calorie malnutrition did not predict the presence of low plasma phylloquinone concentrations in our patient population. The finding that patients in our study whose habitual dietary intake of phylloquinone had significantly lower plasma phylloquinone concentrations corroborates our plasma phylloquinone results. Moreover, the fact that Krasinski *et al* (1985) and Duquette and Ferland (1994) used other methodology besides plasma phylloquinone measurements for determining subtle vitamin K depletion and found a similar prevalence of depletion among other patients lends further strength to our findings and

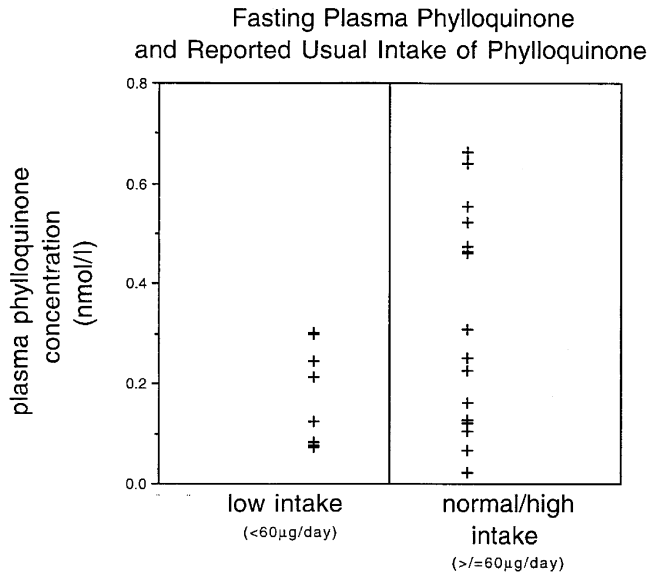

Figure 2 Patients whose phylloquinone intake was $<60\,\mu g/day$ had lower plasma phylloquinone concentrations when compared to those who reported higher intakes: median (range) 0.106 nmol/l (0.022–0.461) *vs* 0.301 (0.067–3.928), respectively ($P=0.023$). An outlier whose plasma phylloquinone level was 3.928 nmol/l and whose reported intake of phylloquinone was $>60\,\mu g/day$ was included in the analysis but excluded from the graph.

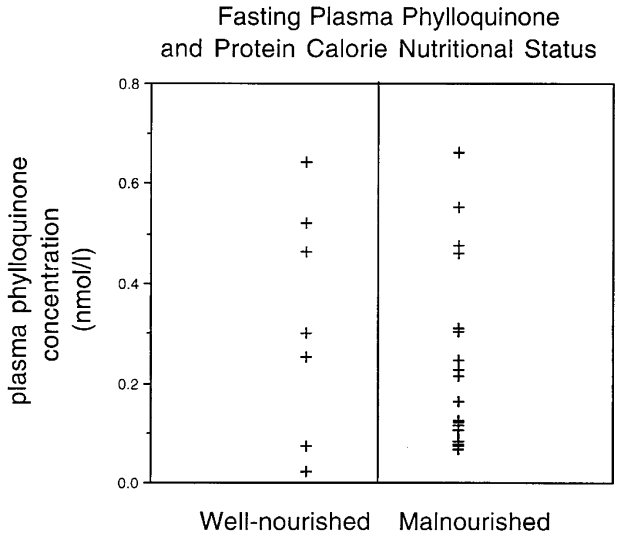

Figure 3 No difference in plasma phylloquinone concentration was seen between patients with protein–calorie malnutrition and those with no evidence of protein–calorie malnutrition: median (range) 0.188 nmol/l (0.067–3.928) *vs* 0.245 (0.022–0.522), respectively ($P = 0.782$). One outlier whose plasma phylloquinone concentration was 3.928 nmol/l and who had evidence of protein–calorie malnutrition was included in the analysis but excluded from the graph.

conclusions about subtle vitamin K depletion and its relationship to nutritional status.

One of the limitations of our study is the lapse between the time of admission to the hospital and the time of blood sampling of up to 72 h. Because some of our subjects may have undergone evaluation of their vitamin K status more than 48 h after hospital admission, one might argue that these low concentrations are a consequence of phylloquinone dietary depletion during hospitalization, as has been demonstrated by other investigators under specific clinical circumstances (Conly *et al*, 1989; Usui *et al*, 1990). However, our dietary data on patients' reported phylloquinone intake one year prior to hospital admission demonstrates that patients whose phylloquinone intake was less than the Recommended Daily Allowance had significantly lower plasma phylloquinone concentrations compared to other patients. These results suggest that marginal vitamin K status often precedes hospitalization. That these patients had significantly lower plasma phylloquinone concentrations during hospitalization reinforces clinical concerns that subtle vitamin K depletion among hospitalized patients may be a long-standing finding and not a transient one that occurs only after patients are admitted to the hospital.

Because the principal dietary source of vitamin K is phylloquinone, plasma phylloquinone concentrations are an appropriate static measure of vitamin K nutritional status. Menaquinones, have previously been reported to provide only a small fraction of bioavailable vitamin K (Olson, 1994). While it was beyond the scope of this study to include more sensitive, functional measures of vitamin K status other than plasma phylloquinone and recall of dietary intake, such as proteins induced in the absence of vitamin K (PIVKA) (Sokoll & Sadowski, 1996), our data demonstrate that subtle vitamin K depletion is prevalent among otherwise well-nourished hospitalized patients; this observation justifies further studies that might utilize these other laboratory measures of vitamin K status.

In conclusion, our study illustrates the difficulty in making a clinical diagnosis of subtle vitamin K depletion and suggests that evaluation for this entity should arise independently of an assessment of protein–calorie nutritional status. The emerging role of vitamin K in health promotion behooves us to seek other clinical screening parameters that might help identify individuals at risk for subtle depletion of this micronutrient in order that we might further explore the clinical consequence of subtle vitamin K depletion.

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