HPLC Method for Plasma Vitamin K\textsubscript{1}; Effect of Plasma Triglyceride and Acute-Phase Response on Circulating Concentrations

MOHAMMED K. AZHARUDDIN,\textsuperscript{1} DENIS ST. J. O’REILLY,\textsuperscript{1} ANDREW GRAY,\textsuperscript{2} and DINESH TALWAR\textsuperscript{1*}

**Background:** The plasma concentration of vitamin K\textsubscript{1} (phylloquinone) is the most reliable index for assessing vitamin K status. Our aim was to analytically validate an HPLC method for quantifying phylloquinone in plasma and to examine the effect of plasma triglyceride concentration on the phylloquinone reference interval. We also examined the effect of acute-phase response on phylloquinone concentration in plasma.

**Methods:** Phylloquinone was extracted from fasting plasma samples by deproteinization and C\textsubscript{18} solid-phase extraction, separated by reversed-phase HPLC, and detected fluorometrically after postcolumn reduction with a platinum catalyst. We synthesized a novel internal calibrator, docosyl naphthoate.

**Results:** The recovery of phylloquinone was >90%. Between-run imprecision was 8.7%–9.0%, and within-run imprecision was 3.8%–7.0%. The linearity was up to 44.8 nmol/L, limit of detection 0.08 nmol/L, and limit of quantification 0.14 nmol/L. The correlation between plasma phylloquinone and triglyceride concentrations was $r = 0.7$ in the reference population. The 95% reference interval for the phylloquinone:triglyceride ratio was 0.20 to 2.20 nmol/mmol. Plasma concentrations of C-reactive protein were significantly increased, whereas triglyceride and phylloquinone but not the phylloquinone:triglyceride ratio were transiently decreased >50% after surgery.

**Conclusion:** Phylloquinone population reference intervals should be expressed as a ratio of the triglyceride concentration. Phylloquinone concentrations in plasma are decreased in acute-phase response and, unless corrected for plasma triglyceride concentration, are unlikely to be a reliable index of vitamin K status.

© 2007 American Association for Clinical Chemistry

Vitamin K is a generic name for a group of quinone compounds with methylated naphthoquinone structure. These include the naturally occurring form phylloquinone (vitamin K\textsubscript{1}), menaquinones, and a synthetic form, menadione. Dietary phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) is the main source of vitamin K (1).

Phylloquinone plays an important role in coagulation, bone metabolism, and vascular health (2–8). Phylloquinone acts as a cofactor for a specific microsomal enzyme, carboxylase, which catalyzes the posttranslational carboxylation of glutamic acid (Glu) to $\gamma$-carboxy glutamic acid (Gla) in vitamin K-dependent proteins (9, 10).

Assessment of phylloquinone status is useful in patients with obstructive liver disease, malabsorption due to celiac disease, or pancreatitis and in patients on long-term parenteral nutrition (11, 12). Phylloquinone status can be assessed by direct measurement of its circulating concentration (13–15) or by functional assays such as prothrombin time (5). The direct quantification of phylloquinone concentration in plasma is the best indicator of vitamin K status and has been associated with dietary intake (15–17). Phylloquinone is present in plasma in nanomole per liter concentrations, however, and its measurement is analytically demanding. Current HPLC methods for measuring phylloquinone in plasma require extensive sample preparation and multiple extraction procedures (18–22).

Highly variable population reference intervals have been reported for phylloquinone, with mean plasma concentrations of 0.22 to 8.88 nmol/L (13, 16, 21, 23, 24). Although this variation is partly due to dietary influences...
(13, 15–17), it also results from differences in the analytical sensitivity and selectivity of analytical methods (20–22) and the effect of variable plasma triglyceride concentrations in the population groups studied (13, 15, 16, 25). The latter effect is relevant because phylloquinone is primarily transported in triglyceride-rich VLDL (25–27).

We developed a reliable method for extraction from plasma of phylloquinone and its separation by isocratic reversed-phase HPLC. We also examined the relationship between phylloquinone and triglyceride concentrations in plasma to establish a phylloquinone population reference interval expressed as a ratio to the triglyceride concentration. Because systemic inflammatory response is associated with a decrease in plasma triglyceride concentrations (28) and may lower vitamin concentrations independently of tissue stores (28, 29), we also examined the effect of acute systemic inflammatory response on phylloquinone concentrations in plasma.

Materials and Methods

CHEMICALS AND REAGENTS

We obtained phylloquinone (vitamin K₁), naphthoic acid, behenyl alcohol (1-docosanol), and platinum on alumina (1.0% and 0.3%) from Sigma. All other reagents and solvents were analytical or HPLC grade. We purchased analytical and guard columns and solid-phase extraction (SPE)³ cartridges from Phenomenex.

BLOOD SAMPLES, CALIBRATOR, AND QC MATERIAL

We obtained blood samples for population reference intervals from patients attending the Cardiovascular Risk Factor clinic for hyperlipidemia and from apparently healthy laboratory staff. The patients were fasting (overnight, 8–14 h), and none were on anticoagulation therapy or vitamin K supplementation or had evidence of a systemic inflammatory response [serum C-reactive protein (CRP) <10 mg/L]; all study participants gave informed consent. The study was approved by the local ethics committee. We collected venous blood into EDTA-containing tubes, centrifuged (500g, 4 °C, 10 min), and removed plasma into plastic tubes, which were stored at −70 °C until analysis.

Elective knee arthroplasty has been shown to induce a large and reproducible systemic inflammatory response (30, 31) and therefore provides a model to examine the relationship between plasma concentrations of phylloquinone and triglyceride and the evolution of systemic inflammatory response. We quantified plasma phylloquinone in 10 patients (8 men and 2 women, ages 60–83 years, median 68 years) who underwent elective knee arthroplasty. All study participants gave informed consent. These individuals received no vitamin supplementation and had no evidence of systemic inflammatory response (CRP <10 mg/L). We collected venous blood samples preoperatively and 12, 24, 48, 72, and 168 h after surgery for analysis of phylloquinone, CRP, and triglycerides.

A single phylloquinone calibrator (4.2 nmol/L) and 2 QC samples (Immunodiagnostics) were reconstituted in distilled water according to manufacturer’s instructions, divided into aliquots, and stored at −70 °C. We used the QC material for internal QC and for generating imprecision data. The assay was also monitored during the study period (May 2003 to June 2005) as part of the External Quality Assurance (EQA) scheme for phylloquinone (KEQAS).

For the purpose of peak identification, we prepared a 100-mg/L (222 nmol/L) solution of phylloquinone in ethanol and checked the concentration by measuring absorbance at 248 nm: E 1% = 420 (19). We diluted this solution in ethanol to obtain 10 working calibrators ranging from 0.35 to 222 nmol/L, which were stored at −70 °C. These were used for linearity and recovery studies.

SYNTHESIS OF DOCOSYL NAPHTHOATE INTERNAL CALIBRATOR

We prepared docosyl naphthoate by esterification of behenyl alcohol (1-docosanol) with naphthoic acid, as described for cetyl alcohol (22). We added behenyl alcohol and naphthoic acid (1 g each) to 25 mL benzene and then added sulfuric acid (5 drops) as catalyst. The mixture was refluxed, and benzene was removed by distillation. We dissolved the remaining solid material in hexane and transferred it to a separating funnel. Deionized water was added to the hexane and inverted several times, and the water was discarded. We repeated this procedure twice and removed the hexane by evaporation in a vacuum chamber. We redisolved the resulting crystalline residue in isopropanol to give a stock solution of docosyl naphthoate (10 mg/L or 20.6 μmol/L). We diluted this stock solution in ethanol to obtain a working solution (206 nmol/L), which was stored in aliquots at −70 °C. At this storage temperature, the stock and working solutions were stable for at least 3 years.

EXTRACTION

We extracted phylloquinone from plasma using C18 SPE columns (500 mg/6 mL; Strata). The columns were placed on a Vac Elut extraction system, activated with methanol (5 mL), and equilibrated with water (2 mL). In a glass tube (16 × 100 mm), 0.5 mL plasma sample, QC, or calibrator was mixed with 100 μL internal calibrator (206 nmol/L), and protein was precipitated with 3 mL ethanol. After centrifugation (3000g, 15 min), we loaded the supernatant onto the water-equilibrated SPE column. The column was washed twice with 2 mL methanol:water (95:5, by volume) under gravity at a flow rate <0.5 mL/min and then dried under full pressure (1 bar) for 30 s. Phylloquinone

³ Nonstandard abbreviations: SPE, solid-phase extraction; CRP, C-reactive protein; EQA, External Quality Assurance; MK6, menaquinone 6.
was eluted with isopropanol (5 mL), dried at 70 °C under air, and reconstituted in 200 μL ethanol. We injected 50 μL of the reconstituted material in the analytical column via an autosampler.

INSTRUMENTATION
The chromatography was based on that described by MacCrehan and Schonberger (21) with some modifications. The HPLC system consisted of a solvent delivery system and fluorometer (Waters). An oxygen scrubber consisting of a stainless-steel column (4.6 × 100 mm; fitted with 0.5-μm frits) dry packed with 1% platinum on alumina was inserted in-line between the pump and the autosampler (model 717). The analytical column was a 5-μm reversed-phase column (Luna C18; 4.6 × 250 mm) protected with a guard column (3 × 4 mm). A postcolumn reactor (4.6 × 50 mm steel column dry packed with 0.3% platinum on alumina) was connected in series between the analytical column and detector. The packing was performed under pressure (3000 psi) to minimize cavitation. All connections were made with short lengths of 0.010-inch i.d. Peek tubing.

CHROMATOGRAPHY
The isocratic mobile phase for chromatography was a mixture of methanol:ethanol (80:20, by volume) that was filtered through a 0.45-μm nylon filter and pumped through the analytical column at a flow rate of 1.0 mL/min. The mobile phase was continuously sparged with nitrogen. After separation and postcolumn reduction with platinum reactor, we measured the reduced form of phylloquinone (hydroquinone) fluorometrically by use of a programmable fluorescence detector (model 2475) with excitation and emission wavelengths of 244 and 420 nm, respectively. At 15 min, we changed the sensitivity setting from 2000 to 200 emission units full scale, to detect phylloquinone and internal calibrator. The fluorescence signal data were collected by a data management system (Millennium 2010; Waters).

QUANTIFICATION
We performed quantification by comparing the peak height ratio of phylloquinone to internal calibrator for the unknown against the peak height ratio for the calibrator.

LINEARITY AND RECOVERY
To evaluate linearity, we extracted and analyzed 9 phylloquinone calibrators (0.5 mL) over the concentration interval 0.14 to 44.8 nmol/L as described for samples. We evaluated linearity by plotting peak height ratio of calibrator to internal calibrator against concentration of calibrator.

For recovery studies, we enriched 5 aliquots (2 mL) of a plasma pool to increase the basal concentration of phylloquinone by 0.55, 1.11, 2.22, 5.55, and 11.1 nmol/L. We extracted each aliquot in duplicate and analyzed them by HPLC to obtain mean recoveries. We calculated phylloquinone extraction efficiency by comparing net chromatographic peak heights obtained by subtraction of peak height after and before phylloquinone addition with that obtained from recovery reference solutions, which we prepared by evaporating 500 μL of an appropriate phylloquinone calibrator containing internal calibrator (100 μL), reconstituting with 200 μL ethanol, and analyzing by HPLC. We also determined extraction efficiency for the internal calibrator. We calculated phylloquinone recovery by dividing the concentration measured by the concentration added to the sample.

TRIGLYCERIDES AND CRP
We measured plasma triglyceride and CRP concentrations using manufacturer’s reagents on an ADVIA 1650 analyzer (Bayer).

STATISTICS
We performed statistical analyses with Minitab statistical software (release 13). We tested data from different time periods for statistical significance using the Friedman test, and where appropriate, we compared data from different time periods using the Wilcoxon signed-rank test.

Results
CHROMATOGRAPHY
Chromatographic profiles for the calibrator and a human plasma extract are shown in Fig. 1. The phylloquinone and internal calibrator peaks were well resolved, with K’ = 5.0 and 9.4, respectively. We identified the peaks by comparing retention times with those of the calibrator solutions. There was no interference in subsequent chromatograms from late-eluting peaks.

INTERFERENCE STUDIES
To investigate possible interfering factors, lipemic (triglyceride; 14.8 mmol/L), hemolysed (hemoglobin; 22 g/L), uremic (creatinine; 560 μmol/L), and icteric (bilirubin; 169 μmol/L) plasma samples and a plasma sample from a patient on warfarin were exposed to UV light in a safety cabinet for 24 h to destroy endogenous phylloquinone (24). We observed no interference from other plasma endogenous substances when these phylloquinone-depleted plasma samples were extracted and analyzed by HPLC (Fig. 1C, for lipemic sample).

SPE
Sample cleanup and enrichment of phylloquinone was successfully achieved using C18 SPE columns. Mean extraction efficiency values for phylloquinone were between 89% and 94% over the range of concentrations studied and 94% to 99% for the internal calibrator. Mean
Phylloquinone recoveries were between 90% and 100% (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue9).

**LINEARITY AND LIMITS OF DETECTION AND QUANTIFICATION**

Fig. 1A (inset) in the online Data Supplement shows that the method is linear from 0.14 to 5.6 nmol/L, the interval that covers most of the 95% reference interval values for phylloquinone. The least-squares regression was $y = 0.36x + 0.0012$, with $R^2 = 0.999$. Fig. 1b in the online Data Supplement shows that this linearity is retained up to at least 44.8 nmol/L ($R^2 = 0.999; y = 0.35x + 0.036$).

The limit of detection, defined as 3 times the chromatographic baseline noise, was 0.08 nmol/L. The lower limit of quantification was 0.14 nmol/L for 0.5 mL plasma and an injection volume of 50 μL. This was defined as the lowest concentration of the extracted calibrator for which the mean value was within 15% of the target concentration and for which the imprecision (CV) around the mean was <20%.

---

Fig. 1. Chromatographic profile of a plasma-based calibrator containing 4.2 nmol/L phylloquinone (A), a plasma sample containing 1.1 nmol/L phylloquinone (B), and a phylloquinone-depleted lipemic plasma sample that had been exposed to UV light for 24 h (C). The baseline was autozeroed at 15 and 25 min.
Between-run and within-run imprecision data are shown in Table 1. During the study period, the performance of the method in the EQA scheme for phylloquinone measurement in plasma was satisfactory (mean $Z$ score, 1.0; 14 observations). The overall error rates (CV) for all laboratories and our laboratory were 23% and 13%, respectively. No systematic bias was observed with our method.

**Population Reference Interval**

Plasma phylloquinone and triglyceride concentrations were measured in 121 fasting individuals (60 men, 61 women) ages 21–88 years (median 58 years). Because the population reference interval for phylloquinone showed a nongaussian distribution, its 95% CI was calculated after log transformation of the data. The geometric mean and 95% reference interval are shown in Table 2. There were no significant differences between sexes or age groups. In the population group studied, there was a wide range of plasma triglyceride concentrations (0.7 to 15 mmol/L), which also showed a nongaussian distribution. Because there was a strong positive correlation between phylloquinone and triglyceride concentrations in plasma ($r = 0.70$, $P < 0.001$; Fig. 2), we also report the phylloquinone population reference interval corrected for triglyceride concentration (Table 2).

**EFFECT OF ACUTE-PHASE RESPONSE ON PLASMA PHYLLOQUINOONE**

The baseline characteristics of the patients who underwent elective knee arthroplasty are shown in Table 3. On analysis of serial postoperative values over the study period of 0 to 168 h, we found a significant increase in circulating CRP concentrations (peak 48 h, $P < 0.001$; Fig. 3; Table 3) and a significant decrease in the median plasma concentrations of phylloquinone (59%, $P < 0.001$) and triglyceride (54%, $P < 0.001$). The nadir of the trough for both was 24 h. CRP ($P = 0.008$), phylloquinone ($P = 0.011$), and triglyceride ($P = 0.076$) had not fully returned to preoperative concentrations on the 7th day postoperation. The time course of the change in concentrations of plasma phylloquinone and triglyceride was the inverse of that of CRP (Fig. 3). Given that both phylloquinone and triglyceride concentrations decreased during the postoperative period, we calculated the ratio of phylloquinone to triglyceride concentration. When adjusted for triglyceride, the reduction in the plasma concentration of phylloquinone in the postoperative period was no longer statistically significant ($P = 0.24$).

**Table 1. Imprecision (CV) of phylloquinone measurements.**

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD), nmol/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between-run</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>0.54 (0.047)</td>
<td>8.70</td>
</tr>
<tr>
<td>Level 2</td>
<td>1.73 (0.156)</td>
<td>9.00</td>
</tr>
<tr>
<td><strong>Within-run</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma pool 1</td>
<td>0.37 (0.026)</td>
<td>7.03</td>
</tr>
<tr>
<td>Plasma pool 2</td>
<td>0.65 (0.025)</td>
<td>3.85</td>
</tr>
<tr>
<td>Plasma pool 3</td>
<td>5.53 (0.220)</td>
<td>3.98</td>
</tr>
</tbody>
</table>

*Between-run imprecision data were obtained by analysis of QC level 1 and level 2 over a period of 6 months ($n = 19$). Within-run imprecision data were obtained by replicate analysis ($n = 10$) of each plasma pool (1, 2, and 3) on the same day. Plasma pools 1, 2, and 3 contained 3 different concentrations of phylloquinone.

**Table 2. Plasma concentrations of phylloquinone and triglyceride and phylloquinone:triglyceride ratio in a reference population ($n = 121$).**

<table>
<thead>
<tr>
<th></th>
<th>Geometric mean*</th>
<th>95% reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylloquinone, nmol/L</td>
<td>1.50</td>
<td>0.28–8.30</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>2.40</td>
<td>0.60–9.60</td>
</tr>
<tr>
<td>Phylloquinone:triglyceride, nmol/mmol</td>
<td>0.63</td>
<td>0.20–2.20</td>
</tr>
</tbody>
</table>

*Antilog10 of log10 mean.
nique has been shown to be inherently more selective, sensitive, and reproducible (19–21). Postcolumn chemical reduction of phylloquinone is usually achieved by using either platinum catalysts (21, 34) or zinc metal in the presence of zinc ions (19–20, 24, 35). The zinc reducer system suffers from a serious drawback in that upon exhaustion of the supply of zinc in the reducer column, the shrinking zinc particles pass through the reducer column frits (21). This limits the useful life of the reducer column and releases damaging particles into the HPLC system, leading to shifts in baseline. In our experience, the zinc reducer column has to be replaced after every batch run to maintain optimal reduction and chromatographic performance. In contrast, the use of platinum on alumina catalyst/alcohol reduction for measurement of phylloquinone has been shown to be reliable and robust (21, 34). For our assay, we used a platinum catalyst reducer column and an alcohol mobile phase as reductant.

Reported methods for the extraction of phylloquinone from plasma are labor intensive (19–21, 24), all requiring multiple extraction procedures to remove lipophilic interferences. To our knowledge, the extraction method we have developed is the only one that requires a single SPE procedure for sample cleanup and concentration of phylloquinone, making the assay much simpler. The effectiveness of sample cleanup was demonstrated by the lack of interfering peaks during HPLC separation of phylloquinone-depleted plasma extracts.

A mobile phase consisting of methanol and ethanol (80:20, by volume) was found to be optimal for chromatographic separation and detection. The presence of ethanol was necessary for optimal catalytic reduction efficiency of the postcolumn reactor and resulting intensity of the fluorescence signal. Like MacCrehan and Schonberger (21), we found that a flow rate of 1 mL/min produced reproducible fluorescence signal for phylloquinone and provided reasonable elution times. Flow rates higher or lower than 1 mL/min resulted in decreased fluorescence signal intensity or peak broadening, respectively. For optimal performance of the postcolumn reducer column, oxygen in the analytical system has to be removed in our system by placing an oxygen scrubber filled with platinum catalyst between the pump and autosampler. Under our specified assay conditions, the oxygen scrubber and reducer columns retained full catalytic activity for approximately 6 months (approximately 600 injections), after which time they had to be repacked with platinum.

Another advantage of our method is the use of the novel internal calibrator docosyl naphthoate, which we synthesized chemically. It is chromatographically separated from phylloquinone and has lipophilic and fluorescence characteristics similar to that of phylloquinone. Because docosyl naphthoate is naturally fluorescent, however, it does not monitor the reduction efficiency of the postcolumn reactor. This process was done by measuring the peak height ratio of phylloquinone and internal calibrator, injected after every 6 samples within the same batch, during calibration.

Internal calibrators commonly used include menaquinone 6 (MK6), menaquinone 4 (vitamin K2), and vitamin K1(25), a structural analog of phylloquinone (19–21,

![Table 3. Concentrations of phylloquinone and triglyceride and phylloquinone:triglyceride ratio after elective surgery for knee arthroplasty (n = 10).](http://example.com/table3)

<table>
<thead>
<tr>
<th></th>
<th>Baseline values (0 h)</th>
<th>Peak/trough</th>
<th>Final values (168 h)</th>
<th>Friedman, P (0–168 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td>&lt;0.001 (&lt;6–7)</td>
<td>155 (83–242)</td>
<td>36 (&lt;6–88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phylloquinone, nmol/L</td>
<td>0.68 (0.44–1.90)</td>
<td>0.28 (0.15–0.55)</td>
<td>0.54 (0.29–1.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.60 (0.70–8.80)</td>
<td>1.20 (0.60–4.60)</td>
<td>1.75 (0.90–3.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phylloquinone:triglyceride, nmol/mmol</td>
<td>0.33 (0.22–0.69)</td>
<td>0.26 (0.13–0.57)</td>
<td>0.30 (0.15–0.51)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Fig. 3. Time course of changes in plasma concentration of phylloquinone, phylloquinone:triglyceride ratio, triglyceride, and CRP after elective knee arthroplasty (n = 10).

CRP concentrations were divided by 100 to enable them to be plotted using the same scale. Open squares represent plasma triglyceride concentration in mmol/L (median; range): preoperative (pre-op, 2.60; 0.70–8.80), 24 h (1.20; 0.60–4.60), 48 h (1.40; 0.40–3.90), 72 h (1.45; 0.50–3.80), and 168 h (1.75; 0.90–3.90). Closed triangles represent CRP concentration in mg/L (median; range): preoperative (<6; <6–7), 24 h (80; 21–154), 48 h (155; 83–242), 72 h (153; 75–231), and 168 h (36; <6–88). Closed squares represent phylloquinone in nmol/L (median; range): preoperative (<6; <6–7), 24 h (1.20; 0.60–4.60), 48 h (1.40; 0.40–3.90), 72 h (1.45; 0.50–3.80), and 168 h (1.75; 0.90–3.90). Open circles represent phylloquinone:triglyceride ratio as nmol/mmol (median; range): preoperative (0.33; 0.22–0.69), 24 h (0.26; 0.13–0.57), 48 h (0.26; 0.18–0.63), 72 h (0.30; 0.15–0.61), and 168 h (0.30; 0.15–0.51).
Neither vitamin K₂ nor MK6 is an ideal internal calibrator, because they occur naturally (21, 34) and may potentially be measurable in some plasma samples. Vitamin Kₐ (25) is not known to be present in biological material, but like MK6, it is not commercially available and laboratories are dependent on gifts for their supplies. Although docosyl naphthoate is also not commercially available, unlike vitamin Kₐ (25), it can be easily synthesized in a Clinical Chemistry laboratory.

The limit of detection of our assay is 0.08 mmol/L, which is comparable to that reported by others (19–22, 24, 33). The between-run imprecision of the assay is well below the reported desirable imprecision goal (CV 19%) based on biological variation of phylloquinone (36).

The linear association between plasma phylloquinone and triglyceride concentrations has been reported (13, 16, 25), but over a fairly narrow plasma triglyceride concentration interval (0.9 to 2.89 mmol/L). Because of this association, the phylloquinone:triglyceride ratio may be more reliable than plasma phylloquinone concentration (13, 16). We measured plasma phylloquinone concentrations in individuals with a wide range of plasma triglyceride concentrations (0.70 to 15 mmol/L) to examine effects on the phylloquinone population reference interval. We document for the first time that plasma concentrations of phylloquinone and triglyceride are correlated (r = 0.7) even at higher plasma triglyceride concentrations. However, visual inspection of the data at the extremes of triglyceride concentrations suggests that there may be a curvilinear relationship between phylloquinone and triglyceride (Fig. 2). To establish with confidence whether this is the case will require the acquisition of a large number of data on individuals with extremes of triglyceride values, such as <0.40 and >10 mmol/L. The positive correlation between phylloquinone and triglyceride concentrations in plasma is not surprising, because phylloquinone is transported mainly by triglyceride-rich lipoproteins, particularly VLDL (25–27), a finding that underlines the necessity of adjusting the plasma phylloquinone reference interval for triglyceride concentration. This situation is analogous to assessment of vitamin E status, for which the most valuable measurement is the plasma vitamin E:cholesterol ratio (3, 37, 38), with cholesterol serving as a marker of LDL, the main plasma transport protein for vitamin E.

Our population data for plasma phylloquinone concentrations showed a nons gaussian distribution consistent with published population data (13, 14, 16, 17, 24). Although a high degree of variability appeared among study participants (95% reference interval, 0.28 to 8.5 mmol/L), when plasma phylloquinone concentrations were expressed as a ratio of the triglyceride concentration, this variability was decreased by almost 3-fold (95% reference interval, 0.28 to 8.5 mmol/L), when plasma phylloquinone concentrations were expressed as a ratio of the triglyceride concentration, this variability was decreased by almost 3-fold (95% reference interval, 0.28 to 8.5 mmol/L). This result suggests that plasma phylloquinone concentrations are partly determined by plasma triglyceride concentrations as reflected by the correlation between the 2 measurements. One previous study, that of Sadowski et al. (13), reported the geometric mean for the plasma phylloquinone:triglyceride ratio in a population group (geometric mean, 0.7; n = 326; age, 20–92 years). Our population data for this ratio (geometric mean, 0.63) in individuals with extremes of plasma triglyceride concentrations is similar to that reported by Sadowski et al. (13).

In contrast, we found no age-associated difference in the plasma phylloquinone:triglyceride ratio values.

We have recently shown that in apparently healthy individuals undergoing an acute inflammatory insult, circulating concentrations of vitamin E and carotenoids are transiently decreased, but when results were adjusted for the decrease in the lipid component of plasma (cholesterol) there were no significant alterations (28, 29). In the present study we have shown that phylloquinone concentration in plasma responds similarly, falling transiently by almost 50% after an acute inflammatory insult. When results were adjusted for the decrease in triglyceride concentration over the same study period, however, the decrease in phylloquinone concentration was no longer significant over the period of injury. These results suggest that the changes in circulating phylloquinone concentration during acute injury may simply reflect the passive process of inflammation-driven redistribution of the plasma lipid fraction (28). Therefore, in the presence of systemic inflammatory response, plasma phylloquinone concentrations are unlikely to be a reliable measure of status, and during such a response the plasma phylloquinone:triglyceride ratio may provide a more reliable measurement of phylloquinone status. This ratio may not be reliable in patients with conditions such as primary hypertriglyceridemia or those with very high triglycerides secondary to poorly controlled diabetes. Much more work needs to be done to investigate phylloquinone status in patients with these and other conditions.

Grant/funding support: None declared.

Financial disclosures: None declared.

Acknowledgments: We gratefully acknowledge the assistance of Dr. D.C. McMillan (University Department of Surgery, Royal Infirmary, Glasgow, UK) for contribution in the statistical analyses. We also acknowledge A. Bell (Department of Biochemistry, Royal Infirmary, Glasgow, UK) for analyzing plasma samples for cholesterol and triglycerides.

References