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Effect of phylloquinone (vitamin K_1) supplementation for 12 months on the indices of vitamin K status and bone health in adult patients with Crohn's disease

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Abstract

Although epidemiological findings support a role for vitamin K status in the improvement of bone indices in adult patients with Crohn's disease (CD), this needs to be confirmed in double-blind, randomised controlled trials (RCT) with phylloquinone (vitamin K_1). By conducting two RCT, the present study aimed to first establish whether supplementation with 1000 μg of phylloquinone daily near-maximally suppresses the percentage of undercarboxylated osteocalcin in serum (%ucOC; marker of vitamin K status) in adult patients with CD currently in remission as it does in healthy adults and second determine the effect of supplementation with phylloquinone at this dose for 12 months on the indices of bone turnover and bone mass. The initial dose-ranging RCT was conducted in adult patients with CD (*n* 10 per group) using 0 (placebo), 1000 or 2000 μg of phylloquinone daily for 2 weeks. In the main RCT, the effect of placebo *v.* 1000 μg vitamin K/d (both co-administered with Ca (500 mg/d) and vitamin D_3 (10 μg/d)) for 12 months (*n* 43 per group) on the biochemical indices of bone turnover (determined by enzyme immunoassay) and bone mass (determined by dual-energy X-ray absorptiometry) were investigated. At baseline, the mean %ucOC was 47 %, and this was suppressed upon supplementation with 1000 μg of phylloquinone daily (−81 %; $P<0{\cdot}01$) and not suppressed further by 2000 μg of phylloquinone daily. Compared with the placebo, supplementation with 1000 μg of phylloquinone daily for 12 months had no significant effect ($P>0{\cdot}1$) on bone turnover markers or on the bone mass of the lumbar spine or femur, but modestly increased ($P<0{\cdot}05$) the bone mass of the total radius. Despite near maximal suppression of serum %ucOC, supplementation with 1000 μg of phylloquinone daily (with Ca and vitamin D_3) had no effect on the indices of bone health in adult CD patients with likely vitamin K insufficiency.

Key words: Phylloquinone (vitamin K_1): Percentage of undercarboxylated osteocalcin: Bone health indices: Intervention studies: Crohn's disease

Osteopaenia and osteoporosis are common conditions among patients with Crohn's disease (CD)[1,2]. Bone loss resulting from these conditions is a major risk factor for osteoporotic fractures of the spine, wrist and hip[3], which can have a deleterious effect on the quality of life of patients with CD, especially in young patients who have a normal life expectancy. Although the pathogenesis of osteopaenia and osteoporosis in CD is likely to be multifactorial, the existence of nutritional inadequacies of Ca and vitamin D in these patients has been implicated[4–9].

The prevalence of vitamin K deficiency in patients with chronic gastrointestinal disorders has been known for some time[10]. The concentration of undercarboxylated osteocalcin (ucOC) and the percentage of osteocalcin present in an undercarboxylated state (%ucOC) are markers of diminished vitamin K nutritive status[11]. Adult patients with CD, even those in remission and not taking high-dose steroids, have been shown to have higher circulating ucOC concentrations and/or %ucOC compared with age- and sex-matched healthy control subjects[12–14]. The increased serum ucOC concentration

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BAP, bone-specific alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; CD, Crohn's disease; cOC, carboxylated osteocalcin; CTx, C-terminal telopeptide of type I collagen; IBD, inflammatory bowel disease; IQR, interquartile range; NTx, N-telopeptides of type I collagen; PTH, parathyroid hormone; ucOC, undercarboxylated osteocalcin; %ucOC, percentage of undercarboxylated osteocalcin.

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and/or %ucOC in patients with CD in these studies appeared to be positively associated with the rate of bone turnover[13,14] and inversely correlated with the bone mineral density (BMD) of the lumbar spine, but not with that of the femoral neck or total body[12]. In a multiple regression analysis, Kuwabara *et al.*[15] showed that in addition to BMI, plasma phylloquinone concentration is a significant determinant of the BMD of the lumbar spine and distal one-third radius, but not of that of the total hip and femoral neck in patients with adult inflammatory bowel disease (IBD). However, in terms of proof of causality, there is a need for data from double-blind, randomised controlled trials of phylloquinone supplementation in patients with CD to confirm these positive observational findings. This is important in terms of informing nutrition-based guidelines for the prevention of osteoporosis in IBD patients, which at present focus only on vitamin D and Ca[6,16].

If there is a causal relationship between high %ucOC and higher bone turnover and lower BMD in adult patients with CD, then suppression of %ucOC by an increased intake of phylloquinone should lead to bone health benefits in this 'at-risk' patient group. In their short-term, dose-related (ranging from 250 to 2000 μg/d) phylloquinone supplementation study, Binkley *et al.*[17] showed that a supplemental intake of 1000 μg phylloquinone/d was required to near-maximally suppress %ucOC (i.e. >70 %) in healthy subjects. However, with possible differences in the efficiency of intestinal absorption of phylloquinone in adult patients with CD[13], it is not clear whether 1000 μg of phylloquinone daily will maximally suppress %ucOC in these at-risk patients. The aims of the present study were to carry out two double-blind, randomised, placebo-controlled trials in adult patients with CD currently in remission to first establish whether phylloquinone supplementation at a dose of 1000 μg/d or at a higher dose of 2000 μg/d near-maximally suppresses ucOC and %ucOC in adult patients in CD and second investigate the effect of supplementation with the required dose of phylloquinone, in addition to Ca and vitamin D, for 12 months on bone turnover and bone mass in patients with CD.

Subjects and methods

Subjects

Patients (aged 18–70 years) visiting the Cork University Hospital IBD Clinic, Cork, Ireland, who were diagnosed with CD and were in clinical remission were randomly asked to participate in the study. There was no selection bias other than that those selected to participate in the study had to be in relatively close geographical proximity to the research centre in consideration of patient inconvenience. CD was previously diagnosed on the basis of consistent clinical findings, Ba radiology and histology. Clinical remission was defined at the time of the study as the absence of gastrointestinal symptoms and not requiring therapeutic doses of corticosteroids. In addition, disease activity was assessed according to the Harvey–Bradshaw index[18]. Of the sixty-eight patients who completed the main RCT (study B, described below), two (3 %) had a Harvey–Bradshaw index of 5 (erythrocyte sedimentation rates of 5 and 17 mm/h, respectively), whereas the remaining sixty-six (97 %) who participated in study B and all patients who participated in the short-term dose-ranging RCT (study A, described below) had inactive disease.

A total of thirty adult patients with CD (eighteen males and twelve females; mean age 45·4 (SD 12·6) years) participated in study A (a 2-week phylloquinone intervention trial). Consenting adult Caucasian men and women aged 18–70 years with long-standing (>5 years of duration) CD in remission at the time of inclusion were eligible to participate in this trial. Reasons for exclusion were severe medical illness, hypercalcaemia, known intestinal malabsorption syndrome, excessive intake of alcohol, use of medications known to interfere with vitamin D and/or vitamin K metabolism, pregnancy, diagnosis of osteoporosis before inclusion in the study, and use of bisphosphonates or vitamin/mineral supplements. As regards medication use, fifteen patients with CD were on maintenance 5-aminosalicylate (400–1200 mg/d) therapy, eight were taking 6-mercaptopurine (50 mg/d) and one was taking azathioprine (100 mg/d). Furthermore, two patients were taking infliximab either monthly or bimonthly. None of the patients was taking steroids, warfarin or methotrexate during the study. At the time of inclusion, none of the patients was taking activated forms of vitamin D (25-hydroxyvitamin D (25(OH)D) or 1,25-dihydroxyvitamin D) or calcitonin, while nine patients were regular cigarette smokers. None of the patients was taking vitamin K supplements during the study. Among the patients who participated in study A, only 20 % had small bowel involvement, 20 % had inflammation of the colon and 60 % had inflammation of both sites. Furthermore, 47 % of the patients had undergone a previous terminal ileal resection (<50 cm), and none ever had steatorrhoea or short bowel syndrome.

A total of eighty-six adult patients with CD (forty-eight males and thirty-eight females; mean age 41·3 (SD 10·2) years) participated in study B (a 12-month phylloquinone intervention trial). Of these, twenty patients had participated in study A. Inclusion criteria and reasons for exclusion were the same as those of study A, with one additional reason for exclusion, i.e. planning to become pregnant during the 12-month intervention trial. As regards medication use, twenty-nine patients with CD were on maintenance 5-aminosalicylate (500–3000 mg/d) therapy, thirty-one were taking 6-mercaptopurine (50–75 mg/d) and four were taking azathioprine (50–100 mg/d). Furthermore, three were taking sulfasalazine (1–2 g/d) and eleven were taking adalimumab (40 mg) either weekly or bimonthly, while five were taking infliximab every 4–6 weeks. None of the patients was taking steroids, warfarin or methotrexate at the time of inclusion or was taking warfarin during the study. At the time of inclusion, none of the patients was taking activated forms of vitamin D (25(OH)D or 1,25-dihydroxyvitamin D) or calcitonin, while twenty-one patients were regular cigarette smokers. None of the patients was taking vitamin K supplements during the study. Among those who participated in study B, only 31 % had small bowel involvement, 25 % had inflammation of the colon and 44 % had inflammation of both sites. Furthermore,

47 % of the patients had a previous terminal ileal resection (< 50 cm), and none ever had steatorrhoea or short bowel syndrome.

During the 12-month intervention trial (study B), thirteen (19 %) of the sixty-eight patients experienced a CD-related disease flare-up: seven were randomised to the placebo group and six were randomised to the phylloquinone treatment group. Of those who experienced a CD-related flare-up, eleven (85 %) reported only one flare-up, while two (15 %) reported more than one flare-up (two and four, respectively) during the intervention period. A range of medical interventions were used to treat flare-ups, including metronidazole (10 mg/kg), oral prednisolone (tapering weekly from 40 mg/d), budesonide (range 3–9 mg/d) and/or prednisolone foam enema (once to twice daily).

Ethical considerations

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving patients were approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Cork, Ireland (October, 2006). Written informed consent was obtained from all patients. The study was also registered at ClinicalTrails.gov (http://clinicaltrials.gov/show/NCT01235325 (ClinicalTrials.gov identifier: NCT01235325)).

Design and conduct of studies

Study A was a short-term, double-blind, placebo-controlled trial in which patients with CD (*n* 10 per group) were randomly assigned to receive 0 (placebo), 1000 or 2000 μg of supplemental phylloquinone daily for 2 weeks. The study was conducted between January and December 2007. The participants took two capsules (each containing 0 or 1000 μg of phylloquinone) daily so as to receive the three assigned doses in addition to their habitual dietary phylloquinone intake amounts. Because phylloquinone is fat soluble, the participants were instructed to take the capsules with their evening meal so as to maximise absorption. Phylloquinone (1000 μg) capsules and matching placebo capsules were produced by Banner Pharmacaps and were identical in appearance and taste. Randomisation was computer-generated and stratified for sex. The phylloquinone content of the capsules was independently confirmed by HPLC analysis. Compliance was assessed by capsule counting. The allocation remained concealed until the final analyses, and all data were reported by individuals who were blinded to the allocation scheme.

All participants were given supplemental vitamin D_3 (10 μg/d) and Ca (500 mg/d) (as Calcichew D3 Forte chewable tablets, kindly supplied by Shire Pharmaceuticals Ireland Limited) for 2 weeks before the phylloquinone intervention trial and throughout the trial, so as to correct any potential serious deficiencies of these nutrients. Each participant attended a screening session at the IBD Clinic during which a blood sample was collected for basic clinical haematology and biochemistry profiles, and anthropometric measurements were taken. Habitual intakes of Ca, vitamin D and phylloquinone were assessed using a FFQ specifically developed for Irish adults[19], which was administered by a research nutritionist. During the screening session, the participants were given the supplemental vitamin D_3 (10 μg/d) and Ca (500 mg/d) tablets and were asked to commence taking them daily. After 2 weeks, each participant returned to the IBD Clinic to provide an overnight fasting blood sample (20 ml; taken between 08.30 and 10.30 hours by a trained phlebotomist) on day 0 (pre-intervention) and again on day 14 (post-intervention). Blood was collected by venepuncture into an evacuated tube without an additive and processed to serum, which was immediately stored at −80°C until analysis.

Study B was a 12-month, double-blind, placebo-controlled trial (conducted between July 2009 and December 2010) in which patients with CD (*n* 43 patients per group) were randomly assigned to receive the placebo or 1000 μg of supplemental phylloquinone daily for 12 months in addition to their habitual dietary phylloquinone intake amounts. The supplemental dose of phylloquinone was chosen on the basis that it was found to be sufficient to near-maximally suppress the degree of under-γ-carboxylation of serum osteocalcin in study A (see the Results section). As in study A, the participants were instructed to take the capsules with their evening meal so as to maximise absorption, and all participants were given supplemental vitamin D_3 (10 μg/d) and Ca (500 mg/d) (Calcichew D3 Forte chewable tablets; Shire Pharmaceuticals Ireland Limited) throughout the intervention period, so as to correct any potentially serious deficiencies of these nutrients. Randomisation was computer-generated and stratified for sex. Compliance was assessed by capsule counting. An *a priori* decision was made to include only those participants with > 80 % compliance in compliance-based analysis to extend the intention-to-treat analysis. The allocation remained concealed until the final analyses, and all outcomes were reported by individuals who were blinded to the allocation scheme.

Each participant made a baseline visit to the IBD Clinic during which a blood sample was collected for basic clinical haematology and biochemistry profiles as well as vitamin and bone biomarker status assessment, and anthropometric measurements were taken. Habitual intakes of Ca, vitamin D and phylloquinone were assessed using a FFQ as in study A. After 6 and 12 months, each participant returned to the IBD Clinic to provide an overnight fasting blood sample (20 ml; taken between 08.30 and 10.30 hours by a trained phlebotomist). Blood was collected by venepuncture into an evacuated tube without an additive and processed to serum, which was immediately stored at −80°C until analysis. In addition, a fasting urine sample was collected, which was immediately stored at −20°C until analysis. Serum osteocalcin markers of vitamin K status and basic clinical haematology and biochemistry profiles were assessed at baseline, midpoint and endpoint. BMD and bone mineral content (BMC) as well as serum- and urine-based markers of bone turnover and serum phylloquinone (subsample only) were assessed at baseline and at endpoint.

Bone densitometry

BMD and BMC were measured by dual-energy X-ray absorptiometry (GE iDXA Lunar Phantom) at the lumbar spine (L2–L4), femur (total hip, neck, Ward's triangle and trochanter) and non-dominant radius (total, ultradistal and distal one-third). All tests were carried out according to a standard protocol on the same machine (using enCore software version 13.4) and by the same trained technician at the Cork University Hospital. The prevalence of osteopaenia and confirmation of the absence of osteoporosis were assessed according to the WHO classification in which osteopaenia is defined as a T score ranging between −1 and −2·5 and osteoporosis as a T score less than −2·5 (normal: T score greater than −1)[20].

Laboratory analysis

Serum 25-hydroxyvitamin D. The concentration of serum 25(OH)D was measured using an ELISA (OCTEIA® 25-Hydroxy Vitamin D; Immunodiagnostic Systems Limited). The intra-assay CV was 5·9 %. The quality and accuracy of serum 25(OH)D analysis carried out in the present study was ensured on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital, London, UK).

Serum intact parathyroid hormone. The concentration of serum intact parathyroid hormone (PTH) was measured using an ELISA (OCTEIA® Intact Parathyroid Hormone; Immunodiagnostic Systems Limited). The intra-assay CV was 3·4 %.

Serum carboxylated and undercarboxylated osteocalcin. The concentrations of serum carboxylated osteocalcin (cOC) and ucOC were measured using ELISA (Gla and Glu EIA Kits; Takara Biomedical Group). The intra-assay CV for ucOC and cOC were 4·4 and 4·5 %, respectively. The %ucOC was calculated by expressing serum ucOC as a percentage of total osteocalcin values (serum ucOC + serum cOC). Although a threshold of serum %ucOC defining vitamin K insufficiency has not been established yet, we have previously shown that adult patients with CD in remission had a significantly higher mean %ucOC compared with age- and sex-matched healthy control subjects (47·6 *v.* 37·0 %, respectively[13]).

Serum phylloquinone. The concentration of serum phylloquinone was measured by reversed-phase HPLC with fluorescence detection using the method described by Wang *et al.*[21]. An internal standard compound (a proprietary vitamin K derivative) (Immundiagnostik AG) was also used in this analysis. The intra-assay CV was <6 %. While serum phylloquinone concentration was determined in all patients who participated in study A, it was measured in only a subset of patients (twenty-seven of the sixty-eight) who participated in study B due to resource limitations. Unfortunately, a reference range for serum phylloquinone concentrations in the healthy adult population, aged 18–70 years, in Ireland is not currently available. For the purposes of the present study, we defined a serum phylloquinone concentration <0·4 nmol/l as 'low' on the basis of reference ranges in healthy adults, which has been previously cited as a cut-off value in clinical studies carried out in the USA[22] and the UK[23].

Serum bone-specific alkaline phosphatase. The activity of serum bone-specific alkaline phosphatase (BAP) was measured using an ELISA (Metra™ Osteocalcin EIA Kit; Quidel Corporation). The intra-assay CV was 5·0 %.

Serum C-terminal telopeptide of type I collagen. The concentration of serum C-terminal telopeptide of type I collagen (CTx) was measured using the Serum CrossLaps® ELISA Kit (Nordic Bioscience Diagnostics A/S). The intra-assay CV was 5·0 %.

Urinary N-telopeptides of type I collagen. The concentrations of urinary N-telopeptides of type I collagen (NTx) were measured using the Osteomark ELISA Kit (Unipath Limited). The concentration of urinary creatinine was measured using a quantitative, colorimetric assay from Metra (Quidel Corporation). The intra-assay CV for NTx and creatinine were 8·3 and 3·4 %, respectively. The concentration of urinary NTx is expressed relative to that of urinary creatinine.

Inter-assay variability in the above-mentioned variables was avoided by analysing all samples collected from an individual in the same run.

Statistical analysis

In their short-term, dose-related phylloquinone supplementation study in healthy young adult and older subjects, Binkley *et al.*[17] showed that a supplemental intake of 1000 μg phylloquinone/d is required to near-maximally suppress %ucOC (approximately 74 % reduction). With possible differences in the efficiency of intestinal absorption of phylloquinone in adult patients with CD[13], it was not clear whether this supplemental dose of phylloquinone would near-maximally suppress %ucOC in patients with CD (this was the main objective of study A). On the basis of the distribution of serum ucOC and %ucOC data collected from adult patients with CD from our previous study[13], a study design recruiting ten volunteers per group was found to have 90 % power to detect a ≥70 % reduction in serum ucOC concentration and %ucOC between the groups at $\alpha = 0{\cdot}5$. As the study was very acute, we assumed no dropout. On confirmation of the ability of 1000 μg of phylloquinone to induce near-maximal suppression of serum ucOC concentration and %ucOC in patients with CD in study A, we aimed to determine the effect of this dose of phylloquinone on the markers of bone turnover and bone mass in adult patients with CD after 12 months of supplementation. Using the standardised coefficients from our previous multiple regression analysis of the association between serum ucOC and urinary NTx concentrations in adult patients with CD[13], we expected a 38 % reduction in urinary NTx concentrations arising from the anticipated decrease in serum ucOC concentrations following supplementation with 1000 μg of phylloquinone daily (as observed in study A) for 12 months. On the basis of the distribution of urinary NTx data collected from adult patients with CD from our previous study[13], a study design (study B) recruiting thirty-two volunteers per group was found to have 90 % power to detect a reduction of 35 % in urinary NTx concentrations arising from a ≥70 % reduction in serum ucOC concentrations between vitamin K-supplemented and

unsupplemented groups at $\alpha = 0{\cdot}5$. We assumed a dropout rate ≤25% over the 12-month trial and thus recruited forty-three volunteers per group.

Statistical analysis of the data was conducted using SPSS for Windows software (version 18.0; SPSS, Inc.) and DataDesk for Mac OS software (version 6.0; Data Description, Inc.). Descriptive statistics (means and standard deviations, medians and interquartile ranges (IQR), where appropriate) were determined for all variables. The distributions of all variables were tested using the Kolmogorov–Smirnov test. In study A, serum ucOC, cOC, %ucOC and phylloquinone values as well as dietary Ca intake values were not normally distributed and thus were log-transformed to achieve near-normal distributions. Similarly in study B, serum phylloquinone, PTH, BAP and CTx and urinary NTx/creatinine values as well as dietary Ca, vitamin D and phylloquinone intake values were not normally distributed and thus were log-transformed to achieve near-normal distributions. The baseline characteristics of subjects in the different intervention groups in the two studies were compared separately using the χ^2 test (for the male:female ratio and smoker:non-smoker ratio) and unpaired Student's *t* test (study B) or one-factor ANOVA (study A). In study A, differences in the markers of vitamin D and K status at baseline and endpoint were assessed using one-factor ANOVA followed by Tukey's *post hoc* tests. In study B, linear models of the response in a repeated-measures analysis for differences in the markers of K status were constructed. The main effects included were dietary treatment (placebo or 1000 μg phylloquinone) and sex. The linear models also included two-way interactions between the main effects. Differences in serum PTH and 25(OH)D concentrations as well as bone measures within a group from baseline to endpoint were assessed using paired Student's *t* tests with Bonferroni adjustment, while differences in these indices between the placebo and phylloquinone treatment groups at baseline were compared using unpaired Student's *t* tests with Bonferroni adjustment. Regression models were run to adjust the BMD and BMC data for possible confounding effects of sex, age, BMI, baseline serum 25(OH)D concentration, smoking status, alcohol consumption, bowel involvement, resection, disease duration, daily Ca intake, infliximab and adalimumab use, disease flare-up during the 12-month intervention period, and medication use for disease flare-up. A *P* value $<0{\cdot}05$ was considered to be statistically significant.

Results

Baseline characteristics of subjects in study A

All subjects recruited to study A completed the trial. The mean age, weight, height, BMI, duration of CD, and habitual intakes of Ca, vitamin D and phylloquinone as well as the male:female ratio of these subjects are given in Table 1. There were no significant differences ($P>0{\cdot}1$) in mean age, weight, height, BMI, duration of CD, or mean habitual intakes of Ca, vitamin D and phylloquinone among the three intervention groups (data not shown). Similarly, there was no significant difference ($P=0{\cdot}4$) in the male:female ratio among the three intervention groups

Table 1. Baseline characteristics of adult patients with long-standing Crohn's disease (CD) in remission who completed the dose-ranging intervention trial (study A)

(Mean values and standard deviations; medians and interquartile ranges (IQR) in the case of non-normally distributed parameters)

	Mean	SD
Final (*n*)	30	
At recruitment (*n*)	30	
Males (*n*)	18	
Females (*n*)	12	
Age (years)	45·5	12·6
Height (m)	1·69	0·10
Weight (kg)	80·9	16·6
BMI (kg/m^2)	28·3	5·2
Duration of CD (years)	15·4	8·8
Dietary Ca intake (mg/d)		
Median	1272	
IQR	1038–1743	
Dietary vitamin D intake (μg/d)	5·4	2·9
Dietary phylloquinone intake (μg/d)	120	57

(data not shown). The median serum phylloquinone concentration was 0·64 (IQR 0·50–1·54) nmol/l.

Effect of phylloquinone intervention on the markers of vitamin K and D status in study A

There were no significant differences in any of the biochemical indices of vitamin D and K status among the three intervention groups at baseline (Table 2). There was good supplement adherence, as determined by capsule counting (median 100 (IQR 96–100)%), and compliance did not differ significantly ($P=0{\cdot}8$) among the three intervention groups. There was a significant ($P<0{\cdot}0001$–$0{\cdot}002$) effect of phylloquinone intervention on mean serum phylloquinone, ucOC and cOC concentrations as well as on serum %ucOC (Table 2). Compared with those in patients who were given the placebo, serum phylloquinone concentrations were significantly ($P<0{\cdot}0001$) increased in a dose-responsive manner in patients who were given 1000 or 2000 μg of phylloquinone daily for 2 weeks (Table 2). Serum cOC and ucOC concentrations were significantly ($P<0{\cdot}01$) higher and lower, respectively, in patients who were given 1000 or 2000 μg of phylloquinone daily for 2 weeks than in those who were given the placebo, with no significant difference being observed between the two phylloquinone treatment groups (Table 2). Compared with that in patients who were given the placebo, serum %ucOC was significantly ($P<0{\cdot}01$) reduced in patients who were given 1000 μg (−81·0%) or 2000 μg (−89·5%) of phylloquinone daily for 2 weeks, with no significant difference being observed between the two phylloquinone treatment groups (Table 2).

Baseline characteristics of subjects in study B

Of the eighty-six subjects recruited to study B, seventy-seven returned for the 6-month assessment session, and sixty-eight completed the intervention phase. The progress of these subjects through the trial is shown in Fig. 1. The male:female ratio and mean age, weight, height, BMI, duration of CD, and

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Table 2. Biochemical measures of vitamin D and K status among the treatment groups before and after the 2-week intervention trial (study A) in adult patients with long-standing Crohn's disease in remission

(Mean values and standard deviations; medians and interquartile ranges (IQR) in the case of non-normally distributed parameters)

	Placebo (*n* 10)		1000 μg phylloquinone/d (*n* 10)		2000 μg phylloquinone/d (*n* 10)		
Treatment groups...	Median	IQR	Median	IQR	Median	IQR	*P**
Serum 25(OH)D (nmol/l)							
Pre-intervention							0·435
Mean		81·8		66·9		76·4	
SD		25·9		19·6		30·9	
Post-intervention							0·492
Mean		85·2		70·0		80·2	
SD		32·6		20·1		31·3	
Serum ucOC (ng/ml)							
Pre-intervention	4·4	3·2–9·5	5·0	3·2–10·0	3·2	1·3–4·6	0·212
Post-intervention	4·5[a]	2·8–9·5	1·1[b]	0·7–2·1	0·7[b]	0·1–1·0	<0·0001
Serum cOC (ng/ml)							
Pre-intervention	4·6	3·4–8·9	6·9	3·5–10·7	3·1	2·3–4·4	0·630
Post-intervention	3·8[a]	2·7–7·5	16·3[b]	13·8–22·4	9·7[b]	8·3–15·1	0·002
Serum %ucOC							
Pre-intervention	48·5	34·4–71·3	41·0	23·1–65·9	46·5	36·1–56·3	0·741
Post-intervention	56·7[a]	27·2–76·6	7·8[b]	6·2–11·8	4·9[b]	0·6–6·2	<0·0001
Serum phylloquinone (nmol/l)							
Pre-intervention	0·62	0·49–0·95	0·67	0·52–0·87	0·88	0·40–2·50	0·525
Post-intervention	0·63[a]	0·54–1·05	3·42[b]	2·58–9·24	16·4[c]	8·55–25·5	<0·0001

25(OH)D, 25-hydroxyvitamin D; ucOC, undercarboxylated osteocalcin; cOC, carboxylated osteocalcin; %ucOC, percentage of undercarboxylated osteocalcin.
[a,b,c] Median values with unlike superscript letters were significantly different among the treatment groups ($P<0·001$; Tukey's *post hoc* tests).
* One-way ANOVA.

habitual intakes of Ca, vitamin D and phylloquinone of patients completing the trial, as an entire group and stratified by the treatment group, are given in Table 3. There were no significant differences in any of the baseline characteristics ($P>0·2$). The median serum phylloquinone concentration in a subset of patients (*n* 27) was 0·43 (IQR 0·25–1·16) nmol/l, and twelve of the twenty-seven patients (44 %) had serum concentrations <0·4 nmol/l[22,23]. There were no significant differences in any of the biochemical indices of vitamin K status (Fig. 2) or markers of bone turnover or serum PTH (Table 4) between the two groups at baseline. Serum 25(OH)D concentrations were significantly ($P<0·05$) lower (by 9 nmol/l, on average) in the placebo group than in the phylloquinone treatment group at baseline (Table 3).

Prior diagnosis of osteoporosis on study entry was an exclusion criterion, and its absence in all patients was confirmed by baseline DXA scan. In this study, five patients (7 %) (three in the placebo group and two in the phylloquinone treatment group) had osteopaenia; the remainder had normal BMD values at baseline. There were no significant differences in unadjusted BMD or BMC at any of the skeletal sites between the two groups at baseline, with the exception of a significantly ($P<0·05$) higher BMD of the 33 % radius in the phylloquinone treatment group than in the placebo group (Table 5).

Compliance and effect of phylloquinone intervention on the markers of vitamin K status in study B

No serious adverse events were reported during study B. A total of four participants reported adverse events due to the co-administration of the Ca/vitamin D supplement with phylloquinone or the placebo, which ranged from cramps and diarrhoea to occasional pain in legs and fingers. Of the eighteen dropouts, eleven and seven were from the placebo and 1000 μg phylloquinone/d groups, respectively. The participants dropped out for a variety of reasons (such as pregnancy, loss of interest, personal reasons, relocation, and illness unrelated to the intervention), and in no instance was dropout related to the intervention. In this study, eight subjects (four in each group) failed to exceed the minimum compliance level of 80 % set, and these were excluded from the main analysis in the compliance-based approach to extend the intention-to-treat analysis. Good supplement

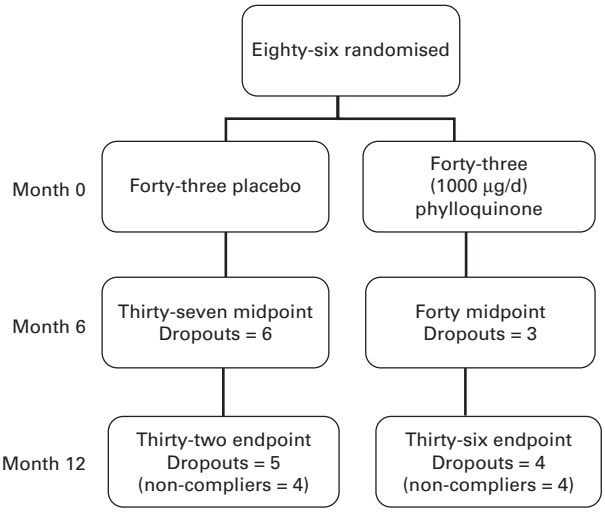

Fig. 1. Progress of the participants of study B through the trial.

Table 3. Baseline characteristics of adult patients with long-standing Crohn's disease (CD) in remission who completed the 12-month intervention trail (study B)

(Mean values and standard deviations; medians and interquartile ranges (IQR) in the case of non-normally distributed parameters)

	Entire sample		Placebo group		Phylloquinone group		
	Median	IQR	Median	IQR	Median	IQR	*P**
Final (*n*)		68		32		36	
At recruitment (*n*)		86		43		43	
n							0·330
Males		37		15		22	
Females		31		17		14	
Age (years)							0·598
Mean		41·3		40·7		42·0	
SD		10·2		10·5		10·0	
Height (m)							0·277
Mean		1·69		1·68		1·70	
SD		0·09		0·09		0·09	
Weight (kg)							0·306
Mean		75·2		73·0		77·1	
SD		16·7		17·6		15·8	
BMI (kg/m^2)	26·2	22·5–29·1	25·0	22·0–28·0	26·5	23·0–30·0	0·454
Duration of CD (years)							0·557
Mean		14·0		14·5		13·5	
SD		7·1		8·4		5·8	
Dietary Ca intake (mg/d)	1337	863–1796	1421	860–1790	1220	887–1895	0·572
Dietary vitamin D intake (μg/d)	4·6	3·0–7·4	5·2	3·3–7·4	4·5	2·8–7·3	0·833
Dietary phylloquinone intake (μg/d)	95·7	58·3–142·3	91·2	55·2–145·4	98·7	60·0–138·0	0·870

* Placebo group *v.* phylloquinone group (determined by the χ^2 or unpaired Student's *t* test).

adherence, as determined by capsule counting, was observed in the remaining subjects (overall median compliance: 93 (IQR 89–97)% and compliance was similar between the two groups; $P>0·7$).

Using a compliance-based approach, it was found that there were no significant differences at baseline in mean age, weight, height, BMI and duration of CD, male:female ratio, habitual intakes of Ca, vitamin D and phylloquinone, and any of the biochemical indices of vitamin K status or markers of bone turnover between the two groups (data not shown). Serum 25(OH)D concentrations tended ($P=0·059$) to be lower in the placebo group (*n* 28) than in the phylloquinone treatment group (*n* 32) at baseline (median 40·9 (IQR 32·6–54·6) *v.* 53·4 (IQR 40·0–74·5) nmol/l, respectively). There were no significant differences in unadjusted BMD or BMC at any of the skeletal sites between the two groups at baseline (data not shown), with the exception of a significantly ($P<0·05$) higher BMD of the 33% radius in the phylloquinone treatment group than in the placebo group (median 0·757 (IQR 0·712–0·816) *v.* 0·670 (IQR 0·620–0·806) g/cm^2, respectively).

Irrespective of using the compliance-based or intention-to-treat approach, a significant ($P<0·001$) 'time × treatment' interaction effect (in a repeated-measures ANOVA model) was observed on serum ucOC and cOC concentrations as well as on serum %ucOC over the 12-month intervention period (Fig. 2). Compared with those at baseline, mean serum cOC and ucOC concentrations at endpoint were significantly higher (76%; $P<0·001$) and lower (−80%; $P<0·001$), respectively, in patients who were given 1000 μg of phylloquinone daily, whereas there were no significant changes from baseline values in those who were given the placebo (Fig. 2). Compared with that at baseline, mean serum %ucOC at endpoint was significantly reduced (−82%; $P<0·001$) in patients who were given 1000 μg of phylloquinone daily, whereas there were no significant changes from baseline values in those who were given the placebo (Fig. 2). The response of serum ucOC, cOC and %ucOC at 12 months was evident in the phylloquinone treatment group and was of a similar magnitude at 6 months (Fig. 2). The median (IQR) serum phylloquinone concentration was significantly increased by

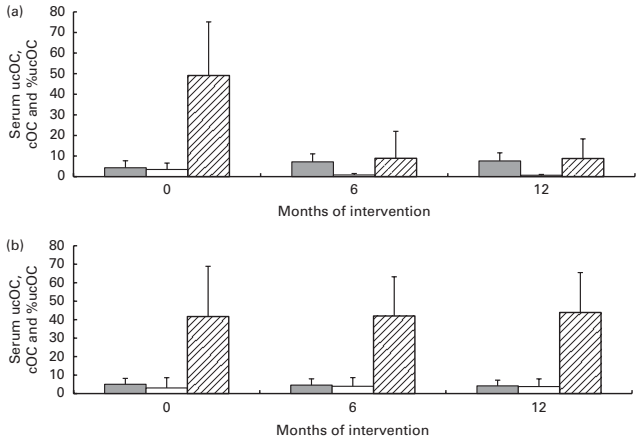

Fig. 2. Serum carboxylated osteocalcin (cOC (ng/ml), ▩) and undercarboxylated osteocalcin (ucOC (ng/ml), □) concentrations and percentage of ucOC (%ucOC, ▨) among adult patients with Crohn's disease before and after the 12-month intervention trial (study B; (a) phylloquinone; (b) placebo). There was a significant 'time × treatment' interaction in the repeated-measures ANOVA ($P<0·001$): phylloquinone intervention had a significant effect (all $P<0·001$) on serum ucOC, cOC and %ucOC values by 6 months, which was of a similar magnitude at 12 months (a), while there were no significant ($P>0·1$) changes from baseline values in those who were given the placebo (a).

Table 4. Biochemical measures of vitamin D status and bone turnover among adult patients with Crohn's disease before and after the 12-month trial (study B)

(Mean values and standard deviations; medians and interquartile ranges (IQR) in the case of non-normally distributed parameters)

	Placebo group (*n* 32)				Phylloquinone group (*n* 36)			
	Baseline		12 months		Baseline		12 months	
Biochemical indices	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Serum phylloquinone (nmol/l)‡	0·43	0·25–1·30	0·54	0·22–0·91	0·46	0·26–1·13	7·57†††	3·23–11·11
Serum 25(OH)D (nmol/l)								
Mean	48·0		59·0††		57·0*		65·3	
SD	25·4		27·2		22·0		24·0	
Serum PTH (pg/ml)	40·0	32·0–59·4	38·5	30·0–50·0	41·5	28·2–55·0	31·0†	21·5–41·3
Serum BAP (U/l)	25·4	20·1–33·5	22·5	18·5–32·0	27·0	19·0–36·0	26·1	20·0–31·3
Serum CTx (ng/ml)	0·45	0·31–0·67	0·44††	0·29–0·61	0·45	0·3–0·52	0·39††	0·25–0·49
Urinary NTx/Cr (nmol BCE/nmol)	39·0	29·0–47·3	29·2	25·0–44·0	34·2	25·5–44·0	34·0	28·5–39·0

25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; BAP, bone alkaline phosphatase; CTx, carboxy-terminal cross-linked telopeptide of type 1 collagen; NTx, cross-linked N-telopeptides of type I collagen; Cr, creatinine; BCE, bone collagen equivalents.

* Mean value was significantly different from that of the placebo group at baseline ($P<0·05$; Bonferroni-adjusted *t* test).

Value was significantly different from the baseline value within a group: † $P<0·05$; †† $P<0·01$; ††† $P<0·0001$ (Bonferroni-adjusted *t* test).

‡ Analysis performed on a subset of patients: *n* 11 for the placebo group and *n* 18 for the phylloquinone group.

phylloquinone supplementation and remained unchanged in the placebo group (Table 4).

Effect of phylloquinone intervention on the markers of bone turnover in study B

Using an intention-to-treat approach, it was found that the mean serum 25(OH)D concentration increased significantly ($P<0·0001$) or tended to increase ($P=0·072$) in the placebo and phylloquinone treatment groups, respectively (Table 3). Mean serum PTH concentrations decreased significantly ($P<0·01$) or tended to decrease ($P=0·079$) in the phylloquinone treatment and placebo groups, respectively (Table 3). Serum CTx concentrations decreased significantly ($P<0·05$) in both groups (Table 3). There was no significant change in serum BAP or urinary NTx concentrations in either group (Table 3).

On using a compliance-based approach, the same trends as in the intention-to-treat analysis were evident in relation to serum PTH, CTx, BAP and urinary NTx concentrations.

Table 5. Unadjusted bone mineral density (BMD) and bone mineral content (BMC) values of patients with Crohn's disease (*n* 68) before and after the 12-month intervention trial (study B)

(Mean values and standard deviations)

	Placebo group (*n* 32)				Phylloquinone group (*n* 36)			
	Baseline		12 months		Baseline		12 months	
Skeletal site	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Spine								
L2–L4 BMD (g/cm^2)	1·194	0·106	1·209	0·127	1·221	0·125	1·217	0·120
L2–L4 BMC (g)	53·538	10·959	55·112	11·311	57·813	9·622	57·456	9·239
Femur								
Total BMD (g/cm^2)	0·989	0·123	1·006	0·137	1·027	0·170	1·017	0·156
Total BMC (g)	33·407	6·645	34·148	7·124	35·227	6·784	35·324	6·480
Neck BMD (g/cm^2)	0·974	0·121	0·984	0·132	0·992	0·181	0·973	0·160
Neck BMC (g)	5·004	0·899	5·073	0·951	5·212	0·984	5·176	0·947
Ward's BMD (g/cm^2)	0·818	0·139	0·825	0·136	0·832	0·207	0·810	0·173
Ward's BMC (g)	2·418	0·688	2·460	0·697	2·578	0·767	2·581	0·763
Trochanter BMD (g/cm^2)	0·783	0·124	0·805	0·139	0·832	0·159	0·833	0·146
Trochanter BMC (g)	10·898	3·070	11·369	3·387	11·748	3·348	11·957	3·145
Radius								
Total BMD (g/cm^2)	0·540	0·084	0·549	0·085	0·567	0·063	0·572†	0·099
Total BMC (g)	8·609	2·424	9·001	2·478	9·405	2·113	9·610	2·102
33 % BMD (g/cm^2)	0·705	0·110	0·725	0·099	0·751*	0·071	0·752	0·072
33 % BMC (g)	1·930	0·450	1·969	0·451	2·091	0·389	2·074	0·399
Ultradistal BMD (g/cm^2)	0·378	0·095	0·372	0·012	0·386	0·062	0·389	0·060
Ultradistal BMC (g)	1·493	0·373	1·527†	0·386	1·587	0·345	1·599	0·338

L2–L4, lumbar spine.

* Mean value was significantly different from that of the placebo group at baseline ($P<0·05$; Bonferroni-adjusted *t* test).

† Mean value was significantly different from the baseline value within a group ($P<0·05$; Bonferroni-adjusted *t* tests).

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The mean serum 25(OH)D concentration increased significantly ($P \leq 0{\cdot}02$) in both the placebo and phylloquinone treatment groups (data not shown).

Effect of phylloquinone intervention on bone mineral density and bone mineral content in study B

Using an intention-to-treat approach, it was found that there was no significant change ($P > 0{\cdot}7$) in the weight or BMI of the participants over the 12-month intervention period in either the placebo or phylloquinone treatment group (data not shown). There was no significant effect of phylloquinone intervention on unadjusted BMD or BMC at any of the spine or hip skeletal sites (Table 4). The BMD of the total radius, but not of the ultradistal or 33 % radius, increased significantly ($P < 0{\cdot}05$) over the 12-month intervention period in the phylloquinone treatment group (Table 4).

Using a compliance-based approach, it was found that phylloquinone intervention had no effect on unadjusted BMD or BMC at any of the spine, hip and radius skeletal sites (data not shown). Furthermore, there was no significant increase ($P > 0{\cdot}1$) in the BMD of the total radius over the 12-months intervention period in the phylloquinone treatment group (data not shown).

Multiple regression models were run to adjust the BMD and BMC data for possible confounding effects of sex, age, BMI, baseline serum 25(OH)D concentration, smoking status, alcohol consumption, bowel involvement, resection, disease duration, daily Ca intake, infliximab and adalimumab use, disease flare-up during the 12-month intervention period, and medication use for disease fare-up, but the data trends remained the same (data not shown).

Discussion

In the present study carried out in adult patients with long-standing CD, in clinical remission on study entry and with adequate intakes of vitamin D and Ca, supplementation with 1000 μg of phylloquinone for 12 months had no significant effect on the indices of bone turnover and bone mass, with the possible exception of a modest beneficial effect on total forearm BMD. These findings were recorded despite the fact that the patients had, on average, a relatively high proportion of their serum osteocalcin in an under-γ-carboxylated state (approximately 48 %) and 44 % had a serum phylloquinone concentration $< 0{\cdot}4$ nmol/l, indicating the presence of vitamin K insufficiency in this at-risk group, who upon supplementation with phylloquinone exhibited a near-maximised γ-carboxylation status of serum osteocalcin.

It is unclear whether the lower vitamin K status in patients with CD is associated with insufficient dietary intake or with some degree of malabsorption of the vitamin, or indeed both. The group of patients with CD who participated in the 12-month intervention trial had a median habitual daily intake of phylloquinone of 95·7 μg vitamin K_1/d, with 39 % having intake values below the European Union and UK recommendation of 1 μg vitamin K_1/kg body weight per d[24,25] and 60 % with intake values below the North American sex-specific adequate intake values for vitamin K[26]. Whatever be the exact underlying reasons for the lower vitamin K status among the patients with CD, it was not surprising to find significant and rapid changes in the indices of vitamin K status upon supplementation with phylloquinone, even after 2 weeks (study A), which were of a magnitude similar to that observed after 6 and 12 months of intervention (study B). For example, the median serum phylloquinone concentration increased by about 11-fold in patients with CD who were supplemented daily with 1000 μg of phylloquinone for 12 months. In their 2-year trial in postmenopausal women, Bolton-Smith *et al.*[27] showed that the mean serum phylloquinone concentration increased by about 2-fold with 200 μg/d phylloquinone supplementation. Serum ucOC and cOC concentrations were significantly reduced and increased, respectively, and consequently serum %ucOC was dramatically reduced (> 80 %, on average), in the patients with CD on supplementation with 1000 μg of phylloquinone daily for 2 weeks, with no further significant changes being observed in these indices in the 2000 μg phylloquinone/d group. These findings in patients are in line with those reported by Binkley *et al.*[17], who in their short-term, dose-related phylloquinone supplementation study (in the range of 250–2000 μg/d) in healthy young adult and older subjects found that a supplemental intake of 1000 μg phylloquinone/d was required to near-maximally suppress %ucOC and thus optimise bone vitamin K status. While in the study carried out by Binkley *et al.*[17] phylloquinone was provided alone, in the present study, it was provided together with Ca and vitamin D, so as to ensure adequacy in all patients. In their 2-year trial in postmenopausal women, Bolton-Smith *et al.*[27] showed that %ucOC reduced from approximately 50 % to approximately 25 % with the more likely dietary attainable supplemental dose of phylloquinone (200 μg/d) and, importantly, the response was similar whether phylloquinone was given alone or with Ca and vitamin D. The suppression of serum %ucOC in patients with CD in the present study achieved after supplementation with 1000 μg of phylloquinone daily for 2 weeks proved to be of a magnitude similar to that achieved after 6 and 12 months in our year-long trial.

The circulating concentration of ucOC (or %ucOC) has been reported to be a predictor of hip fracture risk in healthy individuals[28,29]. Moreover, in observational studies of adult patients with CD, increased serum ucOC concentration and %ucOC have been shown to be positively associated with the rate of bone turnover[13,14] and inversely correlated with the BMD of the lumbar spine[12,30]. However, in the present study, despite the near-maximal suppression of %ucOC by supplementation with phylloquinone for 12 months, no significant effects were observed on urinary NTx concentrations and other biochemical markers of bone turnover or on the BMC and BMD of the spine or femur in adult patients with CD who had adequate intakes of vitamin D and Ca, but probably had vitamin K insufficiency. Intention-to-treat (but not compliance-based) analysis showed that the BMD of the total radius increased modestly, but significantly, in patients supplemented with 1000 μg of phylloquinone daily together with Ca and vitamin D for 12 months.

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The decision to determine the effect of increased phylloquinone intake, and thus greatly enhanced vitamin K status, on bone health while ensuring nutritional adequacy of Ca and vitamin D intakes in the patients with CD was made with the following in mind: dietary reference intake values for any one nutrient (e.g. phylloquinone) presuppose that requirements for energy and all other nutrients (such as Ca and vitamin D) are met; the available data from RCT in healthy adults or adults with osteopaenia seem to suggest that in those studies where a beneficial effect with phylloquinone on bone was evident[27,31,32], and some studies reported no benefit[32,33,34], it was, in general, due to phylloquinone being given with vitamin D and Ca rather than alone. For example, Bolton-Smith *et al.*[27] reported a beneficial effect of phylloquinone supplementation (200 μg/d) for 2 years on the BMD and BMC of the ultradistal radius (but not of the mid-distal radius, femoral neck or trochanter) in older healthy women but only in conjunction with vitamin D and Ca and not when provided alone and not when vitamin D and Ca were provided alone. Braam *et al.*[31] showed that reduction of the BMD of the femoral neck (but not of the lumbar spine) was significantly less in a group of postmenopausal women supplemented with phylloquinone (1000 μg/d) plus Ca, vitamin D and Zn than in the group who were given the same micronutrient mix without phylloquinone or a placebo for 3 years. Kanellakis *et al.*[32] have recently shown that low-dose phylloquinone (100 μg/d) together with vitamin D and Ca, in the form of enriched dairy products, significantly lowered a marker of bone resorption (urinary deoxypyridinoline) and increased lumbar spine BMD in postmenopausal women over 12 months compared with those who were given the same dairy products enriched with vitamin D and Ca.

The amounts of vitamin D and Ca provided in the present study were nutritional rather than therapeutic in nature. For example, 500 mg/d of Ca and 10 μg/d of vitamin D_3 were provided to ensure that the relevant adequate intake levels (1000–1200 mg/d and 5–10 μg/d, respectively) for these nutrients were met in all patients as defined at the time of the study[35], which was before the publication of the 2011 Institute of Medicine Dietary Reference Intake values for vitamin D and Ca[36]. Interestingly, even with 10 μg/d of supplemental vitamin D_3 and a median habitual intake of 4·6 μg/d, and thus close to the new RDA for vitamin D of 15 μg/d in the general healthy adult population up to 70 years[32], 32 % of the CD patients in the present study had serum 25(OH)D concentrations <50 nmol/l. Once Ca and vitamin D_3 status was ensured, no effect of phylloquinone supplementation was observed on the concentrations of urinary NTx (a marker of bone resorption) or other markers of bone turnover (serum CTx and BAP) in the adult CD patients in the present study; this was so despite reported correlations between serum ucOC and urinary NTx concentrations in previous studies[13,14]. This is the first study, to our knowledge, to determine the effect of improving vitamin K status on bone turnover in adult patients with CD. The effect of phylloquinone supplementation (with and without Ca and vitamin D) on the markers of bone turnover in RCT of healthy adults has been very mixed, with some RCT finding effects on some markers but not others[27,31,32] and effects at some time points but not others[33], while some studies found no effects at all[32].

It has been suggested that phylloquinone might have more of an influence on more metabolically active skeletal sites including those with a higher proportion of trabecular bone compared with cortical bone[27]. In the present study, several skeletal sites were examined, including those that have been reported to benefit from micronutrient intervention, but no beneficial responses were observed, except for the total radius. Even within the forearm, the ultradistal radius, which has been suggested to be more responsive to intervention effects[27], did not exhibit a beneficial response. However, it should be stressed that while some studies have reported beneficial effects of phylloquinone on bone mass indices after 1[32] or 2[27] years, some have shown that up to 3 years may be needed to detect a positive effect of phylloquinone[31,37]. However, 12 months would have been ample time for changes in bone turnover to have occurred.

Only patients with relatively good bone health were enrolled in the present study and thus the effect of phylloquinone supplementation on bone parameters in CD patients with osteoporosis still deserves attention. Another limitation of the study is the lack of reference serum phylloquinone concentration values from healthy adults to directly ascertain the degree of vitamin K insufficiency in the patients with CD. In relation to the analytical methodology used for the determination of %ucOC, it has been suggested that the enzyme immunoassay method used for determining ucOC concentrations in the present study detects large osteocalcin fragments (possibly arising from proteolysis in the circulation or produced during sample processing and storage but may also be a feature in individuals with high turnover states[38], which has been reported in patients with CD[13,14]). The %ucOC calculated in the present study expressed serum ucOC as a percentage of total osteocalcin values (i.e. serum ucOC + serum cOC), and it is not clear whether the assay for cOC likewise detects large osteocalcin fragments, in which case the impact on %ucOC would be minimised.

In conclusion, the present study showed that phylloquinone at a dose of 1000 μg is sufficient to near-maximise the γ-carboxylation status of osteocalcin in adult patients with CD with long-standing disease and in clinical remission. Although there are various lines of epidemiological evidence to support an association between high under-γ-carboxylated osteocalcin status and poor bone health outcomes in patients with CD[12–14,30], as in some studies in healthy subjects[39], the clinical relevance of maximising the γ-carboxylation status of osteocalcin to skeletal health was not evident in the 12-month randomised, double-blind, placebo-controlled intervention trial carried out in adult patients with CD in the present study.

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The authors' contributions are as follows: K. D. C. was the principle investigator responsible for the conduct of the present study; E. M. O., G. G., J. M., O. C. and A. D. contributed to the execution of the study; E. M. O. and K. D. C. contributed to the analysis of the data, interpretation of the results and writing of the article; F. S. and K. D. C. contributed to the design and execution of the study, interpretation of the data and writing of the article. All authors read and contributed to the finalisation of the manuscript.

None of the authors has any conflicts of interest to declare.

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