The Journal of Nutrition. First published ahead of print January 22, 2014 as doi: 10.3945/jn.113.188144.

The Journal of Nutrition
Nutrition and Disease

Supplementation with Calcium and Short-Chain Fructo-Oligosaccharides Affects Markers of Bone Turnover but Not Bone Mineral Density in Postmenopausal Women[1,2]

Mary M. Slevin, Philip J. Allsopp, Pamela J. Magee, Maxine P. Bonham,[3] Violetta R. Naughton, J. J. Strain, Maresa E. Duffy, Julie M. Wallace, and Emeir M. Mc Sorley*

Northern Ireland Centre for Food and Health, School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland

Abstract

This 24-mo randomized, double-blind, controlled trial aimed to examine whether supplementation with a natural marine-derived multi-mineral supplement rich in calcium (Ca) taken alone and in conjunction with short-chain fructo-oligosaccharide (scFOSs) has a beneficial effect on bone mineral density (BMD) and bone turnover markers (BTMs) in postmenopausal women. A total of 300 non-osteoporotic postmenopausal women were randomly assigned to daily supplements of 800 mg of Ca, 800 mg of Ca with 3.6 g of scFOS (CaFOS), or 9 g of maltodextrin. BMD was measured before and after intervention along with BTMs, which were also measured at 12 mo. Intention-to-treat ANCOVA identified that the change in BMD in the Ca and CaFOS groups did not differ from that in the maltodextrin group. Secondary analysis of changes to BTMs over time identified a greater decline in osteocalcin and C-telopeptide of type I collagen (CTX) in the Ca group compared with the maltodextrin group at 12 mo. A greater decline in CTX was observed at 12 mo and a greater decline in osteocalcin was observed at 24 mo in the CaFOS group compared with the maltodextrin group. In exploratory subanalyses of each treatment group against the maltodextrin group, women classified with osteopenia and taking CaFOS had a smaller decline in total-body ($P = 0.03$) and spinal ($P = 0.03$) BMD compared with the maltodextrin group, although this effect was restricted to those with higher total-body and mean spinal BMD at baseline, respectively. Although the change in BMD observed did not differ between the groups, the greater decline in BTMs in the Ca and CaFOS groups compared with the maltodextrin group suggests a more favorable bone health profile after supplementation with Ca and CaFOS. Supplementation with CaFOS slowed the rate of total-body and spinal bone loss in postmenopausal women with osteopenia—an effect that warrants additional investigation. This trial was registered at www.controlled-trials.com as ISRCTN63118444. J. Nutr. doi: 10.3945/jn.113.188144.

Introduction

Osteoporosis is a chronic disease characterized by low bone mineral density (BMD)[4] resulting in weaker bones and a predisposition to increased risk of skeletal fracture (1). Calcium (Ca) is one of the primary minerals required in the pre-adulthood achievement of peak bone mass and in the prevention of bone loss associated with aging (2). Postmenopausal women have a higher risk of developing osteoporosis because of declining estrogen concentrations associated with menopause (3). Furthermore, menopause is associated with a decreased intestinal Ca absorption, an increase in renal Ca excretion, and overall reduced Ca retention (4). These changes can lead to a negative Ca balance, which is maintained at the expense of bone resorption, and resultant bone mineral loss may occur (5).

It has been reported that dietary intakes of Ca-rich food decreases with age (6). Furthermore, dietary intakes of Ca are often inadequate in postmenopausal women. Intakes are commonly reported to be well below the current U.S. RDA of 1200 mg/d (7) and the U.K. recommended nutrient intake of 700 mg/d (8). The effectiveness of Ca supplementation for the prevention of bone loss or reduction in fracture risk within the general population remains uncertain because of, among other things, limitations in the design of existing studies (9). Evidence

[1] Supported by Marigot (Cork, Ireland) and Corn Products International (Westchester, IL).
[2] Author disclosures: M. M. Slevin, P. J. Allsopp, P. J. Magee, M. P. Bonham, V. R. Naughton, J. J. Strain, M. E. Duffy, J. M. Wallace, E. M. Mc Sorley, no conflicts of interest.
[3] Present address: Department of Nutrition and Dietetics, Monash University, Faculty of Medicine, Nursing, and Health Sciences, 264 Ferntree Gully, Notting Hill, VIC 3168, Australia.
[4] Abbreviations used: BMD, bone mineral density; BTM, bone turnover marker; Ca, calcium; CaFOS, Aquamin calcium plus NutraFlora short chain fructo-oligosaccharide; CTX, C-telopeptide of type I collagen; DPD, deoxypyridinoline; PTH, parathyroid hormone; scFOS, short-chain fructo-oligosaccharide; 25(OH)D, 25-hydroxyvitamin D.
* To whom correspondence should be addressed. E-mail: em.mcsorley@ulster.ac.uk.

Manuscript received November 12, 2013. Initial review completed November 23, 2013. Revision accepted December 18, 2013.
doi: 10.3945/jn.113.188144.

of the effects of Ca supplementation on bone density and fracture risk in vulnerable groups such as postmenopausal women is conflicting with some reporting beneficial effects (10–12), whereas others report no benefit (13,14). Ca absorption primarily occurs in the small intestine, with the colon having a lesser role in the uptake of Ca (14). However, in optimizing Ca absorption, it is becoming apparent that consideration must be given to nutritional cofactors, such as prebiotic fiber, that affect the intestinal absorption of Ca in both the small and large intestine (15). Ensuring optimal dietary Ca intake in postmenopausal women, along with maximizing its intestinal absorption, may have an important cost-effective role in osteoporosis prevention.

Dietary fibers, such as the fructan-type prebiotic inulin and oligofructose, are natural constituents of many foods (16) and are not digested in the small intestine but reach the colon fully intact, where they are selectively fermented. Several animal studies investigated the influence of prebiotics, including short-chain fructo-oligosaccharide (scFOS), on Ca absorption as well as the consequential effect on indices of bone health and strength (17,18). A number of mineral bioavailability studies have identified the potential enhancing effects of prebiotics on intestinal Ca absorption (19–21). A limited number of human intervention studies have been performed, with the majority investigating the effects of prebiotic fiber, including scFOS, on Ca absorption in adolescents (21–23) and postmenopausal women (24–28). However, to date, few studies have investigated the effect of prebiotic fiber on markers of bone health in postmenopausal women (26–28), and only 1 study in 9- to 13-y-old children has investigated the effect on BMD (29). Therefore, we hypothesized that Ca status could be improved by supplementing the diet with scFOS with consequential beneficial effects on markers of bone health in vulnerable groups, such as postmenopausal women.

The aim of this study was to investigate whether long-term, 24-mo supplementation with a natural marine-derived multi-mineral supplement rich in Ca, taken alone or in conjunction with scFOS, would favorably influence changes in BMD and the biochemical indices of bone metabolism [bone turnover markers (BTMs)] in apparently healthy postmenopausal women compared with maltodextrin supplementation.

Methods

Recruitment, first screening. The double-blind, randomized, controlled trial commenced in August 2008 (registered at www.controlled-trials.com as ISRCTN63118444) after the receipt of ethical approval from the Research Ethics Committee of the University of Ulster (REC/08/0083). Postmenopausal women from across the province of Ulster were invited to participate in the study through information leaflets and posters distributed in public places, media appeals, senior citizens groups, and active retirement groups. A total of 693 women who volunteered to participate were screened by telephone using a short questionnaire that gathered information on their medical history, medication, and dietary supplement usage. Women were excluded based on the following: *1)* being premenopausal or perimenopausal; *2)* having a previous diagnosis of osteoporosis; *3)* using medications (hormone replacement therapy, corticosteroids) or dietary supplements (Ca, vitamin D, cod liver oil) known to affect bone metabolism in the 6 mo before measurement of BMD; *4)* having been diagnosed with a bone-degenerative chronic disease (impaired hepatic or renal function, cancer, heart disease, diabetes, celiac disease, hypoparathyroidism, or hyperparathyroidism); and *5)* having experienced menopause before age 40 y (**Fig. 1**).

Second screening. From October 2008 to June 2009, a total of 372 apparently healthy, free-living postmenopausal women aged between 45 and 75 y who met the inclusion criteria after the first screening attended the study center for the second screening, which was focused on determining the individual's BMD (Fig. 1). An additional screening questionnaire was used to gather information relating to risk factors for osteoporosis. The questionnaire collected information on the following: *1)* menstrual history; *2)* parity history; *3)* hormone replacement therapy use; *4)* oral contraceptive use; *5)* operations; *6)* family history of osteoporosis and fragility fractures; *7)* personal history of osteoporosis and fragility fractures; *8)* vitamin supplement use; *9)* smoking status and alcohol use; *10)* syndromes/diseases diagnosed; and *11)* medication use. Participants provided written informed consent according to the Declaration of Helsinki (30).

All participants underwent a DXA measurement using a Prodigy DXA system (Lunar). Those women with an estimated BMD *T*-score lower than −2.5 (osteoporotic) were excluded from taking any additional part in the study. Participants were also excluded if abnormal values were found after an assessment of their hematologic profile and serum concentrations of Ca, phosphorus, alkaline phosphatase, and creatinine. After the second screening, a total of 300 eligible postmenopausal women proceeded to the intervention phase of the study. A total of 6, 34, 74, 59, and 127 women commenced the study in February, March, April, May, and June 2009 and completed the study 24 mo ± 2 wk later (2011).

Randomization and intervention. The 300 participants were randomly assigned to 1 of 3 treatment groups with a total of 100 individuals in each group. Random assignment of participants was performed by an independent researcher within the University of Ulster using a computer-generated code. Three visually identical supplements were formulated as chocolate-flavored chewable supplements and supplied (Primrose Candy) blinded to the University of Ulster. Participants were required to consume 2 of the supplements per day (13 g) for 24 mo. The supplements supplied contained the following: *1)* 800 mg/d Ca (Aquamin) (Ca group); *2)* 800 mg/d Ca (Aquamin) and 3.6 g/d scFOS (NutraFlora) (CaFOS group); or *3)* 9.8 g/d maltodextrin.

NutraFlora prebiotic fiber is an scFOS derived from beet or cane sugar that consists of low-molecular-weight linear chains synthesized by enzymatic fermentation from sucrose. The nomenclature for scFOS chains comprises 44% 1-kestose (GF2), 46% nystose (GF3), and 10% 1F-β-fructofuranosyl nystose (GF4). Bonds between the scFOS monomers are not hydrolyzed between the mouth and the small intestine. A total of 3.6 g/d scFOS was deemed acceptable for long-term consumption, minimizing adverse effects such as flatulence and loose stool associated with increased prebiotic fiber in the diet (31). Aquamin is derived from the Icelandic farmed red marine algae *Lithothamnion* species and is a natural multi-mineral supplement, as described by Frestedt et al. (32). All supplements were balanced for total protein, total fat, vitamins A, E, and C, and energy content, and supplements for the Ca and CaFOS groups were further balanced for phosphorus and trace elements. None of the supplements contained vitamin D. In an attempt to maximize compliance, participants were provided with supplements every 6 mo with each 4-wk supply separated into bags, and participants were asked to return any unused supplements that were recorded by the researchers. Participants were asked about their compliance with supplement taking in a short questionnaire at their 12-mo and 24-mo appointments. Days during the 24 mo in which the participants were not compliant were added up, and the percentage compliance for the total study (24 mo) was calculated for each participant. Participants were considered compliant when they took ≥75% of the supplement supplied. After overnight fasts, early morning blood samples were collected at baseline and 12 and 24 mo, and non-fasting blood samples were collected at the 6-mo time point. Second-morning void urine samples were collected at baseline and the 12- and 24-mo time points. All serum, plasma, and urine samples were aliquoted and stored at −80°C until assayed.

Anthropometric and dietary assessments. Trained personnel obtained height (meters) and weight (kilograms) measurements, along with waist circumference (centimeters) using standardized methodology (33). BMI was calculated as [weight (kilograms)/height^2 (meters^2)]. Dietary intake was assessed in each participant by means of a prospective 4-d semiquantitative food diary at baseline and analyzed as described previously (34). A validated FFQ (35) was administered at baseline to estimate habitual dietary vitamin D and Ca intake.

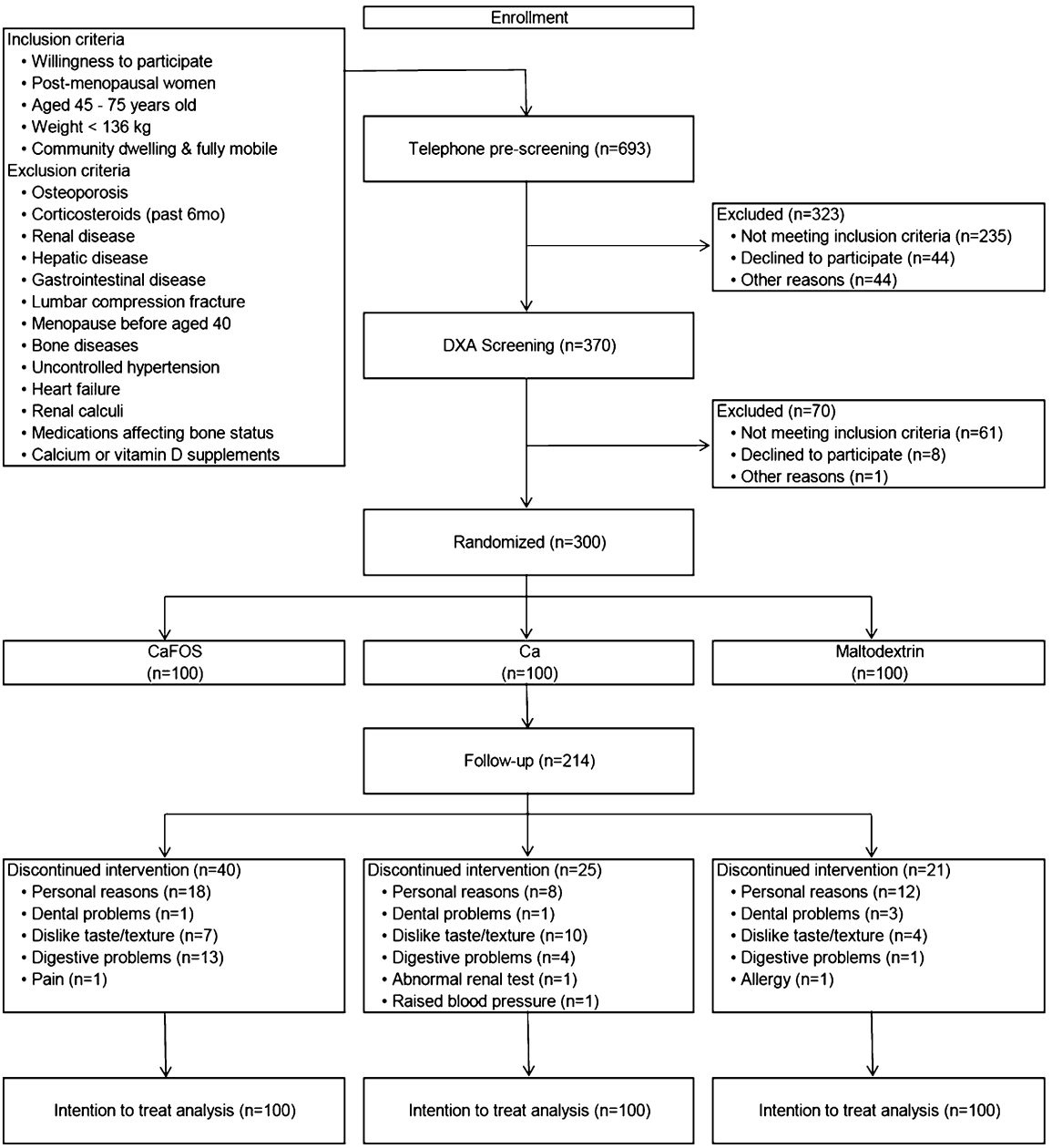

FIGURE 1 Study design: screening, randomization, and follow-up for study participants. Ca, Aquamin calcium; CaFos, Aquamin calcium plus NutraFlora short chain fructo-oligosaccharide.

Physical activity assessment. Physical activity was estimated using the short form of the International Physical Activity Questionnaire (36). Physical activity in metabolic equivalent hours per week was calculated from the participants based on a summation of their frequency and duration of low intensity activity (i.e., home activities), work-based activity, and recreational physical activity.

BMD measurements. BMD (grams per square centimeters) was measured for total body, in lumbar vertebrae 1–4 and left proximal femur by DXA (GE Medical Systems, Madison, WI) at baseline and 24 mo. To minimize participant exposure to radiation, BMD was not measured at 12 mo. In the proximal femur, 3 sites were measured: *1)* the femoral neck at the transcervical position; *2)* the greater trochanter; and *3)* Ward's triangle within the femoral neck. According to the criteria set by the WHO (37), osteoporosis is present when the *T*-score is minus ≥2.5 SDs away from the average BMD for age- and sex-matched controls. Osteopenia is diagnosed when the *T*-score is between −1.0 and −2.5 SDs below the young adult reference mean (37). A *T*-score above −1.0 is considered in the normal range. A daily quality-assurance check was performed using an aluminum phantom provided by the manufacturer. The scans were performed in the morning by the same experienced researcher, who was unaware of the treatment implemented. The CVs estimated for total-body, lumbar spine, and femur BMD were ~0.93%, 0.83%, and 0.99%, respectively. Reported BMD is expressed to 3 decimal places and *T*-scores to 1 decimal place according to the Writing Group for the International Society for Clinical Densitometry Position Development Conference (38).

Biochemical analyses. Serum osteocalcin, serum, and urine C-telopeptide of type I collagen (CTX), urinary deoxypyridinoline (DPD), plasma intact parathyroid hormone (PTH), and 25-hydroxyvitamin D [25(OH)D] were measured at baseline and 12 and 24 mo. Serum osteocalcin was measured using an ELISA (Immunodiagnostics Systems). The intra-assay CV was 1.8%. Serum concentrations of CTX were measured using an ELISA (Serum CrossLaps ELISA; Immunodiagnostics Systems). The intra-assay CV was 1.7%. Urine concentrations of CTX were measured using an

ELISA (Urine CrossLaps ELISA; Immunodiagnostics Systems). The intra-assay CV was 4.7%. Urinary DPD was measured using an ELISA (Urine DPD EIA, Metra osteocalcin EIA kit; Quidel). The intra-assay CV was 4.3%. PTH concentrations were measured using the ARCHITECT i1000SR integrated system (Abbott Diagnostics) at the Biochemistry Laboratory, Altnagelvin Area Hospital (Derry/Londonderry, Northern Ireland). The intra-assay CV ranged from 4.1% to 8.7%. Serum 25(OH)D was measured using an ELISA (25-hydroxy vitamin D; Immunodiagnostics Systems). The intra-assay CV was 5.6%. The quality and accuracy of serum 25(OH)D analysis in our laboratory is ensured on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme as described previously (39). Total Ca and albumin concentrations in serum were measured at baseline and 6, 12, and 24 mo by the Roche modular analyzer. Serum Ca concentrations were adjusted for albumin concentration. The intra-assay CV for serum Ca and albumin were 1.6% and 1.7%, respectively. Urinary creatinine was measured at baseline and 12 and 24 mo using the ILAB 600 chemistry systems analyzer (Instrument Laboratories, Warrington, UK). The intra-assay CV was 1.1%. All urinary parameters were corrected to creatinine excretion. To control for possible side effects of the supplements, liver function and electrolytes were measured at baseline and 6, 12, and 24 mo using the Roche modular analyzer (Biochemistry Laboratory, Antrim Area Hospital, Antrim, Northern Ireland). Batch analysis at the end of the intervention study was performed to reduce inter-assay variability.

Statistical analyses. A priori power calculations to determine the required minimum sample size for the current study were conducted based on a previous study (40) and on *F* test ANCOVA analysis: *1)* α, the probability of type I error (fixed as 0.05); *2)* statistical power, 90%; *3)* effect size, 0.89% difference in percentage change of total-body BMD between treatment and control; and *4)* SE for the outcome in the control group of 1.30%. Power estimation was calculated using the PS program (41). The minimum sample size required for detecting such an effect of the intervention on the rate of change in total-body BMD was 46 for each group. A total of 300 study participants were recruited to the study to maximize the potential of having 46 participants in each treatment group at the end of the 24-mo intervention. Adverse side effects were investigated by comparing between groups the number of participants with out-of-range values for liver function and electrolytes at 6, 12, and 24 mo using χ^2 analysis. The primary outcome of the study was change in BMD in grams per square centimeters. Secondary outcomes were changes in BTMs. Exploratory subgroup analysis was performed on those women classified as osteopenic when they commenced the study.

Statistical analyses of the data were conducted on an intention-to-treat last-observation-carried-forward basis population according to their original randomized assignment using SPSS for Windows (version 19.0; SPSS). Residuals were tested for normality. To ensure the validity of the randomization, differences in baseline characteristics among the 3 supplementation groups were analyzed using 1-factor ANOVA for numerical data or χ^2 analysis for categorical data, and descriptive statistics (means ± SDs) were determined for all variables. ANCOVA (with baseline scores as covariates) was used to assess change over time (time × treatment interaction effects) between groups in the primary outcome, BMD (grams per square centimeters), controlling for age, BMI, and those covariates (physical activity and alcohol use) that differed between groups at baseline, using least significant difference for post hoc comparisons. Analysis of the secondary outcomes, BTMs, was performed using repeated-measures ANOVA to evaluate the change over time (time × treatment interactions effects) after controlling for age, BMI, and those covariates that differed between groups at baseline, using least significant difference for post hoc comparisons. In the exploratory subgroup analyses of participants with osteopenia, there were significant interactions between baseline BMD and supplement groups. Therefore, between-group effects were estimated for 3 representative values at baseline in each of these subgroups: the mean and the observed extremes. Partial correlation analysis was performed to determine the relation between PTH and vitamin D status, controlling for age, BMI, physical activity, and alcohol use. Data are reported as means ± SDs with statistical significance set at $P < 0.05$.

Results

Participant characteristics. Baseline participant characteristics are described in **Table 1**. Overall, randomization was successfully achieved because there were no significant differences between the 3 intervention groups at baseline apart from physical activity (metabolic equivalent hours per week) ($P = 0.04$) and alcohol use ($P = 0.03$), with both parameters being lower in the CaFOS group. There was no significant difference between any group at baseline in the incidence of reported conditions or on reported medication usage (data not shown). Details of dropouts are outlined in Figure 1 with a higher incidence of discontinuation as a result of reported digestive problems in the CaFOS group ($P = 0.01$). A total of 79, 75, and 60 participants completed 24 mo within the maltodextrin, Ca, and CaFOS treatment groups, respectively. Compliance was determined for those who completed the study. Compliance (≥75%) was seen in 76, 73, and 55 of the participants within the maltodextrin, Ca, and CaFOS groups, respectively, with no significant difference in the compliance between the groups of 89.9 ± 1.0%, 88.7 ± 0.9%, and 86.4 ± 1.4%, respectively. We observed no adverse effect from the supplements on liver function or electrolytes, with no significant difference between the number of participants in each group with values outside the reference range at 6, 12, or 24 mo. BMD was not significantly different between the groups at baseline with an osteopenic incidence of 57%, 63%, and 54% in the maltodextrin, Ca, and CaFOS groups, respectively. A high degree of bone turnover was apparent at baseline in all groups with mean bone resorption concentrations in the upper range of the reference values and serum osteocalcin higher than the reference value for premenopausal women. Overall, mean serum 25(OH)D concentrations were adequate at >50 nmol/L in all groups at baseline, and there was no significant change over time or treatment interaction observed for 25(OH)D. None of the 3 groups displayed hyperparathyroidism at baseline, with a mean plasma PTH concentration of <65 ng/mL (42). After adjustment for age, BMI, alcohol use, and physical activity, a significant negative association was observed between plasma PTH and 25(OH)D within the maltodextrin group at baseline ($r = -0.22$; $P = 0.03$), 12 mo ($r = -0.28$; $P < 0.01$), and 24 mo ($r = -0.25$; $P = 0.01$).

BMD. After the intervention, the change in BMD over time at any site after supplementation with Ca or CaFOS did not differ from the change over time in the maltodextrin group (**Table 2**). A smaller decline over time in total-body BMD was observed in the CaFOS group compared with the Ca group ($P = 0.03$).

BTMs. A greater decline in both urinary and serum CTX was observed in the Ca group compared with the maltodextrin group after 12 mo ($P = 0.06$ and $P = 0.04$, respectively), with no significant change over time observed after 24 mo (**Table 3**). A greater decline in serum osteocalcin was observed in the Ca group compared with the maltodextrin group after 12 mo ($P = 0.04$), with no significant change over time observed after 24 mo. There was a greater decline in serum CTX in the CaFOS group compared with the maltodextrin group after 12-mo supplementation ($P = 0.03$), with no significant change over time observed after 24 mo. There was a greater decline in serum osteocalcin observed in the CaFOS group compared with the maltodextrin group after 24 mo ($P = 0.02$). There was no significant change in DPD over time between the groups.

Exploratory subgroup analysis. In the subanalysis of women with osteopenia, the decline over time in total-body BMD was significantly less in those taking CaFOS compared with the

TABLE 1 Baseline characteristics of study participants within the treatment groups Aquamin (Ca), NutraFlora (CaFOS), and MD[1]

	Ca (n = 100)	CaFOS (n = 100)	MD (n = 100)	P^2
Age, *y*	61.3 ± 6.6	61.3 ± 6.4	60.4 ± 6.3	0.53
Age at menarche, *y*	12.9 ± 1.5	13.1 ± 1.5	13.1 ± 1.5	0.45
Age of menopause, *y*	48.2 ± 5.0	49.4 ± 4.8	47.8 ± 5.6	0.06
Surgical menopause, *n*	25	15	19	0.20
Years since menopause	13.1 ± 8.1	12.0 ± 7.2	12.6 ± 7.5	0.67
Height, *m*	1.6 ± 0.1	1.61 ± 0.1	1.61 ± 0.1	0.84
Weight, *kg*	69.6 ± 11.8	72.3 ± 13.5	70.2 ± 12.9	0.31
Waist circumference, *cm*	86.7 ± 11.3	89.5 ± 13.6	87.7 ± 12.3	0.28
BMI, kg/m^2	26.8 ± 4.3	28.0 ± 5.2	27.3 ± 4.8	0.25
Family osteoporosis history, *%*	24	17	26	0.28
Previous hormone replacement therapy use, *%*	39	39	28	0.17
Alcohol use, *%*	65	55	73	0.03
Smoking, *%*				0.78
Never smoked	67	63	61	
Past smoker	28	28	30	
Current smoker	5	9	9	
T-Score				
Lumbar vertebrae 1–4	−0.8 ± 1.2	−0.4 ± 1.4	−0.7 ± 1.1	0.11
Femur	−0.8 ± 0.9	−0.7 ± 1.0	−0.7 ± 0.9	0.59
Total body	0.1 ± 1.0	0.2 ± 1.2	0.1 ± 1.1	0.51
Daily total calcium intake from FFQ, *g*	0.9 ± 0.4	0.9 ± 0.4	0.9 ± 0.4	0.68
Daily vitamin D intake from FFQ, *μg*	4.9 ± 2.6	4.6 ± 2.8	5.1 ± 2.8	0.24
Daily fiber intake from FD[3], *g*	17.2 ± 5.7	18.8 ± 6.4	18.2 ± 5.9	0.17
Estimated osteopenia, *%*	63	54	57	0.42
Physical activity, *MET h/wk*	1.9 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	0.04
Urine DPD, *nmol/mmol Cr*	8.2 ± 2.5	8.2 ± 2.4	7.9 ± 2.0	0.56
Urine CTX, *mg/mmol Cr*	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.16
Serum CTX, *μg/L*	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.32
Serum osteocalcin, *μg/L*	20.6 ± 7.8	18.9 ± 6.7	19.6 ± 8.3	0.35
Serum vitamin D, *nmol/L*	56.2 ± 19.6	55.8 ± 17.7	53.4 ± 17.8	0.47
Insufficient (<50 nmol/L) vitamin D, *%*	40.2 ± 6.0	40.1 ± 6.7	38.3 ± 7.4	0.34
Serum total calcium, *mmol/L*	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	0.87
Plasma PTH, *ng/mL*	61.1 ± 25.5	58.4 ± 18.6	63.1 ± 31.1	0.88

[1] Values are means ± SDs or percentages. Ca, Aquamin calcium; CaFOS, Aquamin calcium plus NutraFlora short chain fructo-oligosaccharide; Cr, creatinine; CTX, C-telopeptide of type I collagen; DPD, deoxypyridinoline; FD, food diary; MD, maltodextrin; MET, metabolic equivalent task; PTH, parathyroid hormone.
[2] P values based on ANOVA (χ^2 analysis used for surgical menopause, BMI status, family osteoporosis history, previous hormone replacement therapy use, smoking, alcohol use, and estimated osteopenia).
[3] Ca, n = 96; CaFOS, n = 97; MD, n = 98.

maltodextrin group. After controlling for baseline interactions between BMD and treatment group, this effect was apparent only in those with a higher total-body BMD at baseline (P = 0.03) (Table 2). The change in spinal BMD was significantly smaller in the CaFOS group compared with Ca (P = 0.02) and maltodextrin (P = 0.03) after 24-mo intervention. After controlling for baseline interactions between BMD and treatment group, this effect was apparent only for those with mean baseline spinal BMD but not at the lower or higher values. Furthermore, there was a greater decline in urine CTX in the Ca group compared with the maltodextrin group after 12- and 24-mo supplementation (P = 0.04 and P = 0.04, respectively).

Discussion

In this randomized, controlled study of postmenopausal women, supplementation with Ca or CaFOS did not significantly alter the rate of bone loss at any site based on intention-to-treat analysis compared with the maltodextrin-supplemented group after 24-mo intervention. Nevertheless, those supplemented with CaFOS had significantly less decline in total-body BMD at 24 mo compared with the Ca group, which is the appropriate control for testing an scFOS intervention. Secondary analysis of BTMs revealed a greater reduction in markers of bone resorption and formation in the Ca group compared with the maltodextrin group at 12 mo; effects that suggest enhanced Ca absorption and a resultant reduction in bone turnover in the first year. Furthermore, a greater reduction in the marker of bone resorption CTX in the CaFOS group compared with the maltodextrin group at 12 mo, together with a greater reduction in the bone formation marker osteocalcin at 24 mo compared with the maltodextrin group, would suggest that less bone turnover is occurring and a more positive Ca balance is evident in the CaFOS treatment group and that it is maintained longer than with Ca supplementation alone. Osteocalcin is the only BTM to be significantly lower in the CaFOS group compared with the maltodextrin group at 24 mo. It may be the case that formation is reduced as the resorption markers are reaching a state of homeostasis in the presence of Ca balance; however, this interpretation is speculative and requires additional investigation.

TABLE 2 Bone mineral density at baseline and after 24-mo supplementation with Aquamin (Ca), NutraFlora (CaFOS), or MD in postmenopausal women[1]

	Ca			CaFOS			MD		
	Baseline	24 mo	Change	Baseline	24 mo	Change	Baseline	24 mo	Change
	g/cm²	*g/cm²*	%	*g/cm²*	*g/cm²*	%	*g/cm²*	*g/cm²*	%
Primary analysis[2]									
Lumbar vertebrae 1–4	1.078 ± 0.141	1.061 ± 0.142	−1.7 ± 3.2	1.121 ± 0.170	1.115 ± 0.171	−1.4 ± 3.1	1.096 ± 0.130	1.082 ± 0.137	−1.7 ± 3.5
Femural	0.885 ± 0.106	0.877 ± 0.106	−1.6 ± 2.1	0.903 ± 0.115	0.897 ± 0.116	−1.5 ± 1.8	0.897 ± 0.112	0.887 ± 0.111	−1.8 ± 1.9
Total body	1.126 ± 0.077	1.119 ± 0.075	−0.7 ± 1.8[a]	1.142 ± 0.093	1.141 ± 0.095	−0.4 ± 1.7[b]	1.130 (0.086)	1.127 ± 0.085	−0.4 ± 1.6[a,b]
Exploratory analysis[3]									
Lumbar vertebrae 1–4	1.003 ± 0.080	0.988 ± 0.083	−1.7 ± 3.2[a]	1.035 ± 0.120	1.033 ± 0.129	−0.9 ± 2.9[b]	1.017 ± 0.069	1.000 ± 0.076	−1.9 ± 3.1[a]
Femural	0.829 ± 0.072	0.822 ± 0.074	−1.4 ± 2.2	0.827 ± 0.062	0.822 ± 0.061	−1.5 ± 1.8	0.832 ± 0.068	0.823 ± 0.072	−1.8 ± 2.0
Total body	1.088 ± 0.054	1.084 ± 0.055	−0.5 ± 1.5[a,b]	1.087 ± 0.055	1.084 ± 0.057	−0.5 ± 1.5[b]	1.087 ± 0.066	1.081 ± 0.063	−0.6 ± 1.6[a]

[1] Values are means ± SDs. Mean values within a row with different superscript letters were significantly different using ANCOVA (least significant difference post hoc comparisons), $P < 0.05$. Ca, Aquamin calcium; CaFOS, Aquamin calcium plus NutraFlora short chain fructo-oligosaccharide; MD, maltodextrin.
[2] Intention-to-treat analysis, $n = 100$ in each group.
[3] Exploratory analysis on those with osteopenia at baseline: $n = 63$, MD; $n = 54$, Ca; and $n = 57$, CaFOS.

Therefore, although no significant difference in BMD was observed between the 2 treatment groups and the MD group, the BTM data would suggest that both Ca and CaFOS supplementation are favorably affecting bone health in these women.

To the authors' knowledge, this is the first study to investigate the long-term effect of scFOS on BMD in postmenopausal women. The only other long-term study of prebiotic fiber (inulin-type fructan group) was performed in adolescents, and showed a beneficial effect on total-body BMD after 12 mo (29). In the only previous study to investigate the effects of supplementation with chicory fiber (for 3 mo) on pre-BMD and post-BMD in postmenopausal women, no beneficial effect was reported (27), although a longer supplementation period would be required to investigate changes in BMD. We hypothesize that the positive effect of scFOS on BTMs is attributable to the enhancement of Ca absorption in the large intestine as reported previously (29), and therefore the coadmission of Ca along with the scFOS may have resulted in the enhanced benefit observed here over and above Ca alone. Although total-body BMD is not used in a clinical setting, a reduction in loss of total-body BMD is useful because it encompasses both cortical and trabecular bone (43) and best reflects total-body bone mineral content and bone area (44), providing useful information for public health. The dose of scFOS used in the current study is lower than that of previous studies of prebiotic fiber on Ca absorption, with most observing effects at 8 g/d or higher (24–29). A higher dose may be required to see an effect on BMD but care must be taken regarding potential side effects because, even with the low dose used in this study, an appreciable number of participants dropped out of the scFOS treatment group as a result of reported digestive problems.

Of interest, the study by Holloway et al. (28) demonstrated significantly lower lumbar spine BMD in those women who responded positively to supplementation with chicory oligofructose and long-chain inulin, with a greater increase in Ca absorption in women with lower initial spinal BMD at baseline. In our subgroup analysis, we observed a similar beneficial effect of scFOS on spinal BMD in those who were classified as osteopenic at baseline, suggesting that response to scFOS may be dependent on the physiologic state at baseline (15). Although this subanalysis was sufficiently powered to test for change in total-body BMD because of the high incidence of osteopenia in this cohort of well-characterized postmenopausal women, it must be noted that this analysis was exploratory and needs additional investigation.

The greater reduction in the amount of BTMs observed between the Ca group and the maltodextrin group would suggest that Ca is the regulating factor responsible for the change over time observed in BTMs. A greater reduction in osteocalcin together with a greater reduction in urine and serum CTX at 12 mo in the Ca compared with the maltodextrin group would suggest that less bone turnover is occurring and a more

TABLE 3 Change in bone turnover markers after 12 and 24 mo of supplementation with Aquamin (Ca), NutraFlora (CaFOS), or MD in postmenopausal women[1]

	Ca			CaFOS			MD		
	Baseline	12 mo	24 mo	Baseline	12 mo	24 mo	Baseline	12 mo	24 mo
Osteocalcin, *μg/L*	20.6 ± 7.8	18.1 ± 8.0	19.9 ± 8.0	18.9 ± 6.7	17.3 ± 6.2	18.0 ± 6.6	19.6 ± 8.3	18.6 ± 7.5	20.1 ± 8.1
% change	—	−10.3 ± 23.5[b]	0.7 ± 33.3[c,d]	—	−5.5 ± 26.1[a,b]	−0.8 ± 32.4[d]	—	−2.3 ± 23.0[a]	−6.5 ± 33.8[c]
Serum CTX, *μg/L*	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.3	0.6 ± 0.2
% change	—	0.3 ± 28.5[b]	−6.4 ± 24.1[c]	—	0.1 ± 21.5[b]	−5.0 ± 23.5[c]	—	6.9 ± 22.6[a]	−3.5 ± 20.7[c]
Urine CTX, *mg/mmol Cr*	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 1.0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
% change	—	−2.3 ± 69.5[b*]	−9.7 ± 50.8[c]	—	−2.9 ± 98.2[a,b]	1.6 ± 101.4[c]	—	−2.3 ± 29.8[a]	−10.3 ± 30.3[c]
DPD, *nmol/mmol Cr*	8.2 ± 2.5	7.2 ± 2.1	7.7 ± 2.2	8.2 ± 2.4	7.2 ± 2.0	8.0 ± 2.3	7.9 ± 2.1	7.2 ± 1.7	8.0 ± 1.9
% change	—	−8.7 ± 22.1	−0.4 ± 29.7	—	−9.86 ± 19.8	−0.1 ± 26.4	—	−6.2 ± 17.9	6.4 ± 30.5

[1] Values are means ± SDs. Intention-to-treat analysis, $n = 100$ in each group. Values within a time point with different superscript letters are significantly different using repeated-measures ANOVA (least significant difference post hoc comparisons), $P < 0.05$ (*$P = 0.059$). Ca, Aquamin calcium; CaFOS, Aquamin calcium plus NutraFlora short chain fructo-oligosaccharide; Cr, creatinine; CTX, C-telopeptide of type I collagen; DPD, deoxypyridinoline; MD, maltodextrin.

positive Ca balance is evident. These findings are similar to what would be seen in normal coupled bone systems in which a decrease in bone resorption is followed by a subsequent decrease in formation (45) and is similar to the biologic action of antiresorptive agents (28). Previous in vivo research with the same Ca supplement (Aquamin) identified its potential to enhance bone mineralization via increased osteoblast activity (46). A significant correlation was observed between plasma PTH and 25(OH)D, the status marker of vitamin D, only in the maltodextrin group and at all time points. These findings lend additional support to the possibility that CaFOS and the Ca-rich supplement had a positive effect on Ca homeostasis in these women.

A particular strength of this study is the length of the intervention period, because at least 24 mo in postmenopausal women might be necessary to detect measurable changes in BMD. However, it is possible that the effects of the Ca supplement on BMD and bone metabolism were not discernible from DXA scans, and use of quantitative computed tomography to measure volumetric BMD as well as stress-strain index could have provided additional information. A limitation of this study is that we did not measure Ca bioavailability, nor did we have a group that was supplemented with scFOS alone; consequently, we cannot conclude whether scFOS works independently of Ca or works in synergy. Therefore, additional investigations are needed to identify the specific effects of scFOS in the absence of Ca and perhaps in those with suboptimal dietary intakes of Ca. Furthermore, because physical activity and dietary intake were only determined at baseline and without a 24-mo measure, it is not possible to determine changes in these parameters that may have affected BMD.

Within postmenopausal women, osteoporosis and the subsequent risk of bone fracture significantly affects morbidity and mortality and has become a major public health problem. The primary goal of nutrition strategies for bone health is aimed at preserving bone density through the provision of sufficient Ca and the maintenance of Ca balance. The change in BMD after 24 mo did not differ between the intervention groups, but we did observe a greater decline in BTMs in the Ca and CaFOS groups compared with the maltodextrin group, which suggests improved Ca balance after supplementation with Ca and CaFOS. Because these benefits were apparent after 12-mo supplementation in both Ca- and CaFOS-supplemented women and maintained through to 24 mo in the CaFOS-supplemented women, the change in BTMs over time observed would suggest that CaFOS has a beneficial effect on bone turnover that is over and above Ca alone. These findings support the need for additional investigation of the effect of using CaFOS supplementation as a strategy to maintain good bone health, particularly in vulnerable groups such as postmenopausal women.

Acknowledgments

The authors thank Alastair Magill (Biochemistry Laboratory, Antrim Area Hospital, Northern Health and Social Care Trust, Northern Ireland) and Mark Lynch (Biochemistry Laboratory, Altnagelvin Area Hospital, Western Health and Social Care Trust, Northern Ireland) for sample analyses and Dr. Ian Bradbury for statistical advice. The authors thank undergraduate students Victoria Gage, Gary Longshawe, Louise Ferguson, and Kirsty Grant, who assisted with the study. This work would not have been possible without the contributions of their late colleague Julie Wallace, who is sadly missed. E.M.M., P.J.M., J.J.S., J.M.W., and M.P.B. were involved in the study design and provided overall study supervision; M.M.S., P.J.A., and E.M.M. were involved in the conduction of the study, and anthropometric, laboratory, and dietary analysis; M.M.S., M.E.D., and E.M.M. were involved in the statistical analyses; and M.M.S., P.J.A., P.J.M., V.R.N., J.J.S., J.M.W., M.E.D., and E.M.M. were involved in data interpretation. All authors read and approved the final manuscript.

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