Lack of effect of supplementation with essential fatty acids on bone mineral density in healthy pre- and postmenopausal women: two randomized controlled trials of Efacal® *v*. calcium alone

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Randomized controlled trials of the effects of the dietary supplement Efacal® (Scotia Pharmaceuticals Plc, Guildford, Surrey, UK) *v*. Ca only on total body bone mineral density (BMD) and markers of bone turnover were conducted in healthy pre- and postmenopausal women separately. Total daily dose for 12 months for the Efacal® groups was: Ca 1·0 g, evening primrose oil 4·0 g and marine fish oil 440 mg; and for the control groups was: Ca 1·0 g. Reported compliance was better than 90 % in both age groups. For the forty-three premenopausal women (age range 25–40 years), initial mean total body BMD values were similar for Efacal® and control groups and both groups showed highly significant mean increases of about 1 %; however, there were no significant between-group differences for the changes in BMD or markers of bone turnover. For the forty-two postmenopausal women (age range 50–65 years), initial mean total body BMD values were again well-matched across treatment groups. Both Efacal® and control groups showed highly significant decreases in total body BMD of about 1 %, but again there were no significant between-group differences in total body BMD or markers of bone turnover. Possible confounding variables such as initial total body BMD were explored but had no effect on the outcome in either age group. Nail quality improved in both age groups and in both Efacal® and control groups. Again, there was no significant difference between treatment groups. No evidence was found to support a beneficial effect of Efacal® on BMD in these women.

Bone mineral density: Menopause: Essential fatty acids

The essential fatty acids (EFA) act as precursors for a variety of components in most cells of the body, and their derivatives are known to be necessary for bone metabolism (Norrdin *et al*. 1990) and for the effects of vitamin D on Ca absorption (Hay *et al*. 1980). It has been suspected that there are rate-limiting steps in the formation of some important derivatives of EFA in the *n*-6 series such as γ-linolenic acid. If so, the rate-limitation might be by-passed with benefit by administering increased amounts selectively. Efacal® (Scotia Pharmaceuticals Plc, Guildford, Surrey, UK) is a preparation which contains both γ-linolenic acid and eicosapentaenoic acid in proportions thought to be optimal for bone tissue. These fatty acids are precursors of prostaglandins which are intermediaries in bone metabolism, and some have been found to have positive influence on Ca absorption from the gut in rats (Coetzer *et al*. 1994). The dietary requirement in man for EFA is small in absolute terms (<3 g/d) and normal diets in Britain today, which contain 8–15 g/d, are assumed to contain sufficient quantities to meet adult needs. In addition there are body reserves of 500–1000 g. However, older people may have reduced intakes and slower rates of metabolic conversion; abnormalities of EFA metabolism are not uncommon. This, coupled with the high prevalence and increasing incidence of osteoporosis in postmenopausal women, which is associated with low bone mineral density (BMD), has led to a hope that supplementation with EFA and Ca might increase BMD and reduce risk of osteoporosis (Horrobin, 1992). Previous studies investigating this hypothesis have produced inconclusive results (Van Papendorp *et al*. 1995; Kruger *et al*. 1996). The purpose of this present trial therefore was to find out whether Efacal® had any such influence in healthy pre- or postmenopausal women on normal diets. The results have not been published except as abstracts and in an MPhil thesis (Littlewood, 1998).

Methods

Women were recruited by postal invitation from staff at Queen's Medical Centre (Nottingham, UK) and a nearby

Abbreviations: EFA, essential fatty acids; BMD, bone mineral density.

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group medical practice. Subjects were screened and excluded for health problems, BMI (BMI = body weight (kg) divided by stature (m^2)) >32 or <18, or BMD outside 2 SD of age-matched norms. They were also excluded for confounding drug therapy, or any pre-existing dietary supplementation, which might have confounded the outcome or posed a remote risk of overdose. Premenopausal women were aged 25–40 years and were excluded for pregnancy or lactation, or irregular menses (less than ten cycles in the previous year). Postmenopausal women were aged 50–65 years and all were checked for oestrogen status using blood assays (see later); they were excluded if they were within 1 year of the last menses, were taking hormone replacement therapy or had hormone levels outside the normal postmenopausal range. Reported occupations ranged from domestic cleaning to university lecturing but the majority of the women were nurses, technicians or secretarial staff. There was a balanced range of occupations across test and control groups in both age groups. Permission for the study was obtained from the Medical School Ethics Committee and each woman gave written informed consent.

Measurements were made of total body BMD, dietary Ca intake, leg extensor power, anthropometry and nail condition; 20 ml venous blood and urine samples were taken, both in the morning after a 12 h fast, for assessment of markers of bone turnover and serum Ca. All assessments were made using standard methodology except for nail condition (see later).

Two initial assessments 1 month apart were made to establish secure baselines. Further assessments were made after 6 and 12 months of dietary supplementation. The 6-month assessment was a precautionary measure to insure against possible loss of numbers and is not reported. The main outcome, therefore, was the difference between the mean of the baseline measurements and the 12-month value. This allows long enough for evident changes in bone to develop and avoids seasonal variation known to affect BMD.

The design was double blind and women were randomized by staff at Scotia Pharmaceuticals Plc to Efacal® (Ca 1·0 g, evening primrose oil 4·0 g and marine fish oil 440 mg) or 1·0 g Ca daily. These doses are greatly in excess of those taken by casual users of these supplements, but are reported to be safe from studies with selected patient groups (Morse *et al*. 1989). These supplements were provided in large capsules, ten to be taken daily in divided doses with meals. The women attended the Medical School at 6-weekly intervals to collect their supplies of capsules. On these occasions a nail inspection was made, body weight was recorded and a brief questionnaire was conducted, in a one-to-one setting. The following information was collected: any new medication, hospitalization or major health problems, change in diet, change in exercise habits, and compliance (from the number of capsules left over). Answers except for the final item were yes or no; if yes the interviewer probed for details.

Bone mineral density

Total body BMD was assessed using dual energy X-ray absorptiometry (Lunar DPX-L software version DPXL 1.3 Z, Lunar Radiation Corp., Madison, WI, USA). The measurements were made without knowledge of the group to which the women belonged and analysis was carried out in accordance with the manufacturer's recommendations. In-house calibration procedures and precision have been reported. *In vivo* reliability from paired measurement of the same group of women within 2 weeks is expressed as a CV calculated from the standard deviation of the paired differences divided by the square root of two (Healy, 1958) and was less than 1 % (Pye *et al*. 1992).

Background dietary calcium intake

This was assessed using a Ca-rich-food frequency questionnaire (Ramsdale *et al*. 1994; Ramsdale, 1995) to ensure that supplementation did not increase intakes over 2500 mg/d. This is considered a safe upper limit to avoid kidney stones (Morse *et al*. 1989).

Leg extensor power

This was assessed using the Nottingham power rig which was designed for and used in the Allied Dunbar national fitness survey (1992). This is sensitive to changes in habitual weight-bearing activity and was included as an objective check on possible spontaneous changes in physical activity levels.

Reported physical activity

Subjects were asked whether or not they were currently regularly engaged in vigorous sport or physical activity, and if so to specify the type of activity, frequency and level of intensity. The interviewer probed for details at this point. Subjects were then classified into sedentary (no vigorous activity reported), moderately active (less than 3 h/week), or vigorously active (at least 3 h/week).

Height and weight were assessed and used to calculate BMI (kg/m^2) for screening.

Nail condition

This was formally assessed on a four-point scale by counting white flecks and inspecting for breaks, chips, tears on all digits of both hands. No blemishes yielded the best score of 'good' and flecks on more than five digits and two tears the worst score of 'very poor'.

Markers of bone turnover

All analyses were done in duplicate using standard methods; intra- and interassay CV are given respectively later. Blood samples were centrifuged, serum decanted into portions and frozen immediately at −80° for later analysis. This was done in duplicate and the 6-month and 12-month samples were batched separately. This included Ca (corrected for albumin), oestrodial, follicle-stimulating hormone, parathyroid hormone, osteocalcin and bone-specific alkaline phosphatase. Commercial kits were used for immunoradiometric assays, Tandem-R Ostase (Hybritech Europe, Liege, Belgium) (6·7 %, 9·4 %) for bone-specific alkaline phosphatase, ELSA-OSTEO (CIS, High Wycombe, Bucks., UK)

Table 1. Characteristics at baseline of premenopausal women randomized to receive Efacal®* (*n* 19) or calcium (*n* 24) supplements
(Mean values with standard errors)

Supplement . . .	Efacal®†		Calcium†	
	Mean	SE	Mean	SE
Age (years)	34	1	35	1
Stature (m)	1·63	0·01	1·64	0·01
Body mass (kg)	60·5	2·1	58·4	1·3
Leg extensor power (W)	146	8	128	5
Dietary calcium (mg/d)	863	47	795	47
Urinary hydroxyproline (μmol/mmol Cr)	15·1	1·45	14·3	1·03
N-telopeptide (nmol BCE/mmol Cr)	24·0	2·2	23·2	1·4
Serum calcium (mmol/l) adjusted for albumin	2·33	0·013	2·33	0·012
Osteocalcin (μg/l)	17·7	1·42	19·5	0·99
Bone-specific alkaline phosphatase (μg/l)	8·9	0·66	9·1	0·43
Parathyroid hormone (ng/l)	22·9	1·38	24·2	1·55
Total body bone mineral density (g/cm^2)	1·154	0·019	1·150	0·013

Cr, creatinine; BCE, Bovine Collagen Equivalent.
* Efacal®, Scotia Pharmaceuticals Plc, Guildford, Surrey, UK.
† There were no significant differences between the groups for any variable.

(3·85 %, 6·01 %) for osteocalcin, INCSTAR (Diasorin Ltd, Wokingham, Bucks., UK) (3·6 %, 10·7 %) for intact parathyroid hormone and Coat-a-count® (Euro/DPC Ltd, Gwynedd, North Wales, UK) (5·6 %, 6·5 %) for oestradiol. In-house assays were used for serum Ca (1·2 %, 1·7 %), and EKTACHEM Kodak dryslide (VITROS) (Ortho-Clinical Diagnostics, Amersham, Bucks., UK) (1·2 %, 1·5 %) for albumin, and an immunoradiometric assay using ^{125}I and fluorescein-labelled monoclonal antibodies (3·5 %, 7·14 %) for follicle-stimulating hormone.

Urine samples were similarly stored and assayed using Osteomark (Shield Diagnostics, Dundee, UK) (8·0 %, 10·5 %) for *N*-telopeptide, and in-house colorimetric methods (6·2 %, 7·2 %) for hydroxyproline, (1·1 %, 1·4 %) for creatinine.

Power calculations and data analysis

The numbers recruited aimed to provide, after an estimated dropout of 25–30 %, at least forty women. This would be adequate to detect as significant at the 5 % level a difference, if it occurred, of 1 % in BMD between test and control (Gore & Altman, 1988). The study provided 90 % power to detect such a difference in BMD. This method is based on calculating a standardized difference from the smallest difference between the two groups which would be medically relevant (1 %) and the standard deviation of the variable on re-test (1 %). Power to detect differences in markers of bone turnover was less since they are more variable.

Interval data were expressed as mean values with standard errors, unless otherwise stated. Changes were assessed using Student's *t* tests for paired means and differences between groups using Student's *t* tests for unpaired means or Mann–Whitney tests for nominal data. Relations were assessed using Pearson's product-moment correlations for interval data or Spearman's rho. Analysis of covariance was used to examine possible interactive effects between variables (Superanova, Abacus Concepts, Berkeley, Inc., CA, USA). Probability levels of 5 % or less were considered significant. All tests were two-tailed.

Results

Premenopausal women

Oestradiol and follicle-stimulating hormone levels confirmed that this group were all premenopausal. There were

Table 2. Changes in variables in premenopausal women after supplementation for 12 months with Efacal®† (*n* 19) or calcium† (*n* 24)
(Mean values with standard errors)

Supplement . . .	Efacal®‡		Calcium‡	
	Mean	SE	Mean	SE
Body mass (kg)	0·37	0·61	0·19	0·32
Leg extensor power (W)	–2	7	0	4
Dietary calcium (mg/d)	172**	62	129**	39
Urinary hydroxyproline (μmol/mmol Cr)	0·76	2·13	–1·52	1·22
N-telopeptide (nmol BCE/mmol Cr	1·08	2·28	2·31	2·01
Serum calcium (mmol/l) adjusted for albumin	0·051***	0·010	0·049***	0·010
Osteocalcin (μg/l)	–2·65**	0·92	–2·69**	0·96
Bone-specific alkaline phosphatase (μg/l)	–2·26***	0·36	–2·64***	0·28
Parathyroid hormone (ng/l)	3·81**	1·61	0·67	1·71
Total body bone mineral density (g/cm^2)	0·008***	0·002	0·011***	0·002

Cr, creatinine; BCE, Bovine Collagen Equivalent.
Mean values were significantly different from those at baseline: ** $P < 0·01$, *** $P < 0·001$.
† Efacal® (Scotia Pharmaceuticals Plc, Guildford, Surrey, UK) provided (per d): Ca 1·0 g, evening primrose oil 4·0 g, marine fish oil 440 mg; the calcium supplement provided (per d): Ca 1·0 g.
‡ There were no significant differences between the groups for any variable.

Table 3. Distribution of nail assessments* in premenopausal women at baseline and after supplementation for 12 months with Efacal®† (*n* 19) or calcium† (*n* 24)

Time . . .	0 months		12 months	
	Efacal®	Calcium	Efacal®	Calcium
Very poor	0	0	0	0
Poor	3	7	0	2
Average	11	11	10	12
Good	5	6	9	10

* For details of nail assessments see p. 630.
† Efacal® (Scotia Pharmaceuticals Plc, Guildford, Surrey, UK) provided (per d): Ca 1·0 g, evening primrose oil 4·0 g, marine fish oil 440 mg; the calcium supplement provided (per d): Ca 1·0 g.

sixty-four women initially and drop-out during the 12 months of the study was 31 %; this was due to ill-health unconnected with the trial, family problems, pregnancy, home removal, or repeated failure to keep appointments. Two women withdrew due to difficulty in swallowing the capsules. One woman's data were excluded from the final analysis due to her excessive weight gain resulting in a BMI of 36 kg/m^2, well above the exclusion threshold of 32 kg/m^2. Nearly half of the women were sedentary and only ten reported vigorous activity and they were distributed evenly across the treatment and control groups, five in each sub-group. None of these women smoked. The characteristics at baseline of the forty-three women who completed the study are given in Table 1; treatment and control groups did not differ significantly from each other for any measured variable. Compliance with the regimen of supplementation was good with a median consumption of over nine capsules per day in both treatment and control groups.

Longitudinal changes after 12 months of supplementation. Total body BMD increased significantly in both treatment and control groups by nearly 1 %, however, there was no significant difference between the two groups in these increases (see Table 2 and Fig. 1). Nail quality improved in sixteen women, nine of them in the control group (see Table 3), and there was no difference between the groups ($P = 0{\cdot}21$). None of the other variables measured showed any significant change, except that both groups reported significant increases in dietary Ca intake of more than 15 %. These increases did not differ significantly across the two treatment groups.

There were no significant changes in the urinary markers of bone resorption (*N*-telopeptide and hydroxyproline) but there were significant decreases in the serum markers of bone formation (osteocalcin and bone-specific alkaline phosphatase), a significant increase in parathyroid hormone in the treatment group and significant increases in serum Ca in both treatment and control groups (see Table 2). There were no significant differences between the treatment and control groups for any of these changes.

Potentially confounding variables were examined as covariates to the change in total body BMD in addition to treatment group in the analysis of variance. These included initial values for total body BMD and changes in body weight. Women who already have high BMD might be less likely to show improvements with any intervention and *vice versa*, and loss or gain in body weight are known to be associated with loss or gain in bone mass. However neither initial total body BMD nor body weight change were found to be significant, nor did they alter the non-significant effect of treatment group.

Postmenopausal women

Oestradiol and follicle-stimulating hormone levels confirmed that this group were all postmenopausal. Five had had hysterectomies, one in the treatment group and four in the control group. There were fifty-seven women initially and drop-out during the 12 months of the study was 23 %; this was mainly due to ill-health including four with stomach problems, one died and one could not swallow the tablets; two with excessive gain in body weight were

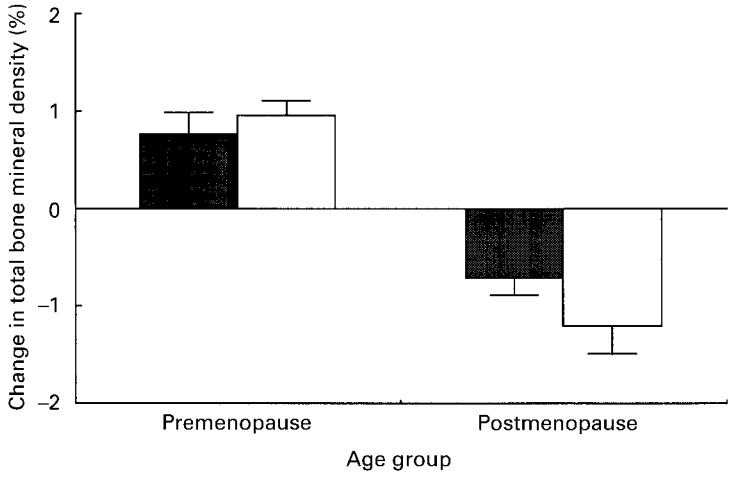

Fig. 1. The mean percentage changes in total body bone mineral density in pre- and postmenopausal women after 12 months of supplementation with Efacal® (■) (Scotia Pharmaceuticals Plc, Guildford, Surrey, UK; supplement provided (per d): Ca 1·0 g, evening primrose oil 4·0 g, marine fish oil 440 mg) or calcium (□) (1·0 g Ca/d). Values are means with standard errors represented by vertical bars.

Table 4. Characteristics at baseline of postmenopausal women randomized to receive Efacal®* (*n* 21) or calcium (*n* 24) supplements
(Mean values with standard errors)

Supplement . . .	Efacal®†		Calcium†	
	Mean	SE	Mean	SE
Age (years)	58	1	55	1
Stature (m)	1·62	0·02	1·62	0·01
Body mass (kg)	64·3	2·1	64·5	1·9
Leg extensor power (W)	122	7	113	5
Dietary calcium (mg/d)	937	54	999	54
Urinary hydroxyproline (μmol/mmol Cr)	19·3	0·92	23·2	1·27
N-telopeptide (nmol BCE/mmol Cr)	52·3	5·5	50·4	2·6
Serum calcium (mmol/l) adjusted for albumin	2·38	0·010	2·37	0·014
Osteocalcin (μg/l)	28·7	1·89	25·4	1·43
Bone-specific alkaline phosphatase (μg/l)	13·80	1·05	13·97	0·77
Parathyroid hormone (ng/l)	28·2	1·67	26·2	1·71
Total body bone mineral density (g/cm^2)	1·114	0·017	1·136	0·017

Cr, creatinine; BCE, Bovine Collagen Equivalent.
* Efacal®, Scotia Pharmaceuticals Plc, Guildford, Surrey, UK.
† There were no significant differences between the groups for any variables.

excluded. There were only two vigorously active women, one in each sub-group. The rest were evenly distributed between sedentary and moderately active and these were evenly distributed across the sub-groups. There were nine women who currently smoked more than two cigarettes per day, five in the control group and four in the treatment group. The characteristics at baseline of the forty-two women who completed the study are given in Table 4; treatment and control groups did not differ significantly from each other for any measured variable. These women differed from the younger group at baseline in expected ways, with slightly lower total body BMD and higher levels of markers of bone turnover.

Longitudinal changes after 12 months of supplementation. Total body BMD decreased significantly in both treatment and control groups by about 1 %, which was expected, however, there was no significant difference between the two groups in these decreases (see Table 5 and Fig. 1).

Nail quality improved in thirty-one women, seventeen in the control group (see Table 6). There was no difference between groups ($P = 0{\cdot}79$). Taking both groups together the improvements were significantly and negatively related to the initial condition ($P < 0{\cdot}001$).

Markers of bone turnover, both for formation and resorption fell in both treatment and control groups and almost all these within-group changes were highly significant (see Table 5); there was no change in serum levels of Ca or parathyroid hormone. There were no significant differences

Table 6. Distribution of nail assessments* in postmenopausal women at baseline and after supplementation for 12 months with Efacal®† (*n* 21) or calcium† (*n* 21)

Time . . .	0 months		12 months	
	Efacal®	Calcium	Efacal®	Calcium
Very poor	1	0	0	0
Poor	5	4	1	0
Average	10	13	7	4
Good	5	4	13	17

* For details of nail assessments see p. 630.
† Efacal® (Scotia Pharmaceuticals Plc, Guildford, Surrey, UK) provided (per d): Ca 1·0 g, evening primrose oil 4·0 g, marine fish oil 440 mg; the calcium supplement provided (per d): Ca 1·0 g.

Table 5. Changes in variables in postmenopausal women after supplementation for 12 months with Efacal®† (*n* 21) or calcium† (*n* 21)
(Mean values with standard errors)

Supplement . . .	Efacal®‡		Calcium‡	
	Mean	SE	Mean	SE
Body mass (kg)	0·74	0·5	0·74	0·3
Leg extensor power (W)	–6	7	2	4
Dietary calcium (mg/d)	114	52	80	54
Urinary hydroxyproline (μmol/mmol Cr)	–6·19***	1·06	–3·95	2·23
N-telopeptide (nmol BCE/mmol Cr)	–22·73***	4·26	–18·38***	2·74
Serum calcium (mmol/l) adjusted for albumin	–0·016	0·012	0·000	0·012
Osteocalcin (μg/l)	–6·77***	1·08	–3·96***	1·24
Bone-specific alkaline phosphatase (μg/l)	–3·12***	0·57	–3·23***	0·70
Parathyroid hormone (ng/l)	1·54	1·71	0·86	1·65
Total body bone mineral density (g/cm^2)	–0·008***	0·002	–0·013***	0·003

Cr, creatinine; BCE, Bovine Collagen Equivalent.
Mean values were significantly different from those at baseline: *** $P < 0{\cdot}001$.
† Efacal® (Scotia Pharmaceuticals Plc, Guildford, Surrey, UK) provided (per d): Ca 1·0 g, evening primrose oil 4·0 g, marine fish oil 440 mg; the calcium supplement provided (per d): Ca 1·0 g.
‡ There were no significant differences between the groups for any variable.

between the treatment and control groups for any of these changes.

As for the younger women and for the same reasons, potentially confounding covariates were examined but not found to be significant, nor did they alter the non-significant effect of treatment group.

Discussion

There was no *a priori* reason why any potential effects of the dietary supplement should be site-specific so total body BMD, which is highly reliable, was considered to offer the best chance of detecting an effect. Changes of less than 1 % were detected as significant, however these changes could not be attributed to Efacal® since they occurred in both treatment and control groups in both pre- and postmenopausal women. Reliability of markers of bone turnover is lower than for BMD, nevertheless, despite significant changes within groups, there was no evidence for a positive effect of Efacal®. This disappointing outcome suggests that these healthy women were already adequately nourished with EFA and that supplementation brought no further benefit. However direct measurements of circulating EFA or background dietary EFA intake were not made; these are difficult assessments which were beyond the scope of this study. The possibility remains that individuals with particular health problems might benefit; however, an earlier study showed that fish oil was just as effective as evening primrose oil for increasing osteocalcin levels (an indicator of bone formation) in osteoporotic patients (Van Papendorp *et al*. 1995).

Some changes which occurred in both treatment groups remain to be explained. The premenopausal groups both improved their total body BMD which was not expected and might be attributed to the greatly increased Ca intake; this must, however, be regarded with caution since there was no non-Ca control group. There was a trend towards a negative relation between initial Ca intake and increase in total body BMD when both groups were considered together, but it did not reach significance ($P = 0{\cdot}11$). Increase in bone density is not found in all supplementation studies and there is debate about requirements, however the consensus from meta-analyses is that BMD can be increased in young women by supplementation (Welten *et al*. 1995). The changes in metabolic markers were reasonably consistent with this interpretation: serum Ca, a tightly controlled variable, rose slightly, and a significant rise in parathyroid hormone was found but only in one subgroup. Most of the markers of bone formation fell, suggesting a small decrease in bone turnover, although this was not apparent in all the markers of resorption. The small decrease of about 1 % in total body BMD, in both the postmenopausal groups, was expected over 1 year in this age group. However, the decrease in markers of bone turnover was not expected but it could also be attributed to the greatly increased Ca intake. This could also explain the improvement in nail quality which occurred in both age groups, and across both subgroups.

In the premenopausal women the supplements were enhanced by spontaneous increases in dietary Ca intake, according to the analysis of the Ca-rich-food frequency questionnaires. This occurred, despite the subjects reporting that they had made no changes in their diets. Assuming good faith, the spontaneous increase may have been due to an increased awareness of dietary issues influencing their behaviour subconsciously. These groups of women all already had mean Ca intakes which were above the recommended nutrient intake of 700 mg/d (Department of Health, 1991), so the Ca supplements approximately doubled the intakes in both age groups. However, since there was no control for Ca supplementation, it cannot be concluded that any of the observed effects were due to this.

In conclusion, in healthy women of 25–65 years on normal diets, supplementation for 12 months with Efacal® had no effect, compared with Ca alone, on their BMD or markers of bone turnover.

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