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Gilbert R. Kaats PhD, FACN, Harry G. Preuss MD, MACN, Sidney Stohs PhD, FACN, CNS & Nicholas Perricone MD, FACN

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Original Research

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Gilbert R. Kaats, PhD, FACN, Harry G. Preuss, MD, MACN, Sidney Stohs, PhD, FACN, CNS, Nicholas Perricone, MD, FACN

Integrative Health Technologies, Inc., San Antonio, Texas (G.R.K.); Department of Biochemistry, Georgetown University Medical Center, Washington, DC (H.G.P.); Creighton University, Frisco, Texas (S.S.); Transdermal Biotechnology, Inc., Meriden, Connecticut (N.P.)

Key words: AlgaeCal, bone mineral density, body composition

Objectives: The objective of this study was to examine the safety and efficacy of a vitamin–mineral enhanced plant-sourced calcium AlgaeCal calcium (AC) in female consumers who had taken the supplement from 1 to 7 years.

Methods: Consumers who had completed at least one dual-energy x-ray absorptiometry (DEXA) bone mineral density (BMD) scan ($N = 172$) and/or blood chemistry test ($N = 30$) and purchased AC from 1 to 7 years were contacted and offered complimentary repeat tests. Safety and efficacy were examined by annualized changes in a 45-measurement blood chemistry panel and changes in BMD.

Results: No adverse effects or safety concerns were found in any of the annualized within-group annualized changes in the 45 blood chemistries or in between-group changes in a similar control group ($n = 5070$) who completed the same measurements. With regard to BMD, consistent and statistically significant within-group increases were found for the 7-year study period and when compared to expected BMD changes in 3 large databases or the combination ($N = 25,885$) of the 3 databases. Data from this study suggest that AC supplement was associated with a significant annualized and linear increase in BMD of 1.04% per year, 7.3% over the 7-year study period. These results stand in marked contrast to normative or expected changes of $-0.4\%/y$ from 3 different databases or in a combination of all 3 databases ($N = 16,289$).

Conclusions: No evidence was found in cardiovascular risk as measured by adverse changes in blood lipids, nor was any evidence found of a diminished efficacy over the 7-year study period because gains in BMD were consistent and linear over the 7-year study period, averaging 1.04% per year over the 7-year study. The results are also consistent with earlier short-term studies suggesting that this supplement can facilitate significant increases in total body BMD in contrast to studies suggesting that calcium supplements can only slow down age-related declines in BMD.

INTRODUCTION

This study is a follow-up to 2 previous comparative effectiveness research (CER) studies [1, 2] that found taking a vitamin/mineral enhanced plant-sourced calcium supplement, AlgaeCal (AC; Table 1), was associated with significant increases in bone mineral density (BMD) over a 6-month study period. In both studies, safety was assessed by examining baseline-ending changes in 45 blood chemistry measurements, a self-reported quality of life scale, and weekly reports of adverse effects and discomforts. No adverse effects were found in any of these 3 safety measures. The increases in age-related BMD stand in marked contrast to a number of studies and literature reviews suggesting that supplemental calcium and vitamin D_3 may slow down age-related declines in BMD but, rarely, if ever, facilitates increases in BMD [3–8].

Although in neither study [1,2] were comparisons made to a randomized controlled trial, both studies employed a

Table 1. Supplement facts of the AlgaeCal bone-health supplement.

Ingredient	Amount
Elemental Strontium (from citrate)	680 mg
Trace Minerals	1608 mg
Calcium	720 mg
Magnesium*	72 mg
Vitamin D3	1,600 IU
Vitamin K as MK-7	100 mcg
Boron	3 mg
Vitamin C	50 mg

*72 mg naturally occurring

number of controls and analyses designed to improve validity. For example, a procedure was employed to estimate volunteer bias by conducting BMD tests on a group of potential volunteers who had expressed interest in participating in a bone health study but without receiving the details of the study length and requirements. Upon completion of the BMD test, all study details, including the 6-month study period, were provided. Almost half of the potential volunteers declined to participate. No differences were found on BMD, fat mass, or fat-free mass between volunteers and non-volunteers. Using a procedure suggested by DeAngelis and Fontanarosa [9] to reduce concerns of bias in industry-sponsored research, an independent academic biostatistician from a local university served as the principal investigator, had full access to all study data and source documents, and took responsibility for the integrity of the data and the accuracy of the data analysis. To encourage candid reporting of product usage, instead of paying an incentive or participation fee, subjects were paid a "reporting fee" that was paid only for reporting daily product usage, even if there was no product used on that particular day. To examine drop-out biases, comparisons were also made between baseline measures of dropouts versus those who completed per protocol. No significant differences were found. Previous studies have concluded that poor compliance is the major obstacle to obtaining full benefit of calcium supplementation, suggesting that high compliance is needed to demonstrate the therapeutic efficacy of calcium supplementation [10]. Therefore, using data obtained from the reporting procedures discussed above, comparisons were made between compliant and partially compliant subjects. Compliant subjects had greater gains in BMD than partially compliant subjects, suggesting a dose-related effect.

Notwithstanding these procedures and controls, there remains the potential study or clinical trial bias existing in all research resulting from the artificial conditions created in explanatory or "biological" clinical trials (BCTs). BCTs examine how treatments or interventions work in carefully controlled settings and with restricted study populations, often to investigate a biological hypothesis or test a drug or device to meet regulatory requirements. One of the difficulties with BCTs is that the controls used to isolate the effects of the product often create relatively short-term conditions very unlike the real-world settings under which the product is likely to be used. In fact, just being in a study often influences behavior and compliance even before controls are implemented. Thus, product and treatment effects often do not transfer to the real world in which consumers are likely to purchase and/or use the product. To reduce the potential influences and artificial conditions of participating in a BCT and approach real-world conditions is at the core of pragmatic clinical trials (PCTs) [11–18] that have been receiving increased attention and funding. PCTs strive to examine the safety and efficacy of products and treatments as close as possible to the conditions under which consumers are likely to purchase and use the product over an extended period of time.

In response to concerns that long-term use of calcium supplements may have adverse cardiovascular effects [19], as a measure of safety, comparisons of baseline-ending changes in lipids and other blood chemistries were made between AC consumers and non-AC users over a 4-year period.

In summary, efficacy was evaluated by comparing 7-year BMD changes in subjects who consumed AC supplements with 7-year changes in 3 different databases. Additionally, the same comparisons were made between the AC subjects and an age-matched group of non-AC supplement users. To evaluate safety, similar comparisons were made using a 45-measurement blood chemistry panel over a 4-year study period.

METHODS

Over the past 22 years, Integrative Health Technologies, Inc. (IHTI) has conducted over 25,000 total body scans using GE Lunar's DEXA DPX-IQ and DPX-NT Bone Densitometers (Madison, WI). The software version for the DPX was DPX-NT enCORE 2003 Version 7.52.002, and for the IQ it was DPX-IQ X-Ray Bone Densitometer with SmartScanVersion 4.7e. Either the DPX or NT was randomly selected for measurement of baseline and ending BMDs. The manufacturer of these 2 versions of dual-energy x-ray absorptiometry (DEXA) units has reported that the interunit variability of these units is between 1.0% and 1.7%, which was confirmed by our own analyses. All tests used in this study were conducted by one of 2 certified technicians to reduce intertechnician variability. All subjects completing these tests executed an informed consent granting permission for their redacted data to be used for research. To obtain subjects for this study, the database was searched to identify healthy female adult women who reported

Table 2. Age, BMD, BMD T and Z scores, number S's with Osteopenia and Osteoporosis for Cohort and Each Study Group Year

Year	BMD Mean	BMD SD	n	Mean Age	SD Age	Mean BMD Z-Score	SD BMD Z-Score	Mean BMD T-Score	SD BMD T-Score	Number Osteopenia	Number Osteoporosis
Total	1.085	0.088	172	65.1	7.8	0.6	1.0	-0.5	1.1	55	3
2007	1.052	0.080	31	64.5	7.1	0.2	0.9	-0.9	1.0	15	1
2008	1.075	0.081	41	63.0	7.1	0.4	1.0	-0.6	1.0	14	1
2009	1.078	0.085	15	68.5	4.3	0.8	1.1	-0.6	1.1	5	0
2010	1.093	0.087	25	65.6	7.2	0.7	1.0	-0.4	1.1	7	0
2011	1.103	0.099	18	67.3	10.5	1.0	1.3	-0.3	1.2	5	0
2012	1.093	0.065	18	65.6	8.7	0.7	0.7	-0.4	0.8	3	1
2013	1.144	0.092	10	62.2	8.2	1.2	0.8	0.2	1.2	2	0
2014	1.108	0.095	14	66.8	7.1	0.9	0.9	-0.2	1.2	4	0

BMD=bone mineral density, SD=standard deviation, osteopenia=T-Score -1.00 to -2.49, osteoporosis=T-Score ≤ -2.50

that they had purchased and used AC for one to 7 years, had completed at least one DEXA total body scan, had not been taking bisphosphonates or other bone building medications, and could verify how much of the AC supplement they purchased and consumed. Only subjects who completed at least one DEXA BMD test at any time during the past 7 years were contacted and asked if they would volunteer to complete another complimentary BMD test and allow us to use redacted data. A total of 172 baseline-ending comparisons were acquired. A description of the ages, average BMDs, and number with osteopenia and osteoporosis for the cohort and each of the 7 years is presented in Table 2. Within-group baseline-ending BMD annualized changes were calculated for all consumers in which the times between the 2 testing periods varied from one to 7 years. Changes for each yearly group were plotted on a scatter diagram along with trend lines and *p* values of the significance of the trend line fit with the raw data. To establish expected or normal changes in BMD against which these data could be compared, BMD data were obtained from 3 databases: the Centers for Disease Control [20], GE Lunar Norms [21], and the IHTI longitudinal database. Between-group comparisons were made between annualized changes in the

treatment group and each of the 3 normative or expected change groups as well as with all 3 databases combined.

To provide a comparison with a reasonably similar control group who had also completed multiple DEXA tests, data were obtained from the same IHTI database of women who consumed a variety of dietary supplements other than AC over the same 7-year period of time. To approximate age-matching, a percentage of subjects of each age was calculated for the AC group. This same percentage of women of this age was then selected from the database. For example, if 10% of the women in the AC group were 54 years of age, the same percentage of 54-year-old women was then randomly selected from the database to form a non-AC control group. Following this procedure, a total of 2540 age-matched women were incorporated into this control group compared to 172 in the AC group. However, though the number of subjects of each age in this control group was greater than the numbers in the AC group, the percentages of subjects in each age group were identical.

To assess safety, a total of 125 AC subjects who had completed at least one of the 45-measurement blood chemistry panels shown in Table 3 were contacted and asked if they would complete a complimentary second blood test in return for using their data. The longest time period between tests for these groups was 4 years as opposed to the 7-year study period available for BMD measurements. In addition to analyzing within-group changes, comparisons were made with an aged-match group of females in the IHTI database ($N = 5070$) who had completed the same blood chemistry panels one to 4 years apart and had been regularly using dietary supplements, including calcium supplements, other than AC. Between-group comparisons were made between the 2 groups on all lipid measurements. Additional between-group comparisons were also made for changes in C-reactive protein (CRP) and serum glucose. However, unlike these 8 continuous variables, increases or decreases in the remaining measures could reflect either positive or adverse effects depending upon whether or not the changes in the subject's measurements went from "normal" to "abnormal" or *vice versa*. Measurements that were

Table 3. The Blood Chemistry Panel

|		
Total Cholesterol	Alkaline Phosphatse	Lymphocytes
HDL Cholesterol	ALT	MCH
Non-HDL Cholesterol	AST	MCHC
Chol/HDL Ratio	Basophils	MCV
LDL Cholesterol	Bilirubin, Total	Monocytes
Triglycerides	Bun/Creatine Ratio	Neutrophils
Cardio CRP	Calcium	Platelet Count
Glucose	Carbon Dioxide	Potassium
Absolute Basophils	Chloride	Protein, Total
Absolute Eosinophils	Creatinine	RDW
Absolute Lymphocytes	Eosinophils	Red Blood Cell Count
Absolute Monocytes	Globulin	Sodium
Absolute Neutrophils	GSR	TSH w/Reflex T-4, Free
Albumin	Hematocrit	Urea Nitrogen (BUN)
Albumin/Globulin Ratio	Hemoglobin	White Blood Cell Count
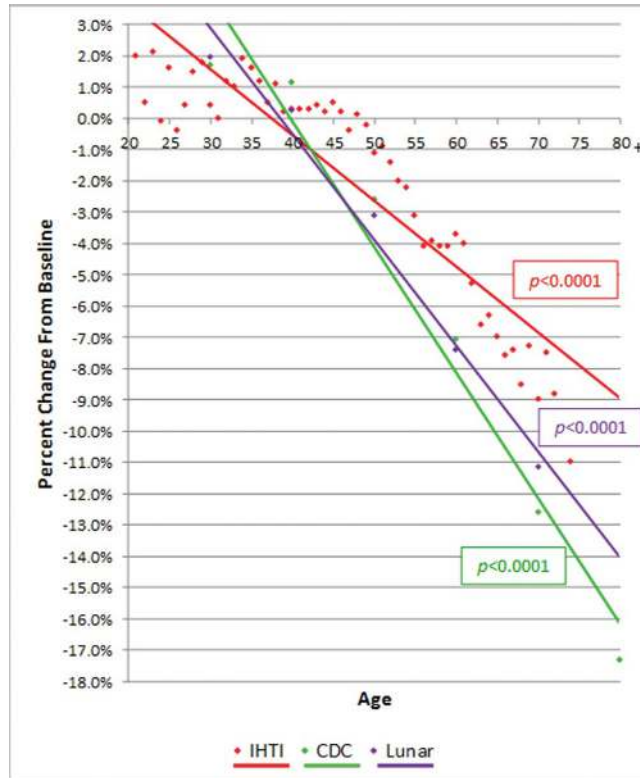
Fig. 1. A Trendline analysis of annualized changes in BMD for women aged 20–80+ from IHTI's longitudinal database ($N = 16{,}289$), the Center for Disease Control's database ($N = 8283$), and GE Lunar's database ($N = 1313$).

in either the normal or abnormal ranges on test 1 and remained in the same ranges on test 2 were deemed irrelevant with regard to positive or negative changes. Measurements that were in the abnormal range on test 1 but were normal on test 2 were assumed to reflect a positive change. Conversely, measurements that were in the normal range on test 1 but were subsequently in the abnormal range on test 2 were deemed to reflect a negative change or an adverse effect.
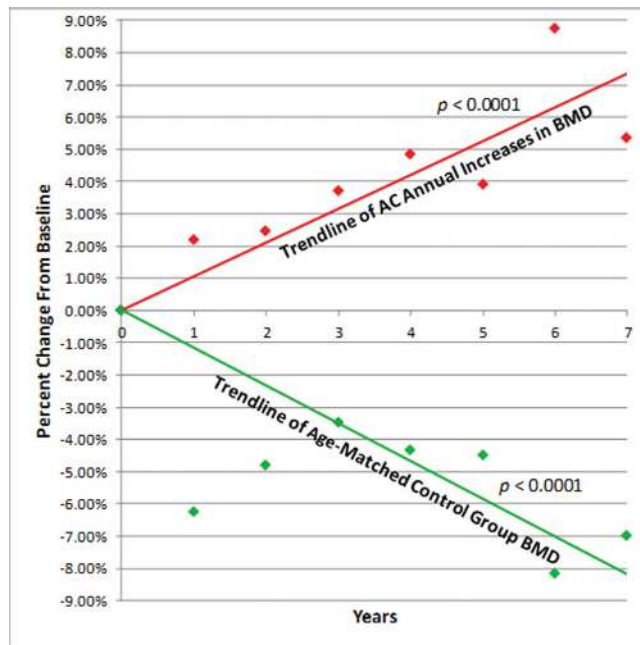

Fig. 2. Trendline comparisons between consumers taking AC and an age-matched control group.
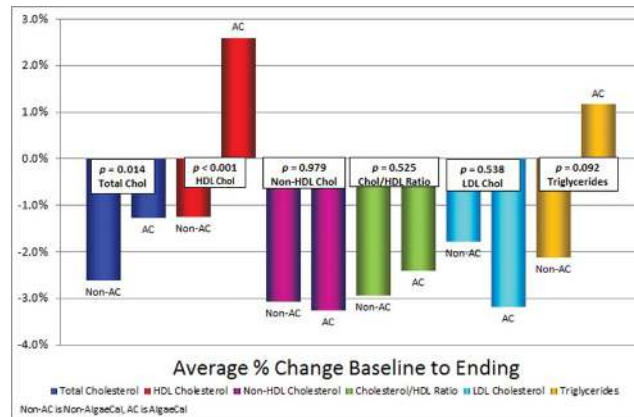
Fig. 3. Average percentage changes from baseline in a blood lipid panel for subjects taking nutritional supplements without AC (non-AC; $N = 5070$) and subjects taking AC supplements (AC; $N = 30$) for 4 years.

To compare changes in the AC group with expected changes for these measures, norms were obtained using the same tests from the IHTI database that were separated by at least one year, but no more than 4 years, for women of ages similar to those in the AC group. Calculations were made to obtain the percentage of measurements that were either normal or abnormal on both test 1 and test 2. The percentage was also calculated for those tests that were normal on test 1 but abnormal on test 2 and those that were abnormal on test 1 but were normal on test 2. These percentages were then compared between this group and the AC group.

RESULTS

Efficacy

To calculate expected average annualized changes in BMD for women, data were obtained from the 3 different databases and are shown in Fig. 1. All significance levels were calculated using a linear trendline statistic. The Centers for Disease Control database contained 8238 women from age 30 to age 80 in 10-year increments and only the plotted trendline was significant ($p < 0.0001$) and reflects an average annual decline of -0.51%/y. GE Lunar's database contained similar data in 10-year increments for 1313 women aged 30 to 79. The plotted trendline was significant ($p < 0.0001$) and reflects an average annual decline of -0.42%/y. The third trendline was derived from the principle researcher's (IHTI) longitudinal database of 16,289 women and are plotted for percentage changes in each year from age 20 through 75. The plotted trendline was significant ($p < 0.0001$) and reflects an average annual decline of -0.35%/y. annualized BMD change. An additional comparison was also made between the AC group and the 2540 age-matched subjects taken from the IHTI database for the same 7-year time period. In contrast to the 7.3% increase found in the AC group,

the age-matched non-AC group had a consistent linear decline of -8.5%, ($p < 0.001$) using a Student's t test—a difference of 15.8% between the 2 study groups. Fig. 2 presents a comparison of trendlines for annual changes in BMD found in the AC cohort and the age-matched control group over the 7-year study period. The AC group had an average linear annual increase of 1.04% per year, a total of 7.3% over the 7-year study period, compared to a progressive and linear decline in BMD of -1.17% a year, a total of -8.19%, for the age-matched control group. The trend lines for both groups were statistically significant ($p < 0.0001$).

Safety

The primary safety measure used in the study was to assess changes from baseline in the 45-measurement blood chemistry panel shown in Table 3. To address concerns suggesting that consumption of calcium supplements may have adverse cardiovascular effects, Fig. 3 provides comparisons of lipid changes from baseline between the AC group and non-AC group for 4 years where data were available for both groups. These data suggest that the non-AC group had a greater reduction in total cholesterol (TC) than the AC group, a difference that was statistically significant ($p = 0.014$). However, the AC group had a statistically significant ($p < 0.001$) increase in high-density lipoprotein (HDL) compared to the non-AC group. Once HDL is removed in the non-HDL cholesterol measurement, the TC measurements for each group are virtually identical ($p = 0.979$). This suggests that after correcting for the effect of HDL, taking AC for this 4-year period had no impact on TC once HDL is removed from the calculation. Despite the graphic representation, no statistically significant differences were found between baseline-ending changes in low-density lipoprotein or triglycerides. Additional within- and between-group comparisons were made between serum glucose levels and CRP. Compared to the non-AC group, greater reductions in

CRP and glucose were found in the AC group but failed to reach statistical significance.

Analyses of the remaining blood chemistry measurements required a different approach because increases or decreases could reflect either no effect, a positive effect, or a negative or adverse effect depending upon what impact the intervention had relative to changes in the normal or abnormal ranges of test 1. Measurements that were normal on test 1 and were also normal on test 2, as well as those that were abnormal on test 1 and also abnormal on test 2, suggested that the product had no effect.

In order to compare changes between the AC and a non-AC control group, baseline-ending changes for one to 4 years for all same-aged women who had also completed the same blood test panel were obtained from the IHTI database. Calculations were then made to determine the percentage of measurements that remained either normal or abnormal for each test pair. Percentages were then calculated for those tests that were normal on test 1 but abnormal on test 2 and those that were abnormal on test 1 but were normal on test 2. These percentages were then compared between the 2 groups. No changes were found in the test 1 ranges for 91.1% of the non-AC group and 93.1% of the AC group—a difference that was not statistically significant. Nor were any statistically significant differences found between the 4.14% of non-AC subjects and the 4.36% of AC subjects who were normal at baseline but became abnormal on the ending test. Finally, there were no significant differences between the 4.70% and 2.56% of subjects who were abnormal at baseline but became normal on the ending tests.

DISCUSSION

Efficacy

Population-based longitudinal studies suggesting that, starting at age 40, there is minor, but significant, annual bone loss [22–24] after which the decline remains about 1% [25]. Other studies suggest that after midlife there is an age-related yearly loss of bone of 1% [26–30], which is accelerated to 2% for up to 14 years around the age of menopause. More recently, another review has suggested that women will lose 35% to 39% of bone after achieving peak bone mass at ages 30–40 years [25–27], after which the decline remains about 1% [27–30]. However, as shown in all 3 databases, the rates of declining BMD for women are considerably below those noted in the above-referenced studies [22–30]. The databases suggest that after reaching a peak BMD between 35 and 40 years of age there is a progressive and generally linear annual decline in BMD at an average rate of ∼0.4%/y. No significant differences were found in this consistent linear change during the menopausal years as the National Osteoporosis Foundation data

suggest [31]. These discrepancies are worthy of further study and clarification.

As with the previous short-term studies of AC [1,2], the data from this study stand in marked contrast to age-related expected changes in BMD. The data suggest that consumption of the AC supplement over the 7-year study period resulted in a significant, consistent, and generally linear increase in BMD. These findings were observed in within-group changes from baseline as well as in the expected between-group comparisons in each of the 3 individual databases as well as in the combination of all 3 databases. Additionally, the comparisons with an age-matched group of women who were regular users of dietary supplements other than AC also revealed a marked contrast with these AC data.

Calcium supplementation is often prescribed with bisphosphonates becausee, as the National Osteoporosis Foundation points out osteoporosis medications do not work without calcium and vitamin D [32]. In view of this close, perhaps synergistic, relationship, questions have been raised as to whether or not 3- to 5-year use of calcium has similar long-term decrements in efficacy and safety. The progressive and linear increases in BMD found in each year of the 7-year study and the absence of adverse effects on blood chemistries discussed below appears to attenuate some of these concerns. These results also stand in contrast to the adverse effects and decrements in efficacy that have been reported with the 3- to 5-year use of bisphosphonates. For example, there is evidence that 3- to 5-year use of bisphosphonate is associated with atypical fractures of the femoral shaft [33]. In a follow-up and larger study, these researchers concluded that the risk of atypical femoral fracture increases progressively with the duration of use and that treating atypical femoral fractures with oral bisphosphonates may do more harm than good [34,35]. Other studies [36,37] also concluded 3- to 5-year use of bisphosphonates has led to heightened interest in interrupting or stopping bisphosphonate therapy after several years of treatment, a recommendation for a "drug holiday" [36–38].

Safety

One of the inherent flaws in using TC as an outcome measure is that increases are considered a negative outcome, whereas increases in HDL, a subfactor of TC, are considered a positive outcome. Thus, a positive HDL increase also results in a negative increase in TC. This conflict is illustrated by the cholesterol changes found in this study. As shown in Fig. 3, compared to the AC group, the non-AC group had a significantly greater decrease in TC ($p = 0.014$), suggesting that the non-AC group had a more positive outcome. However, compared to the non-AC group, the AC group had a positive increase in HDL ($p = 0.001$), resulting in an increase in their TC. Extracting HDL from the calculation of TC shown as

"non-HDL cholesterol" reveals that both groups had identical changes ($p = 0.979$). Thus, the most logical conclusion with regard to changes in TC is that there are no significant differences in TC between the 2 groups. These data also underscore the importance of calculating the non-HDL TC when assessing treatment effects.

An additional concern regarding safety is the potential long-term adverse cardiovascular effects of calcium supplementation reported in some studies [19]. These researchers concluded that calcium supplements, with or without vitamin D_3, are associated with modest increases in risk factors of cardiovascular disease [39]. Though the data that were the subject of this controversy were acquired over multiple decades, it is worth noting that no adverse effects were found in any of the lipid measurements acquired over this 7-year study period.

Future research needs to explore which of the ingredients, or interactions between these ingredients, in the AC supplement contribute to the reversal of age-related declines in BMD. There are data suggesting that each of the ingredients contained in the AC formula can support BMD, most notably strontium citrate [40,41]. Additionally, because AC contains a plant-sourced form of calcium, there are data suggesting that plant-sourced vitamins and minerals may be more easily absorbed than non-plant-sourced forms [42–44]. These studies also suggest that whereas the body was able to absorb only 10% of synthetic minerals contained in many brands of multivitamins, over 80% of minerals derived from plant sources were typically absorbed. Other studies have also reported positive associations between fruit and vegetable consumption and BMD in 5 age- and sex-matched cohorts [45], during the menopausal transition [46] in elderly adults [47,48], adolescents [49], and prepubertal children [50].

The strengths of this study include the use of a pragmatic clinical trial to obtain data under real-world conditions. To achieve this, data were collected from consumers who had purchased and used the product during the study periods without their awareness that their data would be used in a clinical trial. Other strengths include verifying consumer reports of product usage with the retailer from whom they purchased the product, using only subjects who were not taking bisphosphonates or bone-building pharmaceutical products, the 7-year duration of the study with data for each of the 7 years, comparisons with a similar large ($N = 2520$) age-matched control group who were regular users of dietary supplements other than AC, comparisons with expected changes derived from a combination of 3 large independent databases ($N = 25,885$), and the use of the same DEXA technology and technicians for baseline and ending measurements. Another strength of the study is that it provides previously unreported data on annual changes in bone density in a large ($N = 16,289$) national sample of women between the ages of 20 and 90 over a 30-year period of time, very few of whom were taking bone health medications. These data could be useful in estimating expected or normative changes.

Weaknesses of the study include the nonrepresentativeness of the study sample notwithstanding the fact that subjects resided in multiple states throughout the United States. Though the absence of a randomized controlled trial or placebo group for comparisons is another weakness, it should be noted that getting subjects to enroll in a 7-year study with a 50–50 chance of receiving a placebo poses a major challenge and may even raise some ethical issues.

CONCLUSIONS

This 7-year study of consumers who had taken the AC supplement was designed as a PCT in which safety and efficacy are studied under conditions as close as possible to the conditions under which consumers are likely to purchase and use the product. The evidence suggests that taking the AC supplement was associated with a significant, annual, and generally linear increase in BMD of 1.04% per year, 7.3% over the 7-year study period. These results stand in marked contrast to normative or expected changes of $-0.4\%/y$ from 3 different databases and a combination of all 3 databases ($N = 16,289$). Although not as dramatic as the initial short-term studies reported above, the evidence found in this study suggests that the AC supplement facilitated an increase, not a decrease, in age-related BMD. No evidence was found in cardiovascular risk as measured by adverse changes in blood lipids. Nor was any evidence found of a diminished efficacy over the 7-year study period, with data suggesting that the gains in BMD were consistent and linear over the 7-year study period. It also confirms earlier short-term studies suggesting that this supplement can facilitate significant increases in total body BMD in contrast to studies suggesting that calcium supplements can only slow down age-related declines in BMD.

FUNDING

REFERENCES

1. Michalek JE, Preuss HG, Croft HA, Keith PL, Keith SC, Dapilmoto M, Perricone NV, Leckie RB, Kaats GR: Changes in total body bone mineral density following a common bone health plan with two versions of a unique bone health supplement: a comparative effectiveness research study. Nutr J 10:32, 1–10, 2011.
2. Kaats GR, Preuss HG, Croft HA, Keith SC, Keith PL: A Comparative effectiveness study of bone density changes in women over 40 following three bone health plans containing variations of the same novel plant-sourced calcium. Int J Med Sci 8: 180–191, 2011.

3. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A: Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet 370:657–666, 2007.

4. Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, Bassford T, Beresford SAA, Black HR, Blanchette P, Bonds DE, Brunner RL, Brzyski RG, Caan B, Cauley JA, Chlebowski RT, Cummings SR, Granek I, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell FA, Johnson KC, Judd H, Kotchen JM, Kuller LH, Langer RD, Lasser NL, Limacher MC, Ludlam S, Manson JE, Margolis KL, McGowan J, Ockene JK, O'Sullivan MJ, Phillips L, Prentice RL, Sarto GE, Stefanick ML, VanHorn L, Wactawski-Wende J, Whitlock E, Anderson GL, Assaf AR, Barad D: Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 354:669–683, 2006.

5. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE: Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 337:670–676, 1997.

6. Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Amaud S, Garnero P, Meunier PJ: Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study. Osteoporos Int 13:257–264, 2002.

7. Zhu K, Bruce D, Austin N, Devine A, Ebeling P, Prince RL: A randomized controlled trial of the effects of calcium with or without vitamin D on bone structure and bone related chemistry in elderly women with vitamin D insufficiency. J Bone Miner Res 23:1343–1348, 2008.

8. Chel V, Wijnhoven HA, Smit JH, Doms M, Lips P: Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. Osteoporos Int 19:663–671, 2008.

9. DeAngelis CD, Fontanarosa PB: Ensuring integrity industry-sponsored research: primum non nocere, revisited. JAMA 303:1196–1198, 2010.

10. Prince RL, Devine A, Satvinder S, Dhaliwal S, Dick I: Effects of calcium supplementation on clinical fracture and bone structure: results of a 5-year, double-blind, placebo-controlled trial in elderly women. *J Arch Intern Med* 166:869–875, 2006.

11. Maclure M: Explaining pragmatic trials to pragmatic policy-makers. CMAJ 180:1001–1003, 2009.

12. Tunis SR, Stryer DB, Clancy CM: Practical clinical trials: increasing the value of clinical research for decision making in clinical and health policy. JAMA 290:1624–1632, 2003.

13. Glasgow RE, Riley WT: Pragmatic measures: what they are and why we need them. Am J Prev Med 45:237–243, 2013.

14. Johnson KE, Tachibana C, Coronado GD, Dember LM, Glasgow RE, Huang SS, Martin PJ, Richards J, Rosenthal G, Septimus E, Simon GE, Solberg L, Suls J, Thompson E, Larson EB: A guide to research partnerships for pragmatic clinical trials. BMJ 349: g6826, 2014.

15. Glasgow RE: What does it mean to be pragmatic? Pragmatic methods, measures, and models to facilitate research translation. Health Educ Behav 40:257–265, 2013.

16. Chalkidou K, Tunis S, Whicher D, Fowler R, Zwarenstein M: The role for pragmatic randomized controlled trials (pRCTs) in comparative effectiveness research. Clin Trials 9:436–446, 2012.

17. Macpherson H: Pragmatic clinical trials. Complement Ther Med 12:136–140, 2012.

18. Patsopoulos NA: A pragmatic view on pragmatic trials. Dialogues Clin Neurosci 13:217–224, 2011.

19. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR: Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ 341:c3691, 2010.

20. Centers for Disease Control and Prevention: Total body bone area, bone mineral content, and bone mineral density for individuals aged 8 years and over: United States, 1999–2006. Vital Health Stat Ser 11 253: 17–18, 2013.

21. Blake GM, Wahner HW, Fogelman I: "The Evaluation of osteoporosis: dual energy x-ray absorptiometry and ultrasound in clinical practice." Martin Dunitz, 1999.

22. Emaus N, Berntsen GK, Joakimsen RM, Fønnebø V: Longitudinal changes in forearm bone mineral density in women and men aged 25–44 years: the Tromsø Study: a population-based study. Am J Epidemiol 162:633–643, 2005.

23. Chapurlat RD, Garnero P, Sornay-Rendu E, Arlot ME, Claustrat B, Delmas PD: Longitudinal study of bone loss in pre- and peri-menopausal women: evidence for bone loss in perimenopausal women. Osteoporos Int 11:493–498, 2000.

24. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PW, Kiel DP: Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res 15:710–720, 2000.

25. Jones G, Nguyen T, Sambrook P, Kelly PJ, Eisman JA: Progressive loss of bone in the femoral neck of elderly people: longitudinal findings from the Dubbo Osteoporosis Epidemiology Study. BMJ 309:691–695, 1994.

26. Bainbridge KE, Sowers M, Lin X, Harlow SD: Risk factors for low bone mineral density and the 6-year rate of bone loss among premenopausal and perimenopausal women. Osteoporos Int 15:439–446, 2004.

27. Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK: Bone loss and bone size after menopause. N Engl J Med 349:327–334, 2003.

28. Wilsgaard T, Emaus N, Ahmed LA, Grimnes G, Joakimsen RM, Omsland TK, Bernstsen GR: Lifestyle impact on lifetime bone loss in women and men: the Tromso Study. Am J Epidemiol 169:877–886, 2009.

29. Guthrie JR, Ebeling PR, Hopper JL, Barrett-Connor E, Dennerstein L, Dudley EC, Burger HG, Wark JD: A prospective study of bone loss in menopausal Australian-born women. Osteoporos Int 8:282–290, 1998.

30. Sowers M, Crutchfield M, Bandekar R, Randolph JF, Shapiro B, Schork MA, Jannausch M: Bone mineral density and its change in pre- and perimenopausal white women: the Michigan Bone Health Study. J Bone Miner Res 13:1134–1140, 1998.

31. National Osteoporosis Foundation. Accessed at: http://nof.org/articles/235.

32. National Osteoporosis Foundation. "Clinician's Guide to Prevention and Treatment of Osteoporosis." Washington, DC: National Osteoporosis Foundation, 2005.

33. Schilcher J, Michaëlsson K, Aspenberg P: Bisphosphonate use and atypical fractures of the femoral shaft. N Engl J Med 364:1728–1737, 2011.

34. Schilcher J, Koeppen V, Aspenberg P, Michaëlsson K: Risk of atypical femoral fracture during and after bisphosphonate use. N Engl J Med 371:974–976, 2014.

35. Schilcher J, Koeppen V, Aspenberg P, Michaëlsson K: Risk of atypical femoral fracture during and after bisphosphonate use: full report of a nationwide study. Acta Orthop 86:100–107, 2015.

36. Gourlay ML, Ensrud KE: Bone density and bone turnover marker monitoring after discontinuation of alendronate therapy: an evidence-based decision to do less. JAMA Intern Med 174: E1–E2, 2014.

37. Bauer DC, Schwartz A, Palermo L, Cauley J, Hochberg M, Santora A, Cummings SR, Black DM: Fracture prediction after discontinuation of 4 to 5 years of alendronate therapy: the FLEX Study. JAMA Intern Med 174:1126–34, 2014.

38. Diab DL, Watts NB: Bisphoshonate drug holiday: who, when and how long. Ther Adv Musculoskelet Dis 5:107–111, 2013.

39. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR: Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. BMJ 19;342:d2040, 2011.

40. Meunier PJ, Roux C, Seeman E, Ortolani S, Badurski JE, Spector TD, Cannata J, Balogh A, Lemmel EM, Pors-Nielsen S, Rizzoli R, Genant HK, Reginster JY: The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med 350:459–468, 2004.

41. Reginster JY, Seeman E, De Vernejoul MC, Adami S, Compston J, Phenekos C, Devogelaer JP, Curiel MD, Sawicki A, Goemaere S, Sorensen OH, Felsenberg D, Meunier PJ: Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) Study. J Clin Endocrinol Metab 90:2816–2822, 2005.

42. Lanham-New SA: Fruit and vegetables: the unexpected natural answer to the question of osteoporosis prevention? Am J Clin Nutr 83:1254–1255, 2006.

43. New S, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee SJ, Reid DM: Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 71:142–151, 2000.

44. New SA: Intake of fruit and vegetables: implication for bone health—a review [published correction appears in Proc Nutr Soc 63:187, 2004]. Proc Nutr Soc 62:889–899, 2003.

45. Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, Prentice A, Ginty F: Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. Am J Clin Nutr 83:1420–1428, 2006.

46. MacDonald HM, New SA, Golden MH, Campbell MK, Reid DM: Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am J Clin Nutr 79:155–165, 2004.

47. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PW, Kiel DP: Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 69:727–736, 1999.

48. Tucker KL, Chen H, Hannan MT, Cupples LA, Wilson PW, Felson D, Kiel DP: Bone mineral density and dietary patterns in older adults: the Framingham Osteoporosis Study. Am J Clin Nutr 76:245–252, 2002.

49. McGartland CP, Robson PJ, Murray LJ, Cran GW, Savage MJ, Watkins DC, Rooney MM, Boreham CA: Fruit and vegetable consumption and bone mineral density: the Northern Ireland Young Hearts Project. Am J Clin Nutr 80:1019–1023, 2004.

50. Jones G, Riley MD, Whiting S: Association between urinary potassium, urinary sodium, current diet, and bone density in prepubertal children. Am J Clin Nutr 73:839–844, 2001.