

Phylloquinone (vitamin K₁) intakes and serum undercarboxylated osteocalcin levels in Irish postmenopausal women

Aoife Collins^{1*}, Kevin D. Cashman^{1,2} and Máiréad Kiely¹

¹Department of Food and Nutritional Sciences, University College, Cork, Ireland

²Department of Medicine, University College, Cork, Ireland

(Received 26 September 2005 – Revised 20 December 2005 – Accepted 5 January 2006)

Low phylloquinone (vitamin K₁) intakes have been associated with low bone mineral density in older adults. Phylloquinone intakes and serum undercarboxylated osteocalcin (ucOC) levels were assessed in ninety-seven apparently healthy, free-living Irish women aged 50–75 years. Phylloquinone intakes were estimated using a detailed dietary history, which measured habitual food intakes from a typical 14 d period, and recently published food composition data for phylloquinone. Fasting serum ucOC was measured using an enzyme immunoassay. The median daily intake of phylloquinone in the group from all sources was 108.8 µg and from food sources only was 106.6 µg, indicating that approximately 99 % of the phylloquinone came from food. Vegetables and vegetable dishes contributed 67 % of the total phylloquinone intake, but further analysis showed that broccoli, cabbage and lettuce were the primary sources, making a total contribution of 44 %. Twenty per cent of the women had a phylloquinone intake below the UK recommendation of 1 µg/kg body weight per day and 34 % failed to meet the US Adequate Intake value of 90 µg/day. Mean serum ucOC levels in the women were 6.2 (SD 1.7) ng/ml and were predicted by phylloquinone intake ($\beta = 2.20$, generated from log-transformed phylloquinone intake data; $P = 0.04$). On the basis of comparisons with both UK recommendations and US Adequate Intakes for phylloquinone, the habitual intakes of phylloquinone in a high proportion of Irish postmenopausal women may not be adequate.

Phylloquinone intake: Undercarboxylated osteocalcin: Bone health

The function of vitamin K is to serve as a co-factor for vitamin K-dependent carboxylase, a microsomal enzyme that facilitates the post-translational conversion of glutamyl to γ -carboxyglutamyl residues (Esmon *et al.* 1975). Its classic role in this respect involves the synthesis of several coagulation factors, including plasma procoagulants, prothrombin (factor II), factors VII, IX and X, and anticoagulants (proteins C and S) (Price, 1988; Shearer, 1990, 2000; Institute of Medicine, 2001). The maintenance of plasma prothrombin concentration was the basis for the recommended dietary intake value of 1 µg phylloquinone (vitamin K₁) per kilogram body weight per day, set by the National Research Council (1989) in the USA and by the Department of Health (1991) in the UK. More recently, the identification of γ -carboxyglutamyl residue-containing proteins in bone, notably osteocalcin and matrix carboxyglutamyl residue-protein, has generated much interest in the role of vitamin K in bone metabolism and bone health (Binkley & Suttie, 1995; Vermeer *et al.* 1995; Institute of Medicine, 2001; Weber, 2001).

The findings of two large prospective cohort studies (the Nurses' Health Study and the Framingham Heart Study) suggest an association between the relative risk of hip fracture and phylloquinone intake (Feskanich *et al.* 1999; Booth *et al.* 2000). Furthermore, low dietary phylloquinone intakes have

recently been associated with low bone mineral density (BMD) in elderly subjects (Booth *et al.* 2003). The serum concentration of undercarboxylated osteocalcin (ucOC) is a sensitive indicator of vitamin K status, as high serum levels of ucOC are indicative of low vitamin K status and vice versa (Vermeer *et al.* 1995; Sokoll *et al.* 1997). In addition, the circulating concentration of ucOC has been reported to be a predictor of low BMD (Szulc *et al.* 1994; Jie *et al.* 1996) and hip fracture risk (Szulc *et al.* 1993, 1996; Vergnaud *et al.* 1997).

Recent evidence has suggested that phylloquinone intakes that are sufficient to maintain normal blood coagulation may be suboptimal for bone health (Kohlmeier *et al.* 1996; Vermeer *et al.* 1996; Sokoll *et al.* 1997). Furthermore, besides its role in bone health, there is also a growing interest in the possible role of vitamin K in atherosclerosis (Jie *et al.* 1995; Shearer, 2000; Institute of Medicine, 2001) and cognitive function (Rambeck & Stahelin, 2001).

In this context, the US Food and Nutrition Board has recently upwardly revised its recommendation for the daily dietary intake of phylloquinone, and has established an Adequate Intake of 90 and 120 µg/d for adult females and males, respectively (Institute of Medicine, 2001). This Adequate Intake was based on estimating the amount of phylloquinone habitually consumed by a group of healthy people and on

Abbreviations: BMD, bone mineral density; ucOC, undercarboxylated osteocalcin.

Contributors: A. C. contributed to the design, execution, analysis and writing of the study. K. D. C and M. K. contributed to the design, analysis and writing of the study.

***Corresponding author:** Dr Aoife Collins, fax +353 21 4270244, email collinsaoife@ucc.ie

the assumption that these amounts were adequate to promote all aspects of health (Institute of Medicine, 2001).

There are limited data on the dietary intake of phylloquinone in European populations. Available data suggest that the mean intake of phylloquinone is about 60–80 $\mu\text{g}/\text{d}$ in countries such as Scotland, the UK and Norway (Price *et al.* 1996; Askim, 2001; Thane *et al.* 2002) and about 250 $\mu\text{g}/\text{d}$ in the Netherlands (Schurgers *et al.* 1999). Using a 7 d food diary, we recently estimated the phylloquinone intake in a representative sample of Irish adults, aged 18–64 years, to be 79 $\mu\text{g}/\text{d}$ (Duggan *et al.* 2004); of this population, only 17% of men and 27% of women met the US Adequate Intake. Some studies have reported a decrease in phylloquinone intake with age, especially in adults over the age of 65 years (Schurgers *et al.* 1999; Thane *et al.* 2002). There are currently no data on biochemical vitamin K status, using indices such as serum ucOC, for healthy Irish adults. As postmenopausal women are at increased risk of osteoporosis, and as Duggan *et al.* (2004) showed that Irish women have a lower intake of phylloquinone than Irish men, it is possible that older women may be a group at increased risk of suboptimal phylloquinone intake and status. Therefore, the objectives of the present study were to estimate the habitual intakes and adequacy of intakes of phylloquinone and to measure circulating ucOC levels in a group of Irish postmenopausal women.

Subjects and methods

Subjects

A convenience sample of ninety-seven apparently healthy, free-living postmenopausal women (mean age 65.0 (range 50–75) years) was recruited by leaflet or direct contact from the Cork region. None of the subjects was suffering from any condition or taking any medications likely to affect vitamin K status.

Ethical considerations

Ethical approval for the study was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Before participation in this study, all subjects signed an informed consent document.

Study design

This was an observational study of phylloquinone intake and status in Irish postmenopausal women. Each participant was invited to provide a fasting blood sample at the University during February–March 2002, August–September 2002 or February–March 2003. After an overnight fast, a blood sample (20 ml) was taken between 08.30 and 10.30 hours from each participant (see later). Anthropometric measurements (weight and height) were taken using a Seca Alpha 770 digital scale (CMS Weighing Equipment Ltd, London, UK) and a Leicester height measure (CMS Weighing Equipment Ltd), respectively. Habitual food intake was assessed by a 14 d dietary history, which consisted of a one-to-one interview detailing usual food and drink intake over a typical 14 d period. A general health and lifestyle questionnaire was administered to each participant, which provided information

on medical history, the use of hormone replacement therapy, visits to hospital, fracture history and smoking history. The questionnaire also detailed the use of nutritional supplements, including vitamin K-containing supplements.

Dietary assessment methodology

Tapsell *et al.* (2000) have described the detailed methodology of the dietary history that was used to collect food and beverage intake data. In brief, the interviewer began by asking about the first meal of the day (usually breakfast) and continued in a sequential order until the last meal of the day was recorded. The dietary history collected a detailed description of the food and/or drink consumed, the brand name (if applicable) and the quantity of the food and/or drink consumed over a typical 14 d period. Food intakes were quantified using a photographic food atlas of food portion sizes (Ministry of Agriculture, Fisheries and Food, 1997), manufacturer's data and standard portion sizes (Ministry of Agriculture, Fisheries and Food, 1997). Information was collected on the time and location of each eating or drinking occasion, and participants were asked about their current use of nutritional supplements. A short food questionnaire was attached to the end of the dietary history to use as a cross-check.

Food intake data were analysed using WISP (Tinuviel Software, Warrington, UK), which incorporated data from the fifth edition of *McCance and Widdowson's The Composition of Foods* (Holland *et al.* 1995) and supplemental volumes. Our research group had previously expanded the fifth edition to include about 1000 new food codes, including recipes, nutritional supplements and new products (Harrington *et al.* 2001). In addition, the phylloquinone composition database compiled by Bolton-Smith *et al.* (2000) had been added to the food composition database (Duggan *et al.* 2004). In the current study, additional recipes provided by the ninety-seven subjects were analysed for their phylloquinone content and were also included in the database. Food and nutrient intake data were exported from WISP and analysed in SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA).

Assessment of adequacy of phylloquinone intake

As there is currently insufficient evidence to establish an average requirement for phylloquinone, it was not possible to use the cut-point method of Carriquiry (1999) to estimate the prevalence of inadequate dietary intakes in the sample. However, in the absence of an average requirement, the US Food and Nutrition Board has recently established Adequate Intakes of 90 and 120 $\mu\text{g}/\text{d}$ for adult women and men, respectively (Institute of Medicine, 2001), based on reported intakes in apparently healthy US population groups. In the UK, the recommended intake for phylloquinone is 1 $\mu\text{g}/\text{kg}$ body weight per day (Department of Health, 1991), based on its role in blood clotting. Therefore, to gain some measure of the adequacy of phylloquinone intake, the proportions of postmenopausal women in the present study not meeting the UK recommendation and not consuming the US Adequate Intake were calculated.

Collection and preparation of blood samples

Blood was collected by venepuncture into a Vacutainer tube (BD Vacutainer Systems, Plymouth, UK) with no additive and processed to serum, using a refrigerated centrifuge, then immediately stored at -80°C until required for analysis.

Serum undercarboxylated osteocalcin

Serum ucOC levels were measured in serum samples using a recently developed ELISA (Undercarboxylated (Glu-OC) EIA kits; Takara Biomedical Group, Otsu, Shia, Japan). The intra- and interassay CV were 4.4 and 7.5 %, respectively.

Statistical analysis

Data for all variables, with the exception of estimates of phyloquinone intake, were normally distributed, as assessed using Kolmogorov–Smirnov tests ($P > 0.1$). Data estimating phyloquinone intake appeared to have a slight, borderline significant ($P = 0.08$) positive skew, but the data approached a near-normal distribution ($P > 0.1$) after log-transformation, and these transformed intake data were used for all statistical analyses. Data are presented as means and standard deviations, as well as medians and 5th and 95th percentiles. Differences in phyloquinone intake and serum ucOC level between women aged 64 years or less and over 64 years were evaluated using unpaired Student's *t* tests. Stepwise linear regression analysis was used to investigate the association between phyloquinone intake and serum ucOC level, controlling for age, BMI and energy intake.

Results

The mean age, height, weight and BMI of the women are shown in Table 1. Sixty-seven percent of women were regular users of nutritional supplements, but only 26 % of these supplements contained phyloquinone. Intakes of phyloquinone (as μg and $\mu\text{g}/10\text{MJ}$ food energy) are shown in Table 2. The median daily intake of phyloquinone from all sources was 108.8 μg , and from food sources only was 106.6 μg , showing that approximately 99 % of the phyloquinone intake came from food.

When the women were split into two groups of those aged over 64 years ($n = 42$) and those aged 64 years or less ($n = 55$), there was no significant difference in phyloquinone intake between them (Table 3). Of the ninety-seven women who participated in the study, 20 % had a phyloquinone intake below the UK recommendation of 1 $\mu\text{g}/\text{kg}$ body weight per day (Department of Health, 1991). Comparing the current

Table 1. Characteristics of study participants ($n = 97$)

	Mean	SD
Age (years)	65.0	7.3
Height (m)	1.60	0.1
Weight (kg)	66.9	11.0
BMI (kg/m^2)	26.1	4.3
Supplement users (%)*	67	–

* Only 26 % of these supplements contained phyloquinone.

Table 2. Intakes of phyloquinone from all sources (food and supplements) and food sources only in Irish postmenopausal women ($n = 97$)

	All sources		Food sources	
	$\mu\text{g}/\text{d}$	$\mu\text{g}/10\text{MJ}^*$	$\mu\text{g}/\text{d}$	$\mu\text{g}/10\text{MJ}^*$
Mean	123.5	1.5	120.3	1.5
SD	58.5	0.8	65.7	0.8
Median	108.8	1.3	106.6	1.3
5th percentile	43.5	0.6	43.5	0.6
95th percentile	236	3.1	225	2.9

* Vitamin K intake per 10 MJ food energy.

phyloquinone intake data with the current US recommendations for adult women (Institute of Medicine, 2001), 34 % of all women failed to meet the Adequate Intake of 90 $\mu\text{g}/\text{d}$. Forty per cent of the women aged 65–75 years and 26 % of those aged 50–64 years did not achieve the US Adequate Intake, whereas 25 % of the 65–75-year-olds and 14 % of the 50–64-year-olds failed to achieve the UK recommendation.

On the basis of their contribution to phyloquinone intake, foods were aggregated to eight large food groups that provided 91 % of the total intake (Fig. 1). Overall, vegetables and vegetable dishes contributed 67 % of the total phyloquinone intake. Fats and oils, fruit, and milk and milk products also made minor contributions of 7.6, 4.6 and 4.1 %, respectively. As the vegetable food group provided 67 % of the phyloquinone intake, this group was disaggregated into nine vegetable food groupings to identify the actual sources of phyloquinone intake (Fig. 2). Vegetables provided about 50 % to the total phyloquinone intake; of this, broccoli was the highest single source, providing 22 % of total intake, followed by cabbage (12 %) and lettuce (10 %).

Mean serum ucOC concentration in the group was 6.2 (SD 1.7) ng/ml . There was no significant difference in serum ucOC between women aged over 64 ($n = 41$) and 64 or under ($n = 55$). Serum ucOC levels were significantly inversely related to phyloquinone intakes (Fig. 3). Stepwise linear regression analysis controlling for BMI and energy intake showed that vitamin K intake ($\beta = -0.006$; $P = 0.03$) and age ($\beta = -0.04$; $P = 0.05$) were significant predictors of serum ucOC value ($n = 96$).

Discussion

The current report describes the first assessment of phyloquinone intake using a 14 d dietary history, and vitamin K status using serum ucOC concentrations, in ninety-seven Irish women aged between 50 and 75 years. The mean phyloquinone intake in postmenopausal women in the present study was higher (123.5 $\mu\text{g}/\text{d}$) than that previously reported by our group for Irish postmenopausal women aged 50–64 years (82 $\mu\text{g}/\text{d}$; Duggan *et al.* 2004). There are two probable explanations for the differences observed. First, although the food composition data were identical, the method by which vitamin K intake was assessed in the two studies differed; we used a 14 d dietary history in the present study, whereas Duggan *et al.* (2004) reported data from a 7 d food diary. Second, Duggan *et al.* (2004) reported data from a nationally representative sample of women aged 50–64 years,

Table 3. Intakes of phylloquinone and serum under-carboxylated osteocalcin (ucOC) concentrations in 50–64-year-old and 65–75-year-old Irish women

Age groups	Phylloquinone intake ($\mu\text{g}/\text{d}$)				ucOC (ng/ml)	
	Mean	SD	Median	5th–95th percentile	Mean	SD
50–64 years (<i>n</i> 55)	131	54	134	44–225	6.1	1.9
65–75 years (<i>n</i> 42)*	118	62	100	41–241	6.3	1.4
Total (<i>n</i> 97)	123	59	109	43–236	6.2	1.7

* One of the 65–75-years age group was unable to provide a blood sample so there is one missing ucOC value.

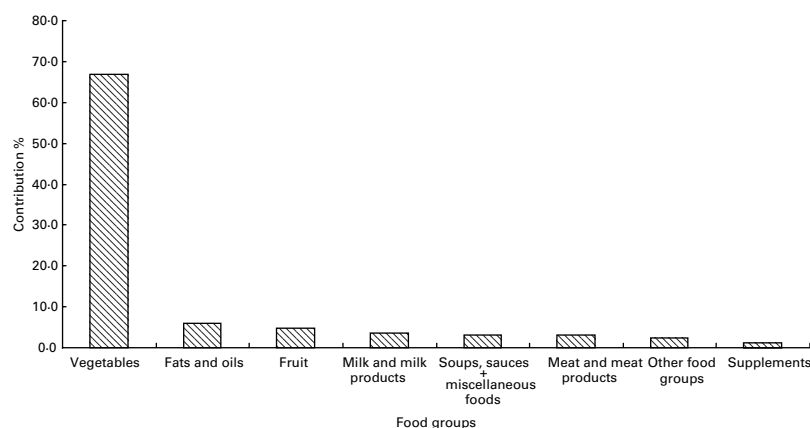
whereas the current sample was self-selected and consisted of highly motivated individuals with an interest in diet and bone health. Lahmann & Kumanyika (1999) found that a high awareness of health and nutrition favourably affected dietary quality in older women. It should be noted that subjects in the current study, although aware that their overall diet was being assessed as part of a diet and bone health study, were not aware we were focusing on vitamin K in particular.

The current phylloquinone intake data were also higher than the value reported by Thane *et al.* (2002) in UK women of the same age (66 $\mu\text{g}/\text{d}$; *n* 325), surveyed during the National Diet and Nutrition Survey of people aged 65 years and over (Finch *et al.* 1998). However, Yan *et al.* (2004) recently reported that British elderly men and women (aged 60–83 years, mean 68 years; *n* 134) recruited from general practice surgeries in Cambridge had a mean phylloquinone intake of 117 $\mu\text{g}/\text{d}$. These studies, in addition to our current and previous (Duggan *et al.* 2004) data, illustrate the importance of considering the impact of sampling method on estimates of dietary intake.

In the US, Booth *et al.* (1995) reported a mean phylloquinone intake of 89 $\mu\text{g}/\text{d}$ for 362 postmenopausal women. McKeown *et al.* (2002) reported that mean daily phylloquinone intakes were around 132–135 $\mu\text{g}/\text{d}$ for adults (aged 50 years and older) participating in the Framingham Offspring Cohort study. In the Netherlands, Schurgers *et al.* (1999) reported mean daily phylloquinone intakes of about 250 $\mu\text{g}/\text{d}$ for adults aged 65 years and older, whereas Jie *et al.* (1995) reported that Dutch postmenopausal women (mean age 66 years; *n* 113) had a mean daily phylloquinone intake of approximately 227 $\mu\text{g}/\text{d}$. The estimates of intake from these latter four studies were

obtained using food-frequency questionnaires, which tend to estimate higher intakes than food-diary methods (Herbert *et al.* 1998; French *et al.* 2001).

Phylloquinone is ubiquitously distributed in the diet; however, the range of concentrations in different food groups is very wide (Shearer *et al.* 1996). The main dietary sources of phylloquinone in the present study are comparable to those reported for studies in the UK (Price *et al.* 1996; Fenton *et al.* 1997; Thane *et al.* 2002; Yan *et al.* 2004) and the only other study of Irish adults (Duggan *et al.* 2004). Foods in the Irish diet that have high concentrations of phylloquinone are green vegetables, such as parsley, spinach and cabbage (400–3000 $\mu\text{g}/100\text{g}$), whereas broccoli, lettuce, coleslaw and vegetable oils have intermediate levels (100–200 $\mu\text{g}/100\text{g}$). Of these, broccoli, cabbage and lettuce were the most important providers of phylloquinone in the current study (44% between them), as they were consumed in the highest quantities. These data were similar to those from the recent report by Yan *et al.* (2004), in which green leafy vegetables contributed 50.4% to mean daily intake of phylloquinone in UK adults aged 60–83 years. In the same report by Yan *et al.* (2004), elderly Chinese adults, who consumed comparatively more green vegetables than their UK counterparts (127 v. 39 g/d) obtained 68.5% of their phylloquinone intake from green vegetables, and they had better vitamin K status. In studies using nationally representative samples in Ireland and the UK, green vegetables made a lower contribution to phylloquinone intake. For example, Duggan *et al.* (2004) reported that green vegetables contributed only 26% to phylloquinone intake in Irish adults aged 50–64 years, and

**Fig. 1.** Percentage contribution of main food groups to mean daily intake of phylloquinone in 50–75-year-old Irish women (*n* 97).

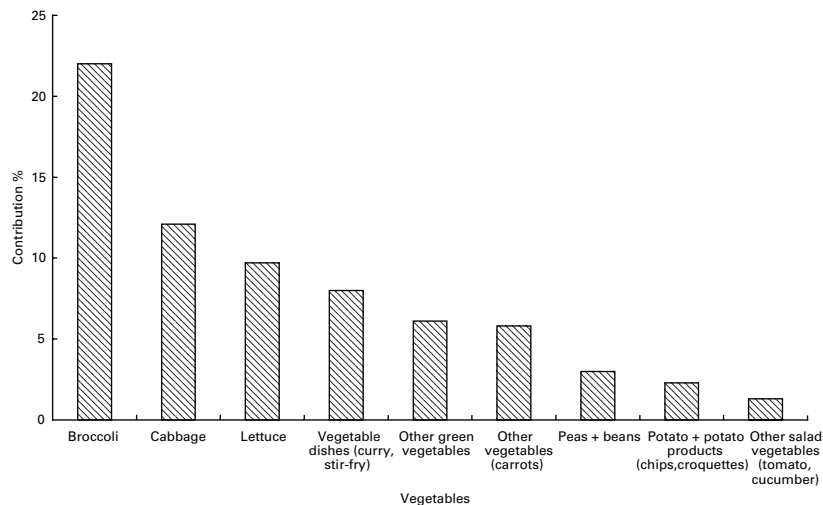


Fig. 2. Percentage contribution of different vegetable groupings to mean daily intake of phylloquinone in 50–75-year-old Irish women (n 97).

Thane *et al.* (2002) showed that only 28% phylloquinone intake in elderly UK adults came from green vegetables.

Some studies have reported a decrease in phylloquinone intake with age, especially in adults over the age of 65 years, and more notably over the age of 85 years (Schurgers *et al.* 1999; Thane *et al.* 2002). In the present study, there was no significant difference in phylloquinone intake between women aged 50–64 years and those aged 65–75 years, but the percentage achieving recommended target intakes was lower in the older women. Contrary to studies that report a decrease in phylloquinone intake with age, Duggan *et al.* (2004) reported that younger Irish adults (aged 18–35 years) had a lower mean phylloquinone intake than older adults (aged 36–64 years). This was probably due to the lower vegetable consumption of younger Irish adults compared with over-35-year-olds, as previously reported by O'Brien *et al.* (2003).

In the present study, over half of the women aged 65–75 years and almost a quarter of those aged 50–64 years were

not consuming the US recommended intake levels. In addition, a quarter of the 65–75-year-olds and 14% of the 50–64-year-olds failed to achieve the UK recommendation. Furthermore, although the mean daily phylloquinone intakes of the women in the present study were similar to or slightly higher than those reported for American and European intakes, there is evidence to suggest that these levels may be insufficient for the maintenance of bone health. In the Nurses' Health Study, phylloquinone intakes of less than 109 $\mu\text{g}/\text{d}$ were associated with an increased risk of hip fracture (Feskanich *et al.* 1999). In the Framingham Heart Study, elderly men and women in the highest quartile of phylloquinone intake (median 254 $\mu\text{g}/\text{d}$) had a significantly lower adjusted relative risk of hip fracture than did those in the lowest quartile of intake (median 56 $\mu\text{g}/\text{d}$; Booth *et al.* 2000). Furthermore, low dietary phylloquinone intakes have recently been associated with low BMD (Booth *et al.* 2003). Women in the lowest quartile of phylloquinone intake (70 $\mu\text{g}/\text{d}$) had significantly lower ($P < 0.005$) mean BMD at the femoral neck and spine than did those in the highest quartile of phylloquinone intake (309 $\mu\text{g}/\text{d}$; Booth *et al.* 2003).

In the present study, ucOC level was inversely associated with phylloquinone intake. This is in agreement with findings in UK-based elderly subjects (Yan *et al.* 2004). Besides acting as a marker of vitamin K nutriture (Sokoll & Sadowski, 1996), serum ucOC has been reported to be an indicator of risk of hip fracture (Szulc *et al.* 1993; Vergnaud *et al.* 1997; Booth *et al.* 2000) and a predictor of BMD (Szulc *et al.* 1994; see reviews by Institute of Medicine, 2001; Weber, 2001). The mean serum ucOC for postmenopausal women in the present study (6.0 ng/ml) was higher than that of a group of Danish postmenopausal women (3.8 ng/ml; mean age 62 years; n 31), which used the same assay (S. Bugel, unpublished results). Furthermore, dietary supplementation of the Danish postmenopausal women with 200 and 500 μg phylloquinone led to a 40% and 68% reduction, respectively, in serum ucOC level after 6 weeks (S. Bugel, personal communication). Therefore, the current data suggest that subclinical vitamin K deficiency may have been present in some of the postmenopausal women, in agreement with the findings of others

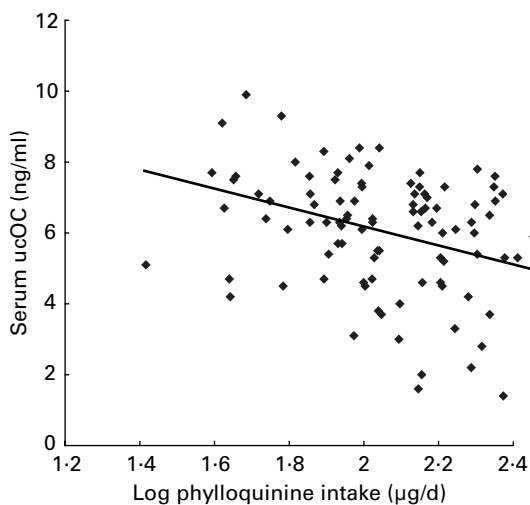


Fig. 3. Relationship between phylloquinone intake (log-transformed) and serum undercarboxylated osteocalcin (ucOC) level in 50–75-year-old Irish women (n 96). $r = -0.288$, $P = 0.004$.

(Knapen *et al.* 1989; Plantalech *et al.* 1990; Binkley *et al.* 2000).

In relation to future work in the area of vitamin K status in postmenopausal women, it is worth emphasising that the direct assays for measuring ucOC (as used in the present study) appear to overestimate values compared with indirect bindings assays, which express ucOC as a percentage of total osteocalcin (Gundberg *et al.* 1998). Notwithstanding this, however, Conway *et al.* (2005) recently pointed to evidence from studies among elderly subjects which showed that the fractional decreases in the percentage of ucOC brought about with vitamin K supplementation were very similar whether measured with direct assays for ucOC (as in the present study) or with indirect bindings assays.

In conclusion, habitual intakes of phylloquinone in a high proportion of relatively well-nourished Irish postmenopausal women may not be adequate to maintain bone health, on the basis of comparisons with both the 1991 UK recommendations and the 2001 US Adequate Intake for phylloquinone. Further research on the impact of vitamin K status on postmenopausal bone health is needed. In addition, investigations of phylloquinone intake in other age groups within the Irish population, particularly younger age groups, are warranted.

References

- Askim M (2001) Vitamin K in the Norwegian diet and osteoporosis. *Tids Den Norske Laege* **121**, 2614–2616.
- Binkley NC, Krueger DC, Engelke JA, Foley AL & Suttie JW (2000) Vitamin K supplementation reduces serum concentrations of under- γ -carboxylated osteocalcin in healthy young and elderly adults. *Am J Clin Nutr* **72**, 1523–1528.
- Binkley NC & Suttie JW (1995) Vitamin K nutrition and osteoporosis. *J Nutr* **125**, 1812–1821.
- Bolton-Smith C, Price RJG, Fenton ST, Harrington DJ & Shearer MJ (2000) Compilation of provisional UK database for the phylloquinone (vitamin K₁) content of foods. *Br J Nutr* **83**, 389–399.
- Booth SL, Broe KE, Gagnon DR, *et al.* (2003) Vitamin K intake and bone mineral density in women and men. *Clin Endocrinol Metab* **89**, 4904–4909.
- Booth SL, Sadowski JA & Pennington JAT (1995) Phylloquinone (vitamin K₁) content of foods in the U.S. Food and Drug Administration's Total Diet Study. *J Agric Food Chem* **43**, 1574–1579.
- Booth SL, Tucker KL, Chen H, *et al.* (2000) Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr* **71**, 1201–1208.
- Carriquiry AL (1999) Assessing the prevalence of nutrient inadequacy. *Public Health Nutr* **2**, 23–33.
- Conway SP, Wolfe SP, Brownlee KG, White H, Oldroyd B, Truscott JG, Harvey JM & Shearer MJ (2005) Vitamin K status among children with cystic fibrosis and its relationship to bone mineral density and bone turnover. *Pediatrics* **115**, 1325–1331.
- Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. Report on Health and Social Subjects No. 41. London: HMSO.
- Duggan P, Cashman KD, Flynn A, Bolton-Smith C & Kiely M (2004) Phylloquinone (vitamin K₁) intakes and food sources in 18–64 year old Irish adults. *Br J Nutr* **92**, 151–158.
- Esmon CT, Sadowski JA & Suttie JW (1975) A new carboxylation reaction: the vitamin K-dependent incorporation of H¹⁴ CO₃ into prothrombin. *J Biol Chem* **250**, 4744–4748.
- Fenton ST, Price RJ, Bolton-Smith C, Harrington D & Shearer MJ (1997) Nutrient sources of phylloquinone (vitamin K₁) in Scottish men and women. *Proc Nutr Soc* **56**, 301A.
- Feskanich D, Weber P, Willett WC, Rockett H, Booth SL & Colditz GA (1999) Vitamin K intake and hip fracture in women: a prospective study. *Am J Clin Nutr* **69**, 74–79.
- Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC (1998) *National Diet and Nutrition Survey: People Aged 65 Years and Over*. Report of the Diet and Nutrition Survey vol. 1. London: Stationery Office.
- French MR, Langdon C & Levy-Milne R (2001) Development of a validated food frequency questionnaire to determine folate intake. *Can J Diet Prac Res* **62**, 82–86.
- Gundberg CM, Nieman SD, Abrams S & Rosen H (1998) Vitamin K status and bone health: an analysis of methods for determination of undercarboxylated osteocalcin. *J Clin Endocrinol Metab* **83**, 3258–3266.
- Harrington KE, Robson PJ, Kiely M, Livingstone MBE, Lambe J & Gibney MJ (2001) The North/South Ireland Food Consumption Survey: survey design and methodology. *Public Health Nutr* **4**, 1037–1042.
- Herbert JR, Hurley TG, Chiriboga DE & Barone J (1998) A comparison of selected nutrient intakes derived from three diet assessment methods used in a low-fat maintenance trial. *Public Health Nutr* **1**, 207–214.
- Holland B, Welch AA, Unwin ID, Buss DH, Paul AA & Southgate DAT (1995) *McCance & Widdowson's The Composition of Foods*, 5th edn. Royal Society of Chemistry/Ministry of Agriculture, Fisheries and Food. London: HMSO.
- Institute of Medicine (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*. Washington, DC: National Academy Press.
- Jie K-SG, Bots ML, Vermeer C, Witteman JCM & Grobbee DE (1995) Vitamin K intake and osteocalcin levels in women with and without aortic atherosclerosis: a population-based study. *Atherosclerosis* **116**, 117–123.
- Jie K-SG, Bots ML, Vermeer C, Witteman JCM & Grobbee DE (1996) Vitamin K status and bone mass in women with and without atherosclerosis: a population-based study. *Calcif Tissue Int* **59**, 352–356.
- Knapen MHJ, Hamulyák K & Vermeer C (1989) The effect of vitamin K supplementation on circulating osteocalcin (bone Gla protein) and urinary calcium excretion. *Ann Intern Med* **111**, 1001–1005.
- Kohlmeier M, Salomon A, Saupé J & Shearer MJ (1996) Transport of vitamin K to bone in humans. *J Nutr* **126**, S1192–S1196.
- Lahmann PH & Kumanyika SK (1999) Attitudes about health and nutrition are more indicative of dietary quality in 50- to 75-year-old women than weight and appearance concerns. *J Am Diet Assoc* **4**, 475–478.
- McKeown NM, Jacques PF, Gundberg CM, Peterson JW, Tucker KL, Kiel DP, Wilson PW & Booth SL (2002) Dietary and nondietary determinants of vitamin K biochemical measures in men and women. *J Nutr* **132**, 1329–1334.
- Ministry of Agriculture, Fisheries and Food (1997) *Food Portion Sizes*. London: Stationery Office.
- National Research Council (1989) *Recommended Dietary Allowances*. 10th ed. Washington, D.C.: National Academy Press.
- O'Brien M, Flynn A & Kiely M (2003) Vegetable intake in the Irish population. *Public Health Nutr* **6**, 711–726.
- Plantalech L, Chapuy MC, Guillaumont M, Chapuy P, Leclercq M & Delmas DP (1990) Impaired carboxylation of serum osteocalcin in elderly women: effect of phylloquinone treatment. In *Osteoporosis*, pp. 345–347 [C Christiansen and K Overgaard, editors]. Copenhagen: Osteopress.
- Price PA (1988) Role of vitamin K dependent proteins in bone metabolism. *Ann Rev Nutr* **8**, 565–583.

- Price R, Fenton S, Shearer MJ & Bolton-Smith C (1996) Daily and seasonal variation in phylloquinone (vitamin K₁) intake in Scotland. *Proc Nutr Soc* **55**, 244A.
- Rambeck WA & Stahelin HB (2001) Emerging scientific evidence. Vitamin K and bone metabolism: effects of vitamins on behaviour and cognition. *Biblio Nutr Diet* **55**, 206–208.
- Schurgers LJ, Geleijnse JM, Grobbee DE, Pols HAP, Hofman A, Witteman JCM & Vermeer C (1999) Nutritional intake of vitamins K₁ (phylloquinone) and K₂ (menaquinone) in the Netherlands. *J Nutr Environ Med* **9**, 115–122.
- Shearer MJ (1990) Vitamin K and vitamin K dependent proteins. *Br J Haem* **75**, 156–162.
- Shearer MJ (2000) Role of vitamin K and Gla proteins in the pathophysiology of osteoporosis and vascular calcification. *Curr Opin Clin Nutr Metab Care* **3**, 433–438.
- Shearer MJ, Bach A & Kohlmeier M (1996) Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health. *J Nutr* **126**, 1181S–1186S.
- Sokoll LJ, Booth SL, O'Brien ME, Davidson KW, Tsaion KI & Sadowski JA (1997) Changes in serum osteocalcin, plasma phylloquinone and urinary γ -carboxyglutamic acid in response to altered intakes of dietary phylloquinone in human subjects. *Am J Clin Nutr* **65**, 779–784.
- Sokoll LJ & Sadowski JA (1996) Comparison of biochemical indexes for assessing vitamin K status in a healthy adult population. *Am J Clin Nutr* **63**, 566–573.
- Szulc P, Arlot M, Chapuy MC, Duboeuf F, Muenier PJ & Delmas PD (1994) Serum undercarboxylated osteocalcin correlates with hip bone mineral density in elderly women. *J Bone Miner Res* **9**, 1591–1595.
- Szulc P, Chapuy MC, Meunier PJ & Delmas PD (1993) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J Clin Invest* **91**, 1769–1774.
- Szulc P, Chapuy MC, Meunier PJ & Delmas PD (1996) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. *Bone* **18**, 487–488.
- Tapsell LC, Brenninger V & Barnard J (2000) Applying conversation analysis to foster accurate reporting in the diet history interview. *J Am Diet Assoc* **100**, 818–824.
- Thane CW, Paul AA, Bates CJ, Bolton-Smith C, Prentice A & Shearer MJ (2002) Intake and sources of phylloquinone (vitamin K₁) variation with socio-demographic and lifestyle factors in a national sample of British elderly people. *Br J Nutr* **87**, 605–613.
- Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K & Delmas PD (1997) Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. *J Clin Endocrinol Metab* **82**, 719–724.
- Vermeer C, Gijsbers BLMG, Craciun AM, Groenen-Van Dooren MMCL & Knapen MHJ (1996) Effects of vitamin K on bone mass and bone metabolism. *J Nutr* **126**, S1187–S1191.
- Vermeer C, Jie KSG & Knapen MHJ (1995) Role of vitamin K in bone metabolism. *Annu Rev Nutr* **15**, 1–22.
- Weber P (2001) Vitamin K and bone health. *Nutrition* **17**, 880–887.
- Yan L, Zhou B, Greenberg D, Wang L, Nigdikar S, Prynne C & Prentice A (2004) Vitamin K status of older individuals in the UK. *Br J Nutr* **92**, 939–945.