Effects of the *AlgaeCal® Bone-Health Program* on Bone Mineral Density (BMD)

EXECUTIVE SUMMARY

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Context

Goals for Nutritional Research from the Office of Dietary Supplements. The Dietary Supplement Health and Education Act (DSHEA) of 1994 addressed growing public interest in the potential value of dietary supplements in maintaining optimal health and reducing the risk of disease. In conjunction with the DSHEA, the Office of Dietary Supplements (ODS) was established in the National Institutes of Health, Department of Health and Human Services. In January 2004, ODS re-stated its goals and strategies in its Strategic Plan, "*Promoting Quality Science in Dietary Supplement Research, Education, and Communication: A Strategic Plan for 2004-2009."* One of ODS's five scientific goals is to *"Evaluate the role of dietary supplements in the prevention of disease and reduction of risk factors associated with disease"* by stimulating research on:

- *"…how dietary supplements moderate, alter, or enhance metabolic, physiological, and psychological processes associated with maintenance or lack of optimal health"*
- *"…validation of the accuracy, sensitivity, and specificity of unique biomarkers of dietary supplement effects on known endpoints and their surrogates associated with specific chronic diseases, optimal health, and improved performance."*

Bone mineral density (BMD) is a specific biomarker related to *"chronic disease, optimal health and improved performance that can enhance metabolic, physiological, and psychological process"*. In the first-ever *Surgeon General's Report on Bone Health*, Surgeon General (SG) Dr. Richard H. Carmona warned that by the year 2020, half of all American citizens older than 50 will be at risk for fractures from osteoporosis and low bone mass if no immediate action is taken by individuals at risk, doctors, health systems, and policy makers. The *Report* also concluded:

- *About 20% of senior citizens who suffer a hip fracture die within a year of fracture*
- *About 20% of individuals with a hip fracture end up in a nursing home within a year*
- *Hip fractures account for 300,000 hospitalizations each year*
- *The direct care costs for osteoporotic fractures alone are already up to $18 billion each year, a number that is expected to increase if action is not taken now.*

The Surgeon General's Call to Action. In conjunction with the issuance of his *Bone Health Report*, the SG issued a "call to action" to the nutritional and healthcare industry to develop bone-health programs that incorporate the three basic components of an effective program:

1. improved nutrition
2. increased physical activity, and
3. improved health literacy.

The SGs recommendation to improve nutrition, increase physical activity and improve health literacy, reflects the National Osteoporosis Foundation's (NOF) recommendations to achieve improved bone health by getting the recommended amounts of calcium and vitamin D, by increasing exercise, and by having a bone density test.

To decrease the likelihood of developing osteoporosis, the SG recommended that people of all ages improve their diets to insure they are getting the recommended amounts of calcium and vitamin D. The SG suggested that for those individuals not getting enough calcium and vitamin D in the diet, *"…supplementation may be helpful."* He concluded that *"America's bone health is in jeopardy"* due to the absence of adequate nutrition during critical bone-building years and that *"… too little of what has been learned thus far about bone health has been applied in practice."* He further stated,

> *"…just 30 years ago when I was a young medical student, we all believed that weak bones and osteoporosis were a natural part of aging. But today we know they are not. We can do a lot to prevent bone disease. Everyone has a role to play in improving bone health, and this report is a starting point for national action on bone health. Let's get started by taking action today in homes, health care settings, and communities across our nation. Remember, you are never too old or too young to improve your bone health."*

In addition to improved diets, the SG recommended that people of all ages increase their daily physical activity levels with a goal of maintaining or increasing lean body mass, particularly bone mass.

The third component of the SGs "call to action" was the recommendation to improve health literacy as a strategy for improving bone health. Health literacy is defined as the degree to which individuals have the capacity to obtain, process, and understand basic information and services needed to make appropriate decisions about their health. Studies have shown that health literacy is a strong predictor of health status. Inadequate health literacy can lead to numerous negative effects on an individual's health and well-being, including poor self-care, increased utilization of health services, worse outcomes, and less likelihood of receiving preventive care and services. The SG suggested that "*Promoting health literacy is perhaps the most important role of any health professional."*

Objective

The objective of this study was to respond directly to the SG's "call to action" by developing a bone health plan (herein named the "Plan") based on the three components suggested by the SG, and to subsequently test the plan by examining changes in BMD during a six-month study period among adults following two different version of the Plan.

Design

Design. This study was designed as an open-label practical clinical trial or pragmatic clinical trial (PCT) in accordance with the guidelines set forth by Tunis et al. in a *Journal of the American Medical Association* manuscript, "Increasing the Value of Clinical Research for Decision Making in Clinical and Health Policy." As these researchers pointed out, PCTs differ from traditional explanatory clinical trials in that:

> "*Clinical trials designed to assist health care decision makers, referred to as pragmatic clinical trials or practical clinical trials (PCTs), are defined as trials for which the hypothesis and study design are formulated based on information needed to make a decision. They are distinguished from explanatory clinical trials, for which the goal is to better understand how and why an intervention works. Explanatory trials are designed to maximize the chance that some biological effect of a new treatment will be revealed by the study. The PCTs address practical questions about the risks, benefits, and costs of an intervention as they would occur in routine clinical practice. The most distinctive features of PCTs are that they select clinically relevant interventions to compare, include a diverse population of study participants, recruit participants from a variety of practice settings and collect data on a broad range of health outcomes.*"

PCTs are often used to decide between non-pharmacological alternatives when decisions are being made as to what recommendations the physician can make to improve patient satisfaction and costs of treatment. If effective in facilitating positive changes in bone density, the results could provide useful information to enhance pharmacological and non-pharmacological treatment programs for people of all ages.

Setting. Participants were free-living adults in their normal living out-patient environments.

Study Participants. Participants were asked to consult with their physicians or healthcare providers before enrolling in the study to insure that they had no medical issues or problems that would have excluded their participation. Enrolled subjects who completed the baseline and 6-month test, who may or may not have completed the 90-day test, and who remained in compliance with the plan, were defined as having completed the study per protocol (PP). Of the 354 screened subjects, 176 completed the study PP. Of the 354 screened, 138 were lost to follow-up and 216 were enrolled. Of the enrolled, 40 dropped after the 90-day test.

Interventions

All participants completed a baseline DEXA screening test and a total of 216 began the study. All participants were provided with (1) a pedometer-based physical activity program, (2) a *Health Literacy Notebook*, (3) a strontium citrate (680 mg) supplement, and (4) one of two versions of the AlgaeCal bone health dietary supplement (AlgaeCal-1 or AlgaeCal-2) containing a plant-sourced form of calcium and other nutrients as shown in Table 1. Although all subjects received the same bone health plan and strontium citrate, they were blinded with regard to the difference between the two supplements. Detailed information on each component of the plan is provided in the study Technical Report.

As opposed to laboratory-derived and inorganic supplements, the *AlgaeCal*® calcium [DN0361 plant mineral complex] occurs naturally in sea algae found on the South American coastline. The sea algae is harvested live and cold-processed to help preserve the characteristics of its phytonutrients. *AlgaeCal* contains 73 minerals, of which 28-31% is calcium and 8-10% is magnesium with a 9.5 pH. Two independent laboratories have provided certificates of analysis supporting the presence and amounts of nutrients listed in the table below, and have certified that the product is free of heavy minerals and contaminating ingredients. In addition to complying with California's Proposition 65 and EPA standards, sea algae has been marketed as a dietary ingredient or supplement in the United States before October 15, 1994. Because it has been present in the food supply and has not been chemically altered, sea algae does not require FDA notification as a "new dietary ingredient."

Table 1. Ingredients and Components of Bone-Health Program

Ingredient or Component	*AlgaeCal-1*	*AlgaeCal-2*
Pedometer-base Activity Program	**YES**	**YES**
Health Literacy "Living @ Goal Weight" Program	**YES**	**YES**
Strontium Citrate (2,230 mg, 680 mg elemental strontium)	**YES**	**YES**
AlgaeCal Bone-health Supplement	**2,400**	**2,520 mg**
Trace minerals from AlgaeCal (mg)	**1,362 mg**	**1,414 mg**
Calcium (mg)*	**672 mg**	**756 mg**
Magnesium (mg)**	**216 mg**	**350 mg**
Vitamin D-3 (IU)	**400 IU**	**1,600 IU**
Vitamin K-2 as MK-4 (mg)	**1.5 mg**	**none**
Vitamin K-2 as MK-7 (mcg)	**none**	**100 mcg**
Boron (mg)	**none**	**3.0 mg**
Vitamin C (mg)	**none**	**50 mg**

***Naturally occuring from AlgaeCal plant source **From AlgaeCal + Magnesium carbonate**

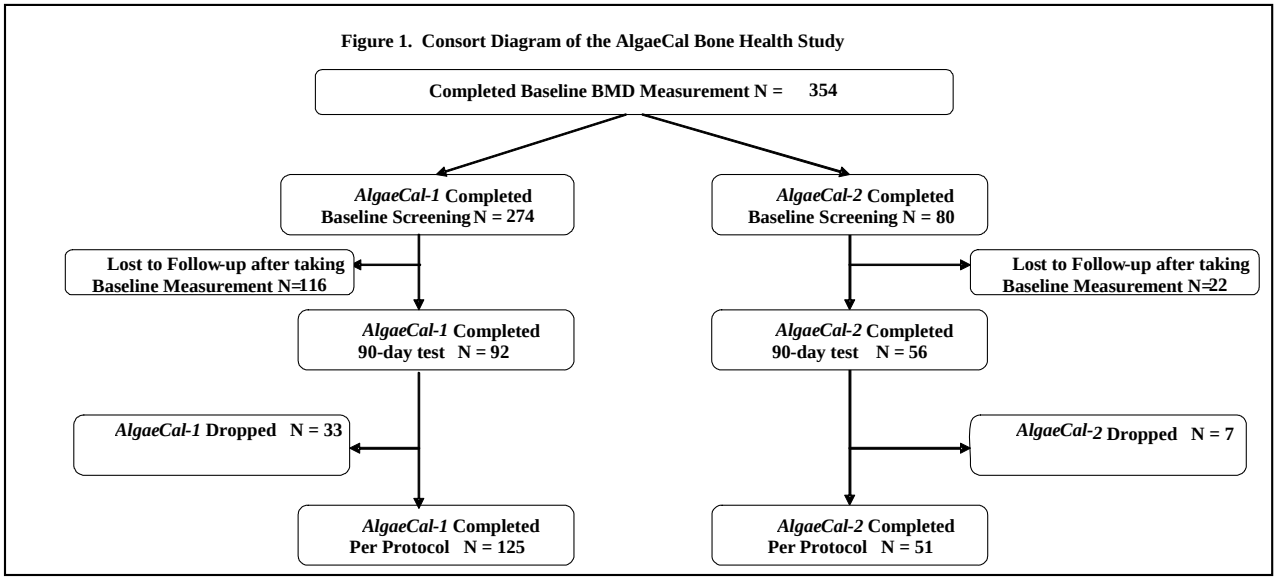
During the study, in the Fall of 2006, the sponsor decided to add additional nutrients and nutrient amounts to the AlgaeCal-1 capsules and replace vitamin K-2 as MK-4 with vitamin K-2 as MK-7 to examine the effect this new AlgaeCal-2 product had on BMD. These changes are shown in Table 1. All other study conditions were held constant. Considerable effort was devoted to

retaining identical conditions for both versions of the AlgaeCal supplements to allow unbiased contrasts between the two. No changes were made in the physical activity plan, the health literary plan, the amounts of strontium citrate participants were provided, in the packaging, or in the recruiting and enrolling procedures.

A total of 354 study participants were screened from April 4, 2006 through May 14, 2007 and flowed through the study as shown in the consort diagram in Figure 1 that depicts:

- total number (N=354) who completed the initial screening test,
- the numbers that took the different versions of the AlgaeCal supplement (AlgaeCal-1, n=274 or AlgaeCal-2, n=80) as described below,
- those who were "lost to follow-up" when they chose not to participate after completing the initial screening test (n=116 in the AlgaeCal-1 group and n=22 in the AlgaeCal-2 group),
- those who continued in the study, some of whom completing a 90-day BMD test (AlgaeCal-1: n=92 and AlgaeCal-2: n=56),
- those who subsequently dropped out after starting the study (AlgaeCal-1: n=33 and AlgaeCal-2: n=7), and
- and those who ultimately completed the study as Per Protocol (AlgaeCal-1: n=125 and AlgaeCal-2: n=51).

Figure 1. Consort Diagram of the AlgaeCal Bone Health Study



Bone Mineral Density (BMD). Changes in body composition and bone mineral content were measured using Dual Energy X-ray Absorptiometry (DXA) at baseline, 90 days, and at six months (180 days) from baseline. Compliance to the product usage was measured using participants' daily tracking forms, a post-study "anonymous" questionnaire to report actual product usage and a blinded subjective evaluation by the research technician(s) with whom the subject had the most frequent contact. For each subject, compliance was rated by the research technician using a five point scale with 5 indicating near-perfect compliance and 1 reflecting poor compliance with the protocol. Participants with a 5 rating were classified as "Highly Compliant" and those with scores of 1, 2, 3 or 4 as "Not Highly Compliant".

Use of Annualized Change in BMD. In order to facilitate ease of comparing changes in BMD during studies of different study periods, a common convention is to annualize changes in BMD to one year. Thus, a two-year study would divide the observed changes by two and a six-month study would double the observed changes. We employed this annualized convention when reporting the results of this study.

Expected Change in BMD. A number of studies have established normal or expected changes in BMD with age. In general, BMD increases with age until about the mid-thirties, remains constant for a few years and then progressively declines. The expected decline for women is about 1% per year and is about one-half a percent for men. Since the participants in this study ranged from 18-85 and were a mixture of males and females, we used norms provided by the National Osteoporosis Foundation (www.nof.org) to calculate the expected decline in BMD for each person in the study based on their age and sex, and calculated the average for all participants.

Hypotheses. The hypotheses of interest are

a) Subjects in both treatment groups (AlgaeCal-1, AlgaeCal-2) would experience increased mean BMD relative to the expected increase based on age- and sex-adjusted national norms
b) Subjects in both groups would experience an increased mean BMD from baseline,
c) Subjects taking AlgaeCal-2 would experience a greater mean increase in mean BMD than those taking AlgaeCal-1
d) Highly Compliant subjects in both groups would experience a greater increase in mean BMD than Not Highly Compliant subjects
e) Among Highly Compliant subjects, those taking AlgaeCal-2 would experience a greater mean increase in mean BMD than those taking AlgaeCal-1
f) Among Highly Compliant subjects, those taking AlgaeCal-2 would experience a greater mean increase in mean BMD over expected than those taking AlgaeCal-1
g) Among subjects Not Highly Compliant subjects, those taking AlgaeCal-2 would experience a greater increase in mean BMD than those taking AlgaeCal-1.

Statistical Methods. Treatment groups (AlgaeCal-1, AlgaeCal-2) were contrasted on the mean of continuously distributed outcomes with analyses of covariance with adjustment for age and sex. Treatment group contrasts with regard to binary outcomes were made with Pearson's chi-square. All statistical testing was 2-sided with a significance level of 5%. SAS Version 9.1.3 for Windows (SAS Institute, Cary, North Carolina) was used throughout.

Results

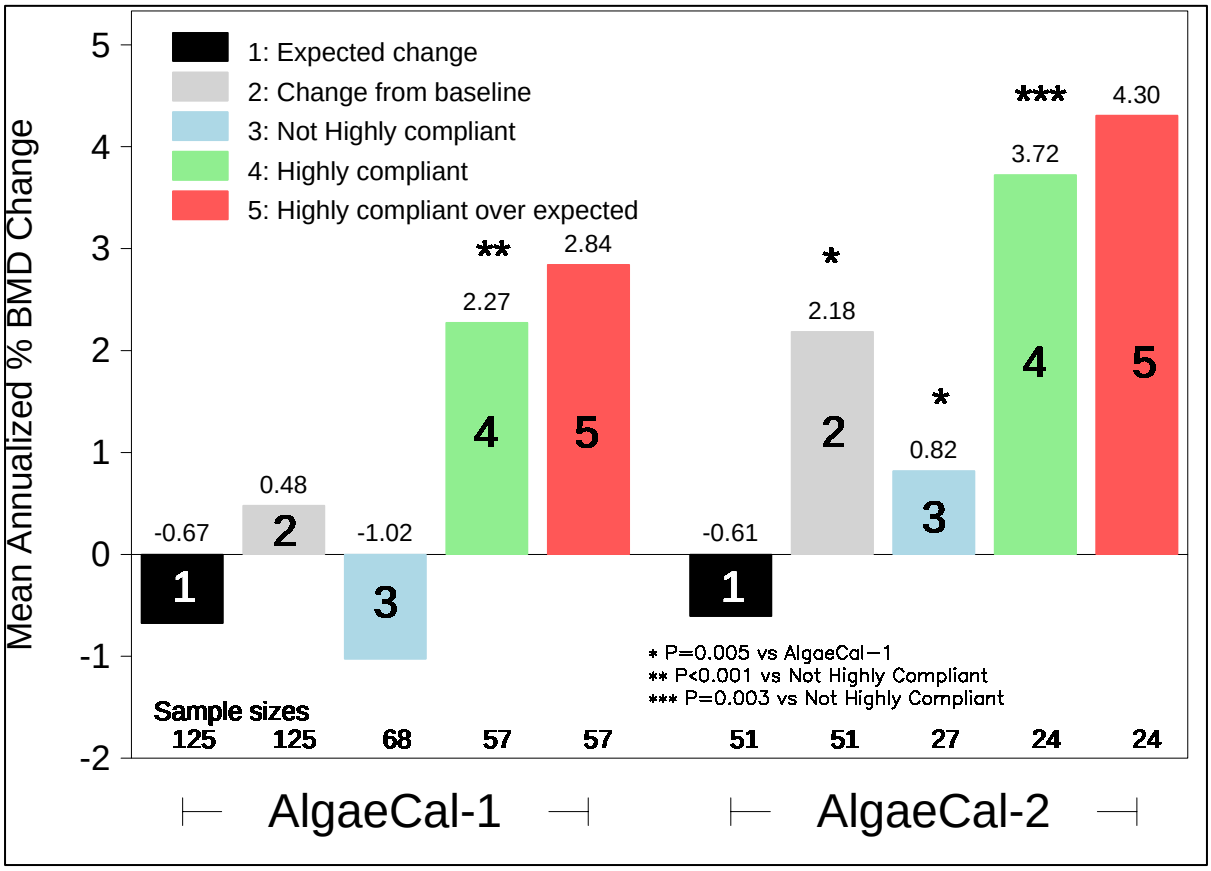
Summary of Findings. The results of this study provide evidence that following the AlgaeCal Bone Health plan per protocol taking either AlgaeCal-1 or Algae-Cal 2 (the version currently being marketed) can facilitate positive increases in mean BMD. The primary findings were as follows:

- Both groups experienced greater mean increases in BMD than expected based on age-adjusted national norms
- The mean increase in BMD over expected in subjects taking AlgaeCal-2 was significantly greater than the mean increase BMD over expected in subjects taking AlgaeCal-1

- Subjects taking AlgaeCal-2 experienced significantly greater mean increases in BMD than those taking AlgaeCal-1
- In both groups, subjects Highly Compliant to the Plan significantly out-performed those Not Highly Compliant to the Plan with regard to the mean change in BMD.

As shown in Figure 2, the mean expected annual change for among Per Protocol subjects was –0.67% for AlgeaCal-1 and –0.61% for AlgaeCal-2. Thus, if participation in the study had no effect on BMD, one could expect that the AlgaeCal-1 group would have an average decline in BMD of 0.67% and the AlgaeCal-2 group would have an average decline in BMD of 0.61%, as depicted in Bar 1 within each treatment group. These two expected annual changes [AlgaeCal-1: –0.67 (0.45), AlgaeCal-2: –0.61 (0.46)] were not significantly different from each other (p=0.73).

Figure 2. Annualized Percent Changes in Bone Mineral Density in Per Protocol Subjects by Treatment Category and Compliance Status



Hypothesis a)

The mean of the annualized percent change in BMD minus the expected annualized percent change in BMD was significantly greater than zero in both groups [AlgaeCal-1: 1.15 (3.62), p<0.001; AlgaeCal-2: 2.79 (3.57), p<0.001).

Hypothesis b)
The mean of the annualized percent change in BMD was significantly increased from baseline in subjects taking AlgaeCal-2 but not in subjects taking AlgaeCal-1 [AlgaeCal-1: 0.48 (3.64), p=0.14; AlgaeCal-2: 2.18 (3.58), p<0.001].

Hypothesis c)
The mean change in BMD from baseline subjects taking AlgeaCal-2 was significantly increased relative to the mean change in those taking AlgaeCal-1 (AgaeCal-1: 0.48 (3.64), AlgaeCal-2: 2.18 (3.58), p=0.005), as represented in Bar 2 within each of the two treatment groups in Fig 2.

Hypothesis d)
Among subjects taking AlgaeCal-1, the mean change in BMD was significantly increased in those who were Highly Compliant relative to those who were not Highly Compliant (Highly Compliant: 2.27 (3.62), Not Highly Compliant: −1.02 (2.92), p<0.001), indicated by Bars 3 and 4 within the AgaeCal-1 group in Figure 2.

Among subjects taking AlgaeCal-2, the mean change in BMD was significantly increased in those who were Highly Compliant relative to those who were Not Highly Compliant (Highly Compliant: 3.72 (3.96), Not Highly Compliant: 0.82 (2.58), p=0.003), indicated by Bars 3 and 4 within the AlgaeCal-2 group in Figure 2.

Hypothesis e)
Among Highly Compliant subjects, the mean change in BMD was increased among those taking AlgeaCal-2 relative to those taking AlgaeCal-1, but this increase did not reach statistical significance (AlgaeCal-1: mean=2.27 (3.62), AlgaeCal-2: mean=3.72 (3.96); p=0.12), as indicated in Bar 4 within each treatment group in Figure 2.

Hypothesis f)
Among Highly Compliant subjects, the mean change in BMD over expected was increased among those taking AlgeaCal-2 relative to those taking AlgaeCal-1, but this increase did not reach statistical significance (AlgaeCal-1: 2.84 (3.68), AlgaeCal-2: 4.30 (3.98); p=0.12), as indicated in Bar 5 within each treatment group in Figure 2.

Hypothesis g)
Among Not Highly Compliant subjects, the mean change in BMD was significantly increased among those taking AlgeaCal-2 relative to those taking AlgaeCal-1 (AlgaeCal-1: −1.02 (2.92), AlgaeCal-1: 0.82 (2.59); p=0.005), as indicated in Bar 3 within each treatment group in Figure 2.

Potential Bias from Selective Enrollment and/or Attrition. A number of previous studies have found that BMD among women typically decreases more rapidly than men and, irrespective of sex, BMD has been found to decrease with age. Thus, it would be more difficult to affect an increase in BMD in older women as compared to younger men which could bias the results if older women were less likely to complete the study per protocol. A similar potential bias exists with body weight, BMI, and lean mass levels, all of which could increase BMD irrespective of effects of the plan. Thus, it is possible that selective attrition could bias the outcome results. Because the assignment of participants into the AlgaeCal-1 or AlgaeCal-2 groups was determined by

enrollment date, it was important to determine if the two groups were, in fact, different with regard to these baseline demographics and body composition measurements.

Referring to Figure 1, group contrasts with regard to baseline demographics (age and sex) and body composition measurements (weight, BMI, BMD) among subjects who completed the plan PP revealed no statistically significant treatment differences between treatment groups (AlgaeCal-1, AlgaeCal-2) with regard to the percent female or mean age, weight, BMI, BMD, or expected BMD change.

There were significant differences at baseline between subjects who were lost (n=138) and those who completed PP (n=176) and between those who were enrolled but dropped (n=40) and those who completed PP. Subjects who were lost or who were enrolled and dropped did not differ significantly from those who completed PP with regard to the percentage female.

- Subjects who were lost and those who were enrolled but dropped were significantly younger than those who completed PP [Lost: 48 (12.4), Dropped: 46.2 (12.7), PP: 55.6 (11.8), Lost vs PP: $p<0.001$, Dropped vs PP: $p<0.001$].
- Subjects who were lost and those who were enrolled but dropped were significantly heavier than those who completed PP [Lost: 187.1 (50.6), Dropped: 189.8 (51.1), PP: 162.9 (44.0), Lost vs PP: $p<0.001$, Dropped vs PP: $p=0.001$].
- Subjects who were lost and those who were enrolled but dropped had a significantly greater mean BMI than those who completed PP [Lost: 31.4 (8.3), Dropped: 32.2 (8.2), PP: 27.0 (7.2), Lost vs PP: $p<0.001$, Dropped vs PP: $p<0.001$].
- Subjects who were lost and those who were enrolled but dropped had a significantly greater mean BMD than those who completed PP [Lost: 1.18 (0.09), Dropped: 1.23 (0.09), PP: 1.13 (0.11), Lost vs PP: $p<0.001$, Dropped vs PP: $p<0.001$].
- Subjects who were lost and those who were enrolled but dropped had a significantly greater mean expected BMD than those who completed PP [Lost: 1.18 (0.1), Dropped: 1.22 (0.09), PP: 1.13 (0.11), Lost vs PP: $p<0.001$, Dropped vs PP: $p<0.001$].
- The percentage of subjects who were lost and of those who were enrolled but dropped who were female did not differ significantly from those who completed PP [Lost: 89.9%, Dropped, 87.5%, PP: 84.1%, Lost vs PP: $p=0.14$, Dropped vs PP: $p=0.59$].

Comments

This study resulted from a decision by the sponsor to change the formulation of AlgaeCal, a dietary supplement designed to increased Bone Mineral Density (BMD), approximately midway through a one arm open label study. The hypothesis of interest was that the second formulation, AlgaeCal-2, would be superior to AlgaeCal-1. The primary endpoint was the average annualized change in BMD. Three hundred fifty four subjects were screened, and 216 completed 180 days of follow-up PP (AlgaeCal-1: n=125, AlgaeCal-2: n=51). During the study, the research staff rated subjects as Highly Compliant or Not Highly Compliant. Both groups experienced a significant increase in BMD relative to the age and sex adjusted expected BMD change. The mean change relative to the expected change was significantly increased in AlgaeCal-2 relative to AlgaeCal-1. On the average, BMD increased significantly more in subjects taking AlgaeCal-2 than in those

taking AlgaeCal-1. In both groups, subjects Highly Compliant to the Plan out-performed those Not Highly Compliant to the Plan.

The study was limited by not being randomized and single-blinded with regard to the type of AlgaeCal supplement (study subjects did not know the difference between the two formulations, but study staff knew the treatment assignments). Subjects who were lost to follow-up and those who dropped out were significantly younger on the average, heavier on the average, had a greater mean BMI, and a greater mean BMD than those who completed PP. It is worth noting that these differences worked against the reported outcome results because mean BMD decreases with age and PP subjects were on the average older than those who were lost or dropped out.

Study strengths included good quality control, a lack of significant baseline differences between treatment groups (AlgaeCal-1, AlgaeCal-2) among those who completed the study PP, and a plausible pattern of increased mean BMD among Highly Compliant subjects relative to Not Highly Compliant subjects regardless of the AlgaeCal supplement taken.

A randomized double-blind trial might be considered to replicate and confirm these results. Any confirmatory trial would necessarily include an active rather than a placebo control. This study was designed to evaluate the combined effects of a three-component Plan (improved nutrition, increased physical activity and increased health literacy) as a pragmatic or practical trial. Therefore, it would not be possible to provide a placebo control for each of the three components of the Plan. Because all components of the plan were held constant when comparing AlgaeCal-1 with AlgaeCal-2, these data suggest that AlgaeCal-2 is superior to AlgaeCal-1. These findings should be confirmed by a randomized non-placebo controlled double-blind trial to isolate the effects of the supplement separate and apart from the potential interactive effects of the other components of the Plan.