



# Efficacy and tolerability of calcium, vitamin D and a plant-based omega-3 oil for osteopenia: A pilot RCT

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## ABSTRACT

**Objectives:** Our pilot study tested the efficacy, acceptability and tolerability of DHA supplementation in addition to calcium and vitamin D in individuals with osteopenia.

**Study design:** 40 participants were randomised to either algal oil containing 400 mg docosahexanoic acid (DHA) daily or placebo. All participants received 1200 mg calcium carbonate with vitamin D<sub>3</sub> 1000 IU daily.

**Main outcome measures:** Bone mineral density (BMD) was measured at baseline and 12 months. Bone turnover was assessed with serum c-terminal telopeptides (CTx) at baseline and 12 months. Tolerability and acceptability were assessed using a validated questionnaire.

**Results:** Mean CTx was suppressed after 12 months for all participants ( $p=0.04$ ) with no difference in effect size between DHA and control groups ( $p=0.53$ ). Changes in CTx at 12 months were significantly correlated with changes in BMD at the lumbar spine ( $p=0.01$ ) and total proximal femur (TPF) ( $p=0.03$ ). There was a non-significant trend towards rising BMD at 12 months. Participants rated the supplements as tolerable and acceptable, with few adverse events.

**Conclusions:** The combination of oral calcium, vitamin D<sub>3</sub> and DHA was safe, tolerable and acceptable when used for 12 months by osteopenic individuals in this pilot study. The combination had a positive effect on bone health as indicated by serum CTx, with no effect demonstrated from the addition of DHA 400 mg. Changes in BMD at the lumbar spine and TPF were significantly correlated with changes in CTx, which may be useful in monitoring bone health and response to treatment.

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## 1. Introduction

Polyunsaturated fatty acids (PUFAs), including omega-3 and omega-6 fatty acids, are essential dietary fatty acids necessary for normal cellular function at all stages from conception to adulthood [1]. The omega-3 fatty acids eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are obtained mainly from fish in the human diet, and to a lesser degree from some plants. The principal omega-6 fatty acid, linoleic acid (LA) is derived from vegetable oils [2]. A diet rich in omega-3 fatty acids may have beneficial effects in a wide range of normal developmental processes and disease states. This list includes cardiovascular disease, infant brain & retinal development, autoimmune disorders, cancer (breast, colon, prostate) arthritis, and potentially bone health [1].

Human bone is subject to a continuous process of remodelling, balancing bone resorption, performed by osteoclasts, with bone formation, performed by osteoblasts. When resorption exceeds

formation, loss of bone mass and alteration of micro architecture occur, resulting in osteopenia or osteoporosis [3].

There is evidence from animal models that omega-3 fatty acids inhibit osteoclast activity and promote osteoblast activity, thus favouring bone formation over bone resorption. Animal studies also suggest that omega-3 fatty acids potentiate the effects of oestrogen on bone, reduce bone loss during oestrogen deficiency, and moderate peripheral peroxisome activated receptor gamma (PPAR- $\gamma$ ), which influences the marrow adiposity which accompanies osteoporosis [2,4]. It appears that the omega-6/omega-3 ratio may be important, in addition to the absolute quantities of omega-3 fatty acids ingested [5]. Lipid metabolism differs between animals and humans, so data cannot always be easily extrapolated from these animals to humans [6]. To date, human studies concerning omega-3 and omega-6 fatty acids and bone health are limited, with some suggesting an effect on calcium absorption, bone turnover, peak bone mass and postmenopausal bone loss [6–9].

Our pilot study aimed to address the paucity of human studies by testing the efficacy, acceptability and tolerability of DHA supplementation in individuals known to have osteopenia, with a view to conducting a future, larger study. Because marine sources of DHA are unacceptable to some individuals, our study is the first to test

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a plant-derived form of DHA supplementation in individuals with osteopenia. Supplemental calcium 1200 mg and vitamin D 1000 IU are recommended in clinical guidelines for use in individuals with osteopenia, and so were provided to all participants. Funding was sufficient to permit recruitment of 40 participants.

## 2. Methods

### 2.1. Study design

#### 2.1.1. Setting

40 participants (36 females, 4 males, mean age 59.2 years) were recruited via a community-based bone mineral density (BMD) testing service in Adelaide, South Australia (Fig. 1).

#### 2.1.2. Eligibility criteria

Inclusion criteria were a BMD *T*-score in the osteopenic range (i.e. between  $-1.0$  and  $-2.4$ ) at the lumbar spine, neck of femur (NOF) or total proximal femur (TPF), being able to provide 5 mL of blood at entry into and conclusion of the study and being able to take the study supplements (2 capsules and 2 tablets each day). Those who were already using medications known to affect bone turnover and/or bone density (e.g. antiresorptive agents, oestrogen, systemic corticosteroids) were excluded, as were those who were already using dietary supplements containing omega-3 fatty acids (e.g. fish oil). BMD in the osteoporotic range (i.e.  $\leq -2.5$ ) at any site resulted in exclusion.

#### 2.1.3. Intervention/outcomes

Twenty consenting participants were randomly allocated to two groups using a random number table. Both investigators and participants remained blinded with regard to all participants' group allocation until after all participant visits were completed. Participants were asked to take two capsules daily containing either DHA ('Life'sDHA<sup>®</sup>', each capsule containing 200 mg DHA derived from algal oil, in a sunflower oil medium), or placebo (corn oil) in a double-blind manner. In addition, all participants were asked to take two tablets per day of 'Ostelin Vitamin D plus Calcium<sup>®</sup>', equivalent to calcium carbonate 1200 mg & vitamin D<sub>3</sub> 1000 IU daily, in keeping with local guidelines [10].

Participants completed a brief questionnaire assessing their dietary calcium intake and sun exposure. Vitamin D is formed by the action of sunlight upon skin, so recording sun exposure provided a means of allowing for this potential confounding factor. Blood was drawn after an overnight fast for measurement of serum c-terminal telopeptides (CTX). Participants were reviewed at 3-monthly intervals until 12 months had elapsed and were asked to keep a study diary which detailed medication compliance, any health issues or side effects experienced and other medications used. At review visits participants were asked to rate the tolerability and acceptability of the study medications using a validated questionnaire [11]. At the conclusion of the study participants again completed the dietary questionnaire, had blood drawn for fasting CTX and had repeat BMD measurement.

Funding was not sufficient to permit measurement of serum vitamin D levels, or incorporate a physical activity or exercise component.

#### 2.1.4. Analysis

Data were analysed using Predictive Analysis Software statistics (SPSS) version 17.0.2 using paired *t*-tests for comparing differences in means, Pearson correlation for examining relationships between changes in variables and two way ANOVA for comparing changes in variables over time.

This pilot study aimed to assess the feasibility of a future, larger trial. Assuming an effect size of a 10–15% suppression of CTX by

DHA, 360 participants would be required to properly examine this relationship.

Approval was obtained from the University of Adelaide Human Research Ethics Committee. The study was registered with the Australia & New Zealand Clinical Trials Registry, ACTRN 2609000238279.

## 3. Results

### 3.1. Participants

The characteristics of the two groups are set out in Table 1. There were no significant differences between the groups at baseline (paired *t*-test). Thirty-seven (92.5%) participants completed the full 12 month study period. Three participants withdrew early. One participant relocated interstate, and was lost to follow-up. Another developed an unrelated illness after randomisation but before commencing study supplements, and chose not to continue. A third participant felt unable to attend for the final study visit, and discontinued the study supplements at some point after 9 months (Fig. 1).

### 3.2. CTx

There was significant suppression of mean CTx at 12 months when compared to baseline, the mean reduction being 41.5 ng/L ( $p=0.04$ , paired *t*-test). There was no significant difference between the effect size in the DHA and control groups ( $p=0.53$ , ANOVA) (Table 1).

### 3.3. Bone mineral density

When all participants were examined using paired *t*-tests, there was no significant increase in mean BMD values at either the lumbar spine ( $p=0.46$ ), total proximal femur ( $p=0.07$ ) or neck of femur ( $p=0.08$ ) at 12 months when compared to baseline. There was no significant difference in effect size between the DHA and control groups ( $p=0.49$ , ANOVA) (Table 1).

### 3.4. Calcium intake from food

Mean calcium intake from food was significantly lower at 12 months than at baseline in both the DHA ( $p=0.01$ , paired *t*-test) and placebo groups ( $p=0.03$ , paired *t*-test).

### 3.5. Sunlight exposure

There was no difference in self-reported average daily sunlight exposure between the DHA and control groups ( $p=0.47$ , proportionate odds model).

### 3.6. Relationships between CTx and BMD

There was a statistically significant, moderately strong relationship ( $p=0.01$ ; Pearson correlation coefficient  $-0.53$ ) between change in lumbar BMD and change in CTx, and a significant but weak relationship ( $p=0.03$ ; Pearson correlation coefficient  $-0.36$ ) between change in TPF BMD and change in CTx (Table 2a). TPF BMD correlated with CTx at baseline ( $p=0.01$ ; Pearson correlation coefficient  $-0.4$ ) and at 12 months ( $p=0.03$ ; Pearson correlation coefficient  $-0.354$ ) (Table 2b).

### 3.7. Adverse events and tolerability

Supplements were rated as either tolerable or very tolerable by 16 participants (80%) in the DHA group, compared to 18

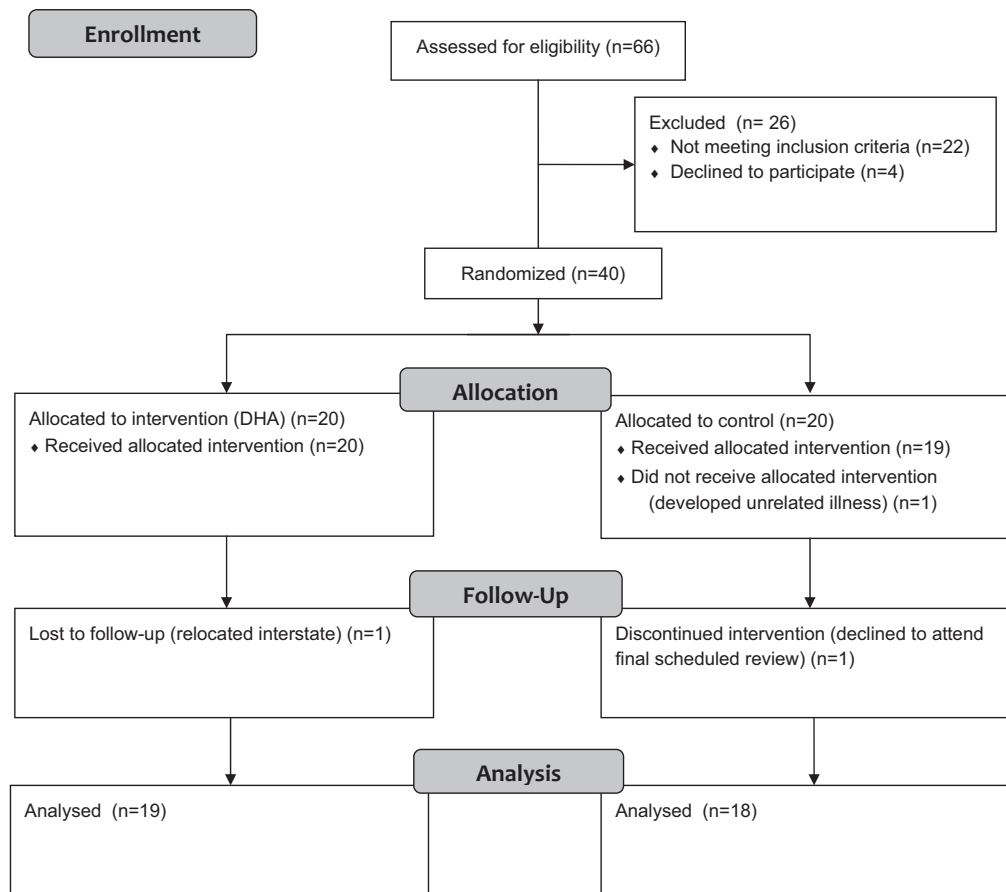


Fig. 1. Study flow diagram.

**Table 1**  
Participants characteristics according to treatment group.

	DHA group		Control group	
	Baseline (n = 20) Mean (SD)	12 months (n = 19) Mean (SD)	Baseline (n = 20) Mean (SD)	12 months (n = 18) Mean (SD)
BMD (g/cm <sup>2</sup> )				
Lumbar spine	0.919 (0.09)	0.967 (0.07)	0.969 (0.08)	0.915 (0.1)
Total proximal femur	0.861 (0.06)	0.895 (0.1)	0.889 (0.1)	0.865 (0.7)
Neck of femur	0.702 (0.08)	0.735 (0.08)	0.732 (0.08)	0.709 (0.06)
Dietary calcium <sup>a</sup> (mg/day)	1014 (576)	1999 (568) <sup>b</sup>	969 (634)	1859 (245) <sup>b</sup>
CTx (ng/L)	390 (158)	357 (173)	401 (185)	351 (184)
Body Mass Index (kg/m <sup>2</sup> )	25.4 (3.3)		25.5 (3.9)	
Sun exposure <sup>a</sup> (min/day)	n (%)			
	7 (35)		5 (25)	
	5 (25)		7 (35)	
	8 (40)		8 (40)	

<sup>a</sup> Self-reported.

<sup>b</sup> 12 month dietary calcium values include 1200 mg/day of supplementation.

participants (90%) in the control group, and as acceptable or very acceptable by 19 participants (95%) in the DHA group, compared to 20 participants (100%) in the control group. There was no significant difference in study withdrawal rate (1 from the DHA group, 2 from the control group). No serious adverse events were reported.

17 participants reported minor adverse events, including constipation (DHA/control group: 3/4), reflux/indigestion (3 in each group), increased joint pain (DHA/control group: 2/1 in the control group), vivid dreams (1 control) & lethargy (1 control), with no significant difference overall between the groups ( $p = 0.52$ , paired  $t$ -test). One

**Table 2a**  
Correlation between change in CTx and change in BMD, all participants.

n = 37	Change in CTx	Change in Lx BMD	Change in TPF BMD	Change in NOF BMD
Change in CTx, baseline – 12 months				
Pearson correlation	1	−0.53	−0.361	−0.138
Significance (2-tailed)	–	0.001	0.03	0.42

**Table 2b**

Correlation between CTx and BMD at baseline and at 12 months.

	Lx BMD Baseline	Lx BMD 12 month	TPF BMD Baseline	TPF BMD 12 month	NOF BMD Baseline	NOF BMD 12 month
Baseline CTx ( <i>n</i> = 40)						
Pearson correlation	−0.148	–	−0.4	–	−0.19	–
Significance (2-tailed)	0.36		0.01		0.24	
12 month CTx ( <i>n</i> = 37)						
Pearson correlation	–	−0.81	–	−0.354	–	−0.206
Significance (2-tailed)		0.64		0.03		0.23

control group participant was unable to continue taking the study oil capsules because of severe indigestion, but persisted with the calcium & vitamin D tablets. One participant was unable to tolerate the full dose (2 tablets) of calcium with vitamin D, but continued in the study at a dose of 1 tablet (i.e. 600 mg calcium with 500 IU vitamin D) daily. A sensitivity test, in which results were re-analysed with these participants' data excluded, did not significantly affect study outcomes. The mean number of missed doses per participant over the 12 month study period was 12.4, indicating excellent adherence.

#### 4. Discussion

Our study demonstrated a fall in CTx values (overall mean reduction 41.5 ng/L, or 10.5%) and a non-significant trend towards a rise in BMD over the course of the study, which can be attributed to vitamin D and calcium supplementation. Supplementation with calcium in combination vitamin D has been shown to have a beneficial effect on BMD and bone turnover markers by others, and generally also on rates of falls and fractures [12,13].

Our study did not demonstrate an effect from the addition of DHA 400 mg daily to calcium & vitamin D supplementation in osteopenic individuals, which may be due to the small sample size in this pilot study. In the existing literature there has not been a consistent pattern of response shown with regard to bone health from PUFA administration. A recent systematic review concluded that studies in humans concerning omega-3 fatty acids and bone health showed mixed results, being summarised as possibly beneficial, but without compelling data [6]. Small sample size, different methodologies and a lack of prospective, interventional studies have resulted in a lack of certainty.

We chose to study the effects of DHA on bone health by supplementation rather than controlled diet because this is potentially more acceptable to many individuals, and we chose a plant-derived DHA source because of its potential acceptability to a wider population than that derived from fish.

A study by Griel et al. [8] suggested a positive effect on bone health, showing a reduction in the bone resorption marker n-terminal telopeptide (NTx) of 11–15% in participants consuming a high PUFA diet compared with those consuming an 'average American diet'. Bone resorption has been shown to be an independent determinant of future fracture risk [14]. The controlled diets also provided a daily calcium intake of approximately 1000 mg and 180 IU of vitamin D, less than the supplementation used in our study. They also aimed for an omega-6/omega-3 ratio rather than a specific intake of a single fatty acid, DHA, as was used in our study.

It is possible that the DHA dose used in our study was inadequate to affect bone health. We chose a dose of 400 mg daily as this is consistent with the recommended dose for cardiovascular [15] and rheumatological [16] health, and is similar to the dose used in a bone health study by van Papendorp et al. [17]. Their study compared the effects of 16 weeks use of 3 different PUFA supplements with a placebo in 40 elderly women with confirmed osteoporosis. Unlike our study, participants received a homogenous diet rather than using vitamin D or calcium supplements. Two of the

preparations included DHA, comprising 440 mg in one preparation and 120 mg in the other. Both preparations produced a statistically significant effect on total alkaline phosphatase (a less specific marker of bone turnover) and procollagen, but only the preparation including 120 mg DHA had a significant effect on osteocalcin (a marker of bone formation). However, the preparation containing 120 mg DHA also included other omega-3 and omega-6 oils. Another study by Kruger et al. [18] gave 65 elderly women known to have low dietary calcium intake an unspecified dose of gamma LA/EPA or placebo, plus calcium, for 18 months and showed increase NOF & lumbar BMD in the active but not placebo oil group.

Participants in these studies did have adequate calcium intake, although the mean calcium and vitamin D intakes achieved in our study were considerably higher. Because of the differences in participant characteristics and interventions it is difficult to compare the results of these studies with our findings, but our findings are in line with others suggesting a positive effect of PUFAs and calcium supplementation on bone health.

In our study, calcium & vitamin D supplementation in osteopenic participants resulted in a 10.5% reduction in CTx after 12 months. This achieved statistical significance ( $p = 0.04$ ), but it is unclear how clinically significant a reduction of this magnitude may be. Reductions of 17% at 12 h after a single dose of calcium carbonate have been reported [19]. Participants in the placebo arm of some osteoporosis studies received calcium and vitamin D as part of the studies' protocols. These studies also reported CTx changes at 12 months (range 0–44.7%) [20–22]. Participants in these studies were older, had lower BMD values and were likely to have prevalent major osteoporotic fractures. Our study is the first to report the effect on CTx in osteopenic individuals, who are present in much greater numbers in the population and for this reason account for a higher absolute number of fractures [23].

The correlation between serum CTx and bone density (lumbar and TPF) found in our study has implications for monitoring bone health and response to treatment. Most of the currently used treatments for osteopenia and osteoporosis have been shown to rapidly suppress bone turnover markers, including CTx [24]. BMD is slower to change, typically showing some response after 6–12 months [25]. Changes in CTx may therefore be useful for predicting changes in BMD over this longer period, in addition to its existing role in monitoring treatment adherence and response, as has been suggested elsewhere in the literature [26,27].

In our study BMD did not change significantly at any of the three sites tested, although there was a non-significant trend towards a rise at the TPF (0.6% mean rise,  $p = 0.07$ , paired *t*-test) and NOF (0.9% mean rise,  $p = 0.08$ , paired *t*-test). BMD is slower to change in response to treatment than CTx, so this finding was consistent with expectations. There was no difference in size of the effect between the two groups, implying that the addition of 400 mg daily of DHA to calcium and vitamin D for 12 months had no detectable additional effect on BMD in our sample.

Our study supplementation consisting of 2 capsules and 2 tablets daily was acceptable and tolerable to participants. This is important because some people will choose taking a supplement rather than attempt sustained dietary manipulations. It also shows

that this regimen can be sustained for a 12 month period. Using DHA which is of plant origin will be permissible for vegetarians and vegans, who may find marine-origin DHA unacceptable. We also found no significant difference in adverse effect rates between DHA and placebo groups.

Mean self-reported calcium intake from food decreased significantly at the 12 month point (348 mg/day,  $p < 0.001$ , paired  $t$ -test), with no difference in effect size between groups. However, when the supplemental calcium intake was added, all participants still achieved an increase in total calcium intake. We speculate that individuals who commence calcium supplementation may become less motivated to maintain their dietary calcium intake (e.g. from dairy products), or that they may experience satiety with respect to calcium when taking a supplement, which may need to be taken into account when recommending calcium supplementation to individuals, as the actual daily intake is likely to be less than the sum of the prior intake and the supplemental calcium.

In conclusion, the combination of oral calcium, vitamin D and DHA was safe, tolerable and acceptable when used for 12 months by osteopenic individuals in this pilot study. The combination had a positive effect on serum CTx, a marker of bone health, with no effect demonstrated from the addition of DHA 200 mg. Changes in BMD at the lumbar spine and TPF were significantly correlated with changes in CTx, which may be useful in monitoring bone health and response to treatment.

### Contributors

Dr Vanlint was the original proposer of the trial, wrote the major portions of grant application, trial documents and the submitted manuscript, and performed the majority of the trial visits with participants.

Dr Ried contributed to development of the original concept, submission of the grant application, trial participant visits, data analysis and preparation of the manuscript.

### Competing interests

Dr Vanlint provides consultant advice to the bone mineral density testing service through which participants were recruited. He has received consultancy fees in the area of bone health from AMGEN (Australia) Pty Ltd. No consultancy fees or other payments were received in connection with this study. Dr Ried declares no competing interests.

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