The Osteoporosis Education Project
Susan E. Brown, Ph.D.
Director

working with nature to regenerate bone health

A Pilot Study Comparing AAACA and Calcium Citrate Supplementation in Menopausal U.S. Women

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SUMMARY

Over the years there has been considerable attention given to the use issue of calcium effectiveness and bioavalability. In this small (N11), four week pilot study, the novel Japanese form of calcium (AAACa) was compared with calcium citrate. The end points of this pilot study were alterations to bone resorption, intact parathyroid hormone and first morning urine pH.

In each case, the subject received 900 mg of elemental calcium in a dosing regiment of 150 mg with each meal, and 450 mg at bedtime. This dosing regimen was likely an important factor in producing a consistent reduction in bone resorption within only four weeks.

In this small study formal statistical analysis was not able to detect a significant difference between the effects of these two forms of calcium on any end point studied.

From a case study perspective, however, there was a trend favoring AAACa over calcium citrate in reducing bone resorption markers. Specifically:

- AAACa subjects as a group began with higher Dpd bone resorption markers than calcium citrate group (M 9.77 as compared to M 9.36) and ended with lower markers (M 5.4 compared to M 5.84).
- The average reduction in Dpd from the beginning of the study to the end was greater in those using AAACa (M - 4.37) than those using calcium citrate (- 3.52).
- The average percentage change was - 43% for AAACa and - 33% for calcium citrate.
- Using a 26% reduction in Dpd as statistically significant, all women using AAACa experienced a significant 26% decrease in Dpd. Only two of the five women on calcium citrate experienced such a 26% reduction.
- While the group given AAACa began with a higher average Dpd reading, three of these six subjects reduced their Dpd level to near or below the ideal pre-menopausal level. On the other hand, none of the women on calcium citrate came to so closely approximate the ideal pre-menopausal level.

In regard to fasting iPTH, neither supplement was able to reduce the parameter in this short four week study. Those using calcium citrate experienced a sizable increase in iPTH, while those using AAACa remained stable. As for first morning urine pH, there was no significant change detected from the use of either supplement.

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INTRODUCTION

Statement of Problem

Numerous, but not all, studies conducted in the U.S. report low calcium intakes to be associated with lower bone density and increased risk of fracture at all ages. Further, studies on various segments of the US population suggest that calcium supplementation can increase bone density and reduce fracture risk. A comprehensive review of published studies suggests that simple calcium supplementation (often accompanied by vitamin D supplements) results in a 30 to 70% reduction in fracture rates over two to four years (Prince, 1997). Those with inadequate calcium intakes appear to benefit most from calcium supplementation (Dawson-Hughes, 1990, Dawson-Hughes, 1991).

While a variety of nutrients are important to bone health (Brown, 2000), calcium is the nutrient most associated with osteoporosis. Given its predominant place in the prevention and treatment of osteoporosis, the bioavailabilty and effectiveness of different calcium sources is a topic of considerable interest. Simply, an important question of concern is, "Which form of calcium is best?"

Distinctions of this Study

This pilot study comparing the Japanese AAACa calcium source with calcium citrate is of special interest for several reasons. First, while AAACa has been widely studied in Japan, this is the first study testing the efficacy of AAACa on an other-than-Japanese population. Second, in the U.S. calcium citrate is generally held as the "gold standard" of calcium sources, having been found more bioavailable than calcium carbonate. Adding a new dimension, this pilot study compares the efficacy of the novel Japanese calcium source, AAACa, with calcium citrate. Thirdly, this study is of note because of its short duration of four weeks. Most commonly changes in bone resorption are studied over six to twelve months. Recently, however, selected researchers have reported rapid reduction in bone resorption with certain calcium supplement programs (Scorpacasa et al., 2000; Fujita et al., '97; Ohgitani et al., '98). This study was designed to uncover the minimal amount of time needed to predictably see a reduction in bone resorption from calcium supplementation in postmenopausal women on low calcium diets. Also this study looked at week by week changes in bone resorption. These close interval, serial measurements provides insights into the reliability and variability of Dpd as a bone resorption marker. Finally, in this study we used a unique scheduling of calcium supplementation which attempts to compensate for the concomitant nocturnal rise in PTH and bone resorption observed in menopausal women (Lakatos, et al., '95; Tohme et al., '90; Ledger et al., '95; Fujita et al., '97; Fujita et al., '95) .

Review of Prior Science

It has long been known that different sources of calcium provide different levels of elemental calcium and have varying degrees of bioavailabity and effectiveness. Calcium carbonate, is the most concentrated, widely used and least expensive form of calcium. Calcium carbonate has been compared with other calcium sources including calcium citrate and the Japanese product known as AAACa.

A series of studies have shown that calcium citrate is more bioavailable and more effective than calcium carbonate. In the mid 1980's Nicar and Pak reported that calcium is better absorbed from citrate than from carbonate (Nicar and Pak, 1985). A mixed calcium citrate-malate salt, known as calcium citrate malate, was also found to be better absorbed that calcium carbonate (Smith et al., '87; Miller et al., '88). Subsequent studies by Bess Dawson Hughes demonstrated that calcium citrate malate was more effective at reducing bone loss than was calcium carbonate (Dawson-Hughes et al., 1990). Further refinement from Recker (1985) demonstrated that in subjects with decreased gastric acid secretion, calcium from carbonate is bioavailable only when it is taken with meals, whereas calcium citrate is absorbed by achlorhydric patients in both fasting and fed states.

More recently a series of studies have shown that the novel Japanese calcium source, AAACa, is both more bioavailable and more effective than the popular calcium carbonate. (AAACa is oyster shell heated under reduced pressure to which has been added a heated algal ingredient). In 1996 a small study of normal males suggested AAACa was more highly absorbed than was calcium carbonate (Fujita, '96 [A]). Later a four-month study by Dr. Fujita and colleagues found AAACa to increase trabecular bone density more effectively than calcium carbonate (Fujita et al., 2000).

In another two year study, 58 hospitalized women, mean age 82, were given 900 mg calcium as either calcium carbonate or AAACa. In this study also, AAACa was more effective at halting bone loss and increasing bone density than was calcium carbonate (Fujita, 1995, Fujita et al., '96 [B]). These two latter studies also documented that AAACa is more effective than calcium carbonate at lowering parathyroid hormone in those with osteoporosis than was calcium carbonate (Fujita, 1995, Fujita et al., '96 [B]). Further, two small, one week studies, one with healthy young females (12 - 19 yrs of age) and the other with healthy males (45 - 59 yrs of age) showed AAACa to lower parathyroid hormone more effectively than milk or placebo respectively (Shigeki et al., '98; Fujita et al., '97).

Finally, over the last several years there has been a growing awareness of the role bone plays in acid-alkaline balance. Specifically, bone has been shown to serve as an important reservoir for alkalinizing compounds. When faced with severe acute or chronic low grade metabolic acidosis, potassium, magnesium, citrate, bicarbonate and calcium are lost from bone (See Brown and Jaffe, 2000 for details and a review of this literature). Further, clinical studies show that the correction of metabolic acidosis results

in a halting of bone loss and the stimulation of bone formation (Sebastian, '94) NEJM article.

Objective of this Study

The purpose of this study was to compare the novel Japanese form of calcium known as AAACA with calcium citrate. (See end note #1 for description of AAACa). Of note also is that this is the first study of AAACa conducted with an other-than-Japanese population.

The objectives of this pilot study are fourfold:

(a) To determine if AAACa could in the short term (4 weeks) effectively lower bone resorption among US postmenopausal women with calcium intakes below 1000 mg per day.

(b) To determine if AAACa is more effective at lowering these bone loss markers than is calcium citrate.

(c) To test the ability of both forms of calcium to bring about a significant reduction in 8 AM fasting parathyroid hormone.

(d) To investigate if either calcium therapy by itself potentially influences net acid load as suggested by first morning urine pH.

METHODS / STUDY DESIGN

This study was a prospective, case controlled, blinded, randomized four-week intervention trial with two intervention arms.

Study End points

(1) Bone resorption as measured by urinary Deoxypyridinoline Crosslinks Assay (Dpd) provided by Great Smokies Diagnostic Laboratory. The second morning urine sample was used (taken before 10 am). Samples were taken at the initial screening, on the morning prior to beginning calcium supplementation, and then weekly at the end of each of the 4 weeks. The study Dpd baseline was established by averaging the two Dpd measurements taken prior to intervention with calcium (the screening and day one measurements). The final end of the study Dpd measurement was the Dpd measurement taken at the end of week four.

(2) Intact parathyroid hormone (iPTH) was the second end point. Fasting iPTH was taken on the day the study began (prior to supplementation) and at the end of the study.

(3) First morning urine pH as self-measured at home was the third end point. Subjects were asked to measure the pH of their first morning urine as much as possible without causing great inconvenience.

Study Population

Study participants were postmenopausal US women at least 5 years since their last period with osteoporosis or osteopenia (as determined by the Achilles Ultrasound measurement) and with high bone resorption (as measured by the Dpd assay). In addition to being osteopenic or osteoporotic, and having high bone breakdown, all women were also consuming suboptimal calcium, with daily intakes of 1,000 mg or less calcium.

Excluded from the study were women on medications for osteoporosis, those on estrogen in any form for any reason and those on any other interfering medications or supplements. Those with secondary osteoporosis were also excluded and participants were in general good health.

The study population was selected by the above criteria and then randomly divided into two groups (group A to be given AAACA and group B to be given calcium citrate). The study was blinded, participants not knowing which supplement they received.

Methods of Analysis

The data from this pilot study is analyzed and presented in two ways. First the research data pertaining to each of the study end points is presented and reviewed from both a group and case study perspective. Second, standard statistical analysis (linear regression, t-tests, etc) is used to assess statistical significance of these findings.

Intervention

Both study groups were supplemented with 900 mg elemental calcium. Group A received the 900 mg calcium in the form of AAACa; Group B was given calcium in the form of calcium citrate. In each group the calcium was given in divided doses according to the following schedule: 150 mg calcium with each meal and 450 mg calcium at bedtime. All participants continued on their typical pre-study diet and supplements, which contained less than 1,000 mg calcium per day.

STUDY RESULTS

End Point A:

The first end point of the study concerned the ability of AAACa to effectively lower bone resorption among a small sample (N6) of postmenopausal US women with sub optimal calcium intakes (less than 1000 mg per day).

As illustrated on Graph 1 and Table 1, as a group and individually, women given AAACa experienced a reduction in bone resorption over the four-week study period.

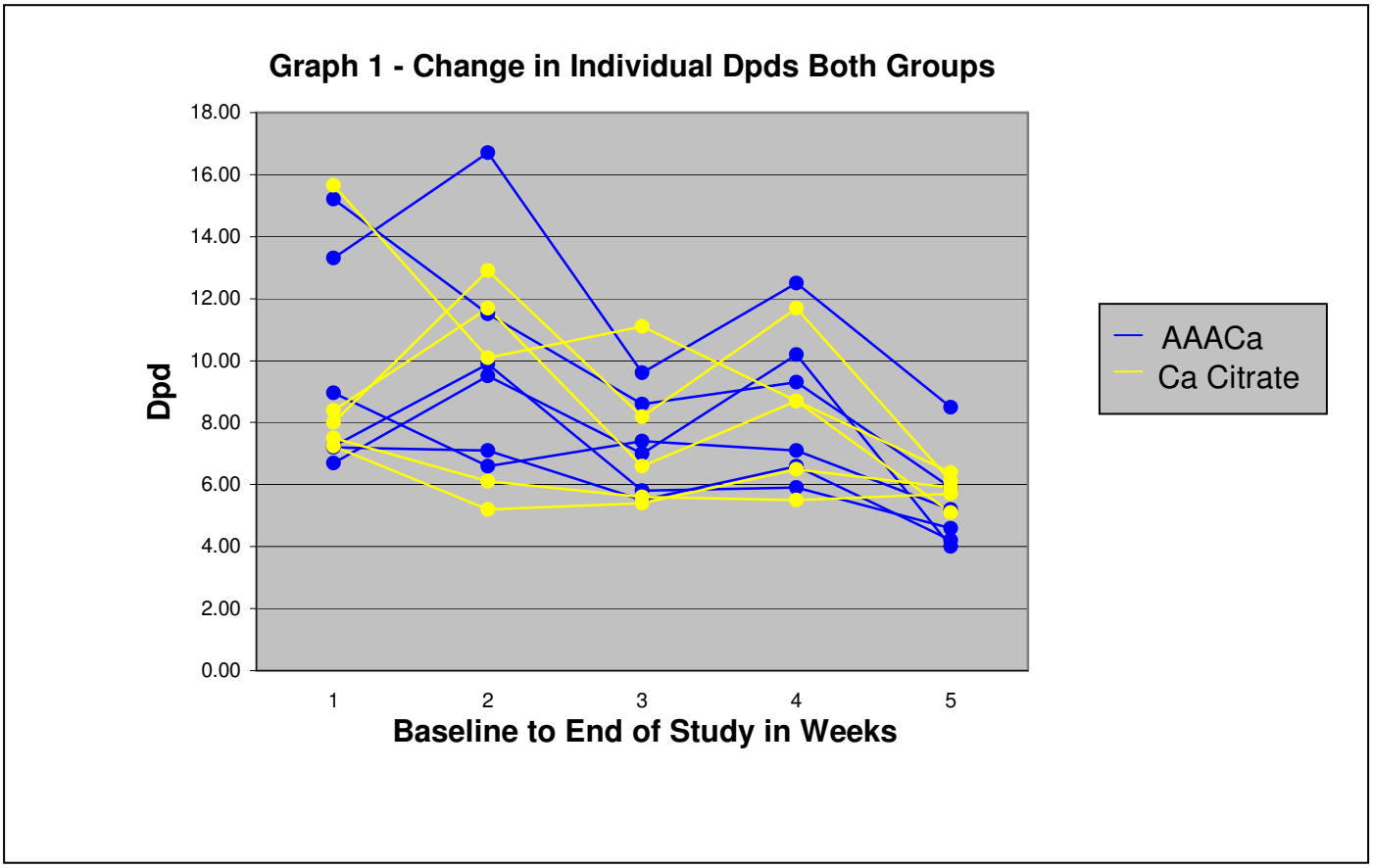


Table 1

AAACa Group: Change in Dpd from Baseline to End of Study (4th week)

AAACa

Name	Baseline	4th Week	Point Change (pT)	% Change
JC	6.70	4.00	- 2.70	- 40.30%
BM	8.95	5.20	- 3.75	- 41.90%
GK	15.20	5.90	- 9.30	- 61.18%
RD	7.25	4.60	- 2.65	- 36.55%
PD	13.30	8.50	- 4.80	- 36.09%
CG	7.20	4.20	- 3.00	- 41.67%
Average	**9.77**	**5.40**	**- 4.37**	**- 42.95%**

Table 2

Calcium Citrate Group: Change in Dpd from Baseline to End of Study (4th week)

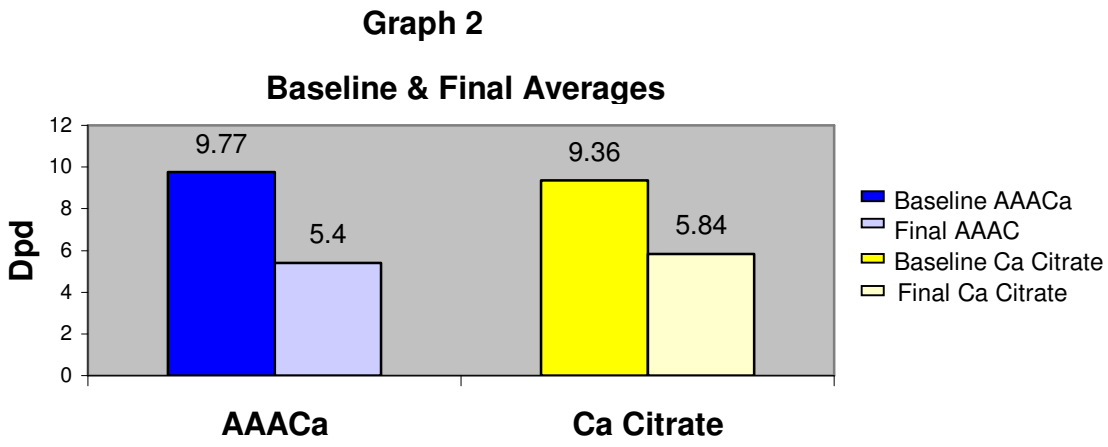
Calcium Citrate

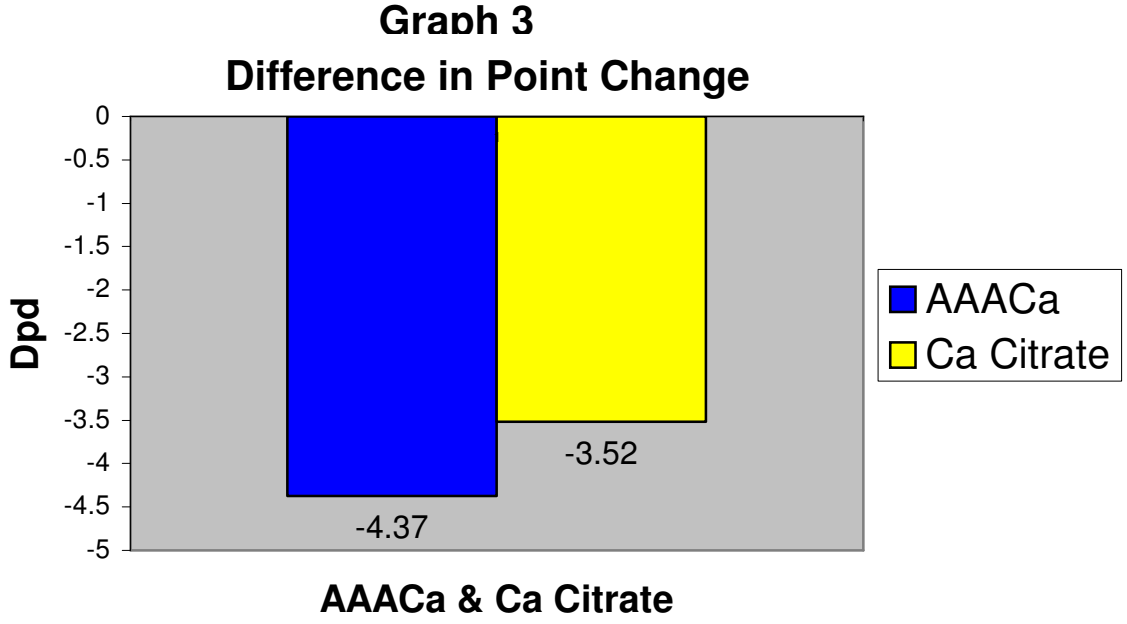
Name	Baseline	4th Week	Point Change (pT)	% Change
MD	7.25	5.90	- 1.35	- 18.62%
AE	8.40	5.10	- 3.30	- 39.29%
ClG	7.50	5.70	- 1.80	- 24.00%
MR	15.65	6.40	- 9.25	- 59.11%
EW	8.00	6.10	- 1.90	- 23.75%
Average	**9.36**	**5.84**	**- 3.52**	**- 33.00%**

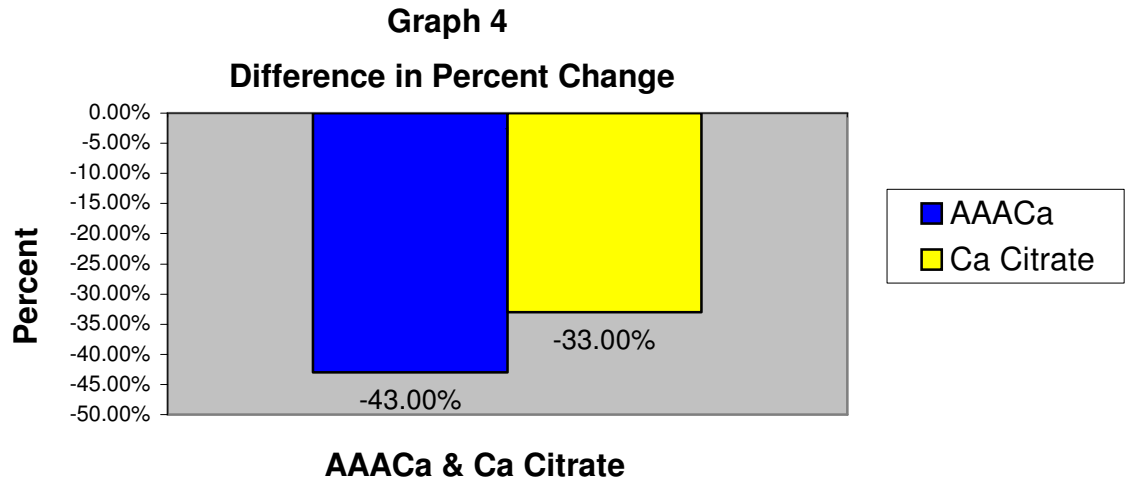
End Point B:

The second end point of the study asked if AAACa was more effective at lowering bone resorption than was calcium citrate.

Graphs 2 - 4 compare the impact of AAACa and calcium citrate on bone resorption. While calcium citrate also lowered bone resorption, it did so to a lesser extent than did AAACa. See Table 2.







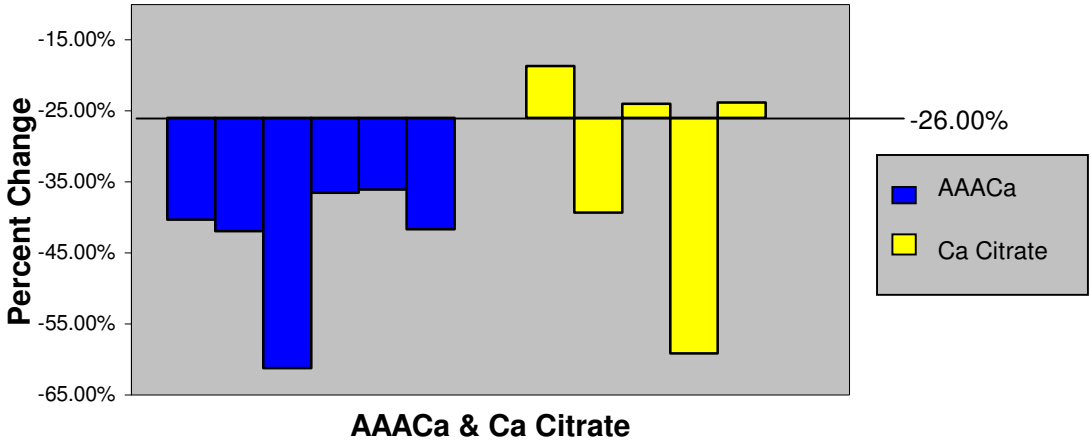
As a group those using calcium citrate had a mean 33% reduction in bone resorption (range - 18 to - 59%) as compared to a 43% reduction for those using AAACa (range - 36% to - 61%). The average reduction in points of Dpd for calcium citrate was - 3.52 and - 4.37 for AAACa.

While there was a trend favoring AAACa, using classical statistical analysis there was no statistically significant difference between the impact on bone resorption of AAACa and calcium citrate. The "Statistical Analysis" section, "AAACa versus Ca Citrate- Version 3. Sample A", details this statistical analysis. Also as detailed in the Statistical Analysis section, the study data comparing AAACa and calcium citrate was additionally organized and analyzed in several different ways.

From a case study perspective the data presented in Tables 1 & 2 is of special interest. It is held that with serial testing a 26% change in Dpd is statistically significant (Hannon et al., 1998). All 6 women given AAACa experienced a significant 26% or more reduction in bone resorption. In contrast only 2 of the five subjects using calcium citrate experienced a significant 26% in Dpd level. Graph 5 illustrations this distinction.

Graph 5

Significant Percent Change



From another case study efficacy perspective, it is interesting that a large (N7598) study found the average pre-menopausal Dpd to be 4.43 (Garnero, et al. 1996). At the same time, elderly women (M 75 years) without hip fractures averaged a Dpd of 5.8, and those with hip fractures averaged 6.41. In this pilot study, 3 of the 6 women on AAACa lowered their Dpd levels to near or below the ideal pre-menopausal average. None of the women on calcium citrate came to approximate the ideal pre-menopausal Dpd level.

From the above data it is clear that the case study analysis consistently finds AAACa more effective at reducing bone resorption than calcium citrate.

End Point C:

The third end point of the study concerned whether such short term (4 week) supplementation with either calcium source could reduce fasting morning intact PTH level in study women.

Pilot Study Finding:

> Over this short four week period neither AAACa nor calcium citrate significantly reduced fasting morning intact PTH level among postmenopausal osteopenia/osteoporotic women on low calcium diets.

In the calcium citrate groups the mean change in iPTH was increased over the four week period (+50.4%). As a group those using AAACa experienced a slight decrease in the final average iPTH over this time period. The formal statistical analysis of this variable is presented in the Statistical Analysis Section, "Analysis of iPTH data from AAACa versus Ca Citrate"

As it appears, a longer study period is needed to adequately test the impact of calcium supplementation on iPTH in postmenopausal women with osteopenia/osteoporosis. Previous Japanese research reported AAACa to lower parathyroid hormone within days among young women and healthy middle aged men (Shigeki et al., '98; Fujita et al., '97) Among elderly women with osteoporosis, however, such a reduction in parathyroid hormone was seen only after eighteen months of AAACa supplementation in osteoporotic women (Fujita et al., '96, Fujita et al., '95)

This short study did not find a significant reduction in iPTH from either calcium intervention program. Several interesting trends, however, did emerge:

a) There was no mean increase in iPTH in the AAACa group, as compared to the calcium citrate group.
b) At the end of this four week study one half of the women using AAACa had a lowering of PTH. Among the calcium citrate users only 2 of 5 showed such a reduction at the end of this four week study.
c) The range of PTH changes was wider among the calcium citrate group (from +220% increase to - 27% decrease) than in the AAACa group (from +79% increase to - 36% decrease).

End Point D:

Would either calcium therapy by itself influence net acid load as suggested by the first morning urine pH measurements.

Pilot Study Finding:

Analysis of the data from this study does not reveal any significant pattern of change in first morning urine pH from the two calcium supplementation regimens. Overall there might be a tendency for pH to rise in both groups, but the effect is small. Comparing the seven days at the beginning and at the end for each subject, neither linear regression, or paired t-tests showed significant differences. Also comparison of beginning to end for both groups also failed to reveal significant differences. See the statistical analysis section "AAACa versus Ca Citrate: Effects on pH" for this analysis.

Limitations of this Study

Being a short pilot study this investigation is limited by both number of participants and duration. With such a small study population (N 11) it is very difficult to establish statistical significance or generalize about the population at large. While trends from this study suggest that AAACa is more effective at reducing bone resorption than is calcium

citrate, a larger study population is needed to test for true statistical significance. In addition, the study population was specifically limited to calcium deficient postmenopausal women, and thus represents only a segment of all older women. The duration of the study was one month, designed to test for short-term changes in bone resorption with calcium supplementation. While short-term changes were noted, this study did not address the long-term effects of such supplementation.

Finally, there is much more we need to learn about urine markers of bone resorption. Day-to-day intra-patient variation can be a significant problem. This variation is such that in large studies, an average 26% or more reduction in Dpd was found necessary for significance (Hannon et al.,1998). This intra-patient variation in urine markers of bone resorption appears both when using calcium supplementation and when not on supplements. Analysis of our data suggests that the Dpd levels in some postmenopausal women seem to vary much more greatly than in others. It may we worthwhile to determine if there are actually two distinguishable patterns; one type who exhibit stable bone resorption, and another type who exhibit labial bone resorption. Also our bone resorption measurements were not batch tested. We now realize that for best accuracy the urine samples should be frozen and batch tested.

CONCLUSIONS

The following conclusions can be made in regard to the major end points of this study :

(1) This small pilot study verifies that the Japanese novel calcium source, AAACa, is effective at reducing bone resorption in postmenopausal women with suboptimal calcium intakes; (2) Calcium citrate was also found to reduce bone resorption in this same four week study period, but to lesser extent than did AAACa; (3) In this small study, however, the difference between AAACa and calcium citrate did not reach statistical significance; (4) Neither form of calcium significantly lower iPTH within the study period; (5) Neither form of calcium supplementation significantly altered first morning urine pH.

While formal statistical analysis failed to find a significant difference between the effect of AAACa and calcium citrate on bone resorption, the case study approach favors AAACa. From a case study perspective we see:

- AAACa subjects as a group began with higher bone resorption markers than the calcium citrate group (M 9.77 as compared to M 9.36) and ended with lower markers (M 5.4 compared to M 5.84).
- The average reduction in Dpd from the beginning of the study to the end was greater in those using AAACa (M - 4.37) than those using calcium citrate (- 3.52).
- The average percentage change was - 43% for AAACa and - 33% for calcium citrate.

- Using a 26% reduction in Dpd as statistically significant, all women using AAACa experienced a significant 26% decrease in Dpd. Only two of the five women on calcium citrate experienced such a 26% reduction.
- While the group given AAACa began with a higher average Dpd reading, three of the six subjects reduced their Dpd level to near or below the ideal pre-menopausal level. On the other hand, none of the women on calcium citrate came to so closely approximate the ideal pre-menopausal level.

At the most basic level this research confirms the widely held notion that postmenpausal US women on low calcium intakes can reduce their rate of bone breakdown by taking supplemental calcium. Further this pilot study suggests that this reduction in bone resorption can occur within a short time period. When using 900 mg elemental calcium in the form of AAACa and in the given dosing regimen, all 6 study participants experienced a significant reduction in bone resorption in only four weeks. Among women using calcium citrate, only two of the five participants experienced a significant reduction in bone resorption during the four weeks.

While all trends suggest AAACa is more effective at reducing bone resorption than calcium citrate, a larger, longer study is needed to test for true statistical significance and long term effect on bone resorption.

ENDNOTES

Endnote #1

AAACa is a unique form of active absorbable algal calcium developed from heated oyster shell with vacuum-heated seaweed ingredient, HAI)

AAACa is created through a process, which involves first the fine mechanical grinding of oyster shell, then the superheating of this powder to 800 degrees centigrade, which creates a fine ash. This superheating process is reported to not only remove heavy metals but also to release calcium from the tighter calcium. In the attempt to further enhance absorption of these calcium compounds, a "Heated Algae Ingredient" derived from super-heated dried and powdered Cystophyllum fusiforme was specially processed (Fujita et al, J Bone Mineral Metabolism, 2000:18:165 - 169) and added to the AAACa compound.

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RAW DATA

AAACa

Name: RD

Test	Date	Result
pTH Inact - 1	26-Jun	14
Calcium - 1	26-Jun	9
pTH Inact - 2	25-Jul	25
Calcium - 2	25-Jul	9
Screening Dpd	5-Jun	5.7
Baseline Dpd	27-Jun	8.8
Dpd – 2	3-Jul	9.9
Dpd – 3	10-Jul	5.8
Dpd – 4	17-Jul	5.9
Dpd – 5	24-Jul	4.6

Date	pH Level
27-Jun	5.5
28-Jun	5.5
29-Jun	6.2
30-Jun	6.2
1-Jul	6
2-Jul	6
3-Jul	5.8
4-Jul	6.2
5-Jul	6.6
6-Jul	6
7-Jul	6.2
8-Jul	5.8
9-Jul	n/a
10-Jul	6
11-Jul	6.4
12-Jul	6
13-Jul	6
14-Jul	6.2
15-Jul	n/a
16-Jul	6.4
17-Jul	6.8

AAACa
Name: GK

Test	Date	Result
pTH Inact- 1	26-Jun	39.4
Calcium- 1	26-Jun	9.3
pTH Inact- 2	25-Jul	34.4
Calcium- 2	25-Jul	9.7
Screening Dpd	5-Jun	6.7
Baseline Dpd	27-Jun	23.7
Dpd- 2	3-Jul	11.5
Dpd- 3	10-Jul	8.6
Dpd- 4	17-Jul	9.3
Dpd- 5	24-Jul	5.9

Date	pH Level
27-Jun	5.5
28-Jun	5.5
29-Jun	n/a
30-Jun	n/a
1-Jul	5.5
2-Jul	5.5
3-Jul	n/a
4-Jul	n/a
5-Jul	6.4
6-Jul	7.2
7-Jul	5.5
8-Jul	7.4
9-Jul	6.4
10-Jul	n/a
11-Jul	6.2
12-Jul	5.8
13-Jul	5.8
14-Jul	5.8
15-Jul	5.8
16-Jul	7
17-Jul	6
18-Jul	5.8
19-Jul	6.8
20-Jul	6
21-Jul	6.2
22-Jul	6
23-Jul	6

AAACa

Name: CG

Test	Date	Result
pTH Inact- 1	26-Jun	48.3
Calcium- 1	26-Jun	9
pTH Inact- 2	25-Jul	31.1
Calcium- 2	25-Jul	9.4
Screening Dpd	6-Jun	7.6
Baseline Dpd	27-Jun	6.8
Dpd- 2	3-Jul	7.1
Dpd- 3	10-Jul	5.5
Dpd- 4	17-Jul	6.6
Dpd- 5	24-Jul	4.2

Date	pH Level
27-Jun	6.2
28-Jun	6.2
29-Jun	6
30-Jun	5.8
1-Jul	5.8
2-Jul	5.5
3-Jul	5.5
4-Jul	5.5
5-Jul	5.5
6-Jul	5.5
7-Jul	5.5
8-Jul	n/a
9-Jul	5.5
10-Jul	5.5
11-Jul	5.5
12-Jul	n/a
13-Jul	n/a
14-Jul	n/a
15-Jul	6.2
16-Jul	5.2
17-Jul	n/a
18-Jul	5.5

AAACa
Name: PD

Test	Date	Result
pTH Inact- 1	26-Jun	19.5
Calcium- 1	26-Jun	9.1
pTH Inact- 2	25-Jul	25.9
Calcium- 2	25-Jul	9.2
Screening Dpd	6-Jun	12.9
Baseline Dpd	27-Jun	13.7
Dpd- 2	3-Jul	16.7
Dpd- 3	10-Jul	9.6
Dpd- 4	17-Jul	12.5
Dpd-5	24-Jul	8.5

Date	pH Level
27-Jun	5.5
28-Jun	6.4
29-Jun	7
30-Jun	6.4
1-Jul	6
2-Jul	6.4
3-Jul	6.2
4-Jul	6
5-Jul	6.6
6-Jul	6.6
7-Jul	6.6
8-Jul	6
9-Jul	6.8
10-Jul	6.2
11-Jul	6.2
12-Jul	6.8
13-Jul	6
14-Jul	6
15-Jul	6.2
16-Jul	6.2
17-Jul	6.4
18-Jul	6.4
19-Jul	6.4
20-Jul	6.4
21-Jul	6.8
22-Jul	7
23-Jul	6.2
24-Jul	6

AAACa
Name: JC

Test	Date	Result
pTH Inact- 1	26-Jun	49.1
Calcium- 1	26-Jun	9.8
pTH Inact- 2	25-Jul	45.8
Calcium- 2	25-Jul	9.6
Screening Dpd	7-Jun	6.1
Baseline Dpd	27-Jun	7.3
Dpd- 2	3-Jul	9.5
Dpd- 3	10-Jul	7
Dpd- 4	17-Jul	10.2
Dpd- 5	24-Jul	4

Date	pH Level
27-Jun	7
28-Jun	n/a
29-Jun	6.4
30-Jun	6.4
1-Jul	n/a
2-Jul	6
3-Jul	6.8
4-Jul	7.4
5-Jul	6.2
6-Jul	n/a
7-Jul	n/a
8-Jul	6.4
9-Jul	n/a
10-Jul	6.8
11-Jul	6.4
12-Jul	6.2
13-Jul	6.8
14-Jul	n/a
15-Jul	n/a
16-Jul	5.8
17-Jul	6.2
18-Jul	6.8
19-Jul	6.8
20-Jul	7.2
21-Jul	6.2
22-Jul	6.4
23-Jul	6.2
24-Jul	6.6
25-Jul	6

AAACa
Name: BM

Test	Date	Result
pTH Inact- 1	26-Jun	15.3
Calcium- 1	26-Jun	9.6
pTH Inact- 2	25-Jul	18.5
Calcium- 2	25-Jul	9.9
Screening Dpd	20-Jun	8.7
Baseline Dpd	27-Jun	9.2
Dpd- 2	3-Jul	6.6
Dpd- 3	10-Jul	7.4
Dpd- 4	17-Jul	7.1
Dpd- 5	24-Jul	5.2

Date	pH Level
27-Jun	5.5
28-Jun	n/a
29-Jun	6.2
30-Jun	6.6
1-Jul	n/a
2-Jul	7.2
3-Jul	7.2
4-Jul	6.8
5-Jul	6.6
6-Jul	7
7-Jul	n/a
8-Jul	n/a
9-Jul	7
10-Jul	7.4
11-Jul	n/a
12-Jul	6.8
13-Jul	6.6
14-Jul	6.8
15-Jul	n/a
16-Jul	n/a
17-Jul	6.8
18-Jul	6.6
19-Jul	n/a
20-Jul	6.6
21-Jul	n/a
22-Jul	n/a
23-Jul	6.8

Calcium Citrate

Name: MD

Test	Date	Result
pTH Inact- 1	28-Jun	10.7
Calcium- 1	28-Jun	9.3
pTH Inact- 2	26-Jul	34.2
Calcium- 2	26-Jul	9.2
Screening Dpd	3-Jun	7.9
Baseline Dpd	28-Jun	6.6
Dpd- 2	4-Jul	5.2
Dpd- 3	11-Jul	5.4
Dpd- 4	18-Jul	6.5
Dpd- 5	25-Jul	5.9

Date	pH Level
28-Jun	6
29-Jun	5.5
30-Jun	6.6
1-Jul	6.6
2-Jul	6.45
3-Jul	6.6
4-Jul	6.6
5-Jul	6.2
6-Jul	5.8
7-Jul	5.8
8-Jul	5.8
9-Jul	5.5
10-Jul	5.5
11-Jul	6.4
12-Jul	6.4
13-Jul	6
14-Jul	5.8
15-Jul	5.8
16-Jul	5.8
17-Jul	6
18-Jul	7
19-Jul	6.2
20-Jul	7.8
21-Jul	6
22-Jul	6.4
23-Jul	5.8
24-Jul	6.8
25-Jul	6.2

Calcium Citrate

Name: EW

Test	Date	Result
pTH Inact- 1	27-Jun	16.3
Calcium- 1	27-Jun	9.3
pTH Inact- 2	26-Jul	24.9
Calcium- 2	26-Jul	9.1
Screening Dpd	5-Jun	6.2
Baseline Dpd	28-Jun	9.8
Dpd- 2	4-Jul	12.9
Dpd- 3	11-Jul	8.2
Dpd- 4	18-Jul	11.7
Dpd- 5	25-Jul	6.1

Date	pH Level
18-Jul	6.2
25-Jul	7

Calcium Citrate
AE

Test	Date	Result
PTH Inact- 1	27-Jun	39.8
Calcium- 1	27-Jun	9.3
PTH Inact- 2	25-Jul	46.6
Calcium- 2	25-Jul	9.4
Screening Dpd	20-Jun	7.4
Baseline Dpd	27-Jun	9.4
Dpd- 2	3-Jul	11.7
Dpd- 3	10-Jul	6.6
Dpd- 4	17-Jul	8.7
Dpd- 5	24-Jul	5.1

Date	pH Level
27-Jun	5.5
28-Jun	5.5
29-Jun	5.5
30-Jun	6.2
1-Jul	6.2
2-Jul	7
3-Jul	7
4-Jul	5.5
5-Jul	6.2
6-Jul	7
7-Jul	5.5
8-Jul	7
9-Jul	8
10-Jul	7
11-Jul	6
12-Jul	6.8
13-Jul	7.2
14-Jul	6
15-Jul	5.5
16-Jul	5.5
17-Jul	6
18-Jul	8
19-Jul	7.4
20-Jul	7.2
21-Jul	6.8
22-Jul	7
23-Jul	8
24-Jul	6.6

Calcium Citrate

Name: MR

Test	Date	Result
pTH Inact- 1	27-Jun	31
Calcium- 1	27-Jun	9
pTH Inact- 2	25-Jul	22.6
Calcium- 2	25-Jul	9
Screening Dpd	6-Jun	18.8
Baseline Dpd	27-Jun	12.5
Dpd- 2	3-Jul	10.1
Dpd- 3	10-Jul	11.1
Dpd- 4	17-Jul	8.7
Dpd- 5	24-Jul	6.4

Date	pH Level
27-Jun	7.2
28-Jun	7.6
29-Jun	7.2
30-Jun	7.2
1-Jul	7.8
2-Jul	n/a
3-Jul	7
4-Jul	n/a
5-Jul	8
6-Jul	7.4
7-Jul	7.4
8-Jul	n/a
9-Jul	7.2
10-Jul	8
11-Jul	7.2
12-Jul	n/a
13-Jul	6.8
14-Jul	7.4
15-Jul	7
16-Jul	7.4
17-Jul	8
18-Jul	n/a
19-Jul	7.4
20-Jul	7.2
21-Jul	8
22-Jul	7
23-Jul	7.2

Calcium Citrate

Name: ClG

Test	Date	Result
pTH Inact- 1	27-Jun	24.1
Calcium- 1	27-Jun	9.4
pTH Inact- 2	25-Jul	21.5
Calcium- 2	25-Jul	9.7
Screening Dpd	7-Jun	5.9
Baseline Dpd	27-Jun	9.1
Dpd- 2	3-Jul	6.1
Dpd- 3	10-Jul	5.6
Dpd- 4	17-Jul	5.5
Dpd- 5	24-Jul	5.7

Date	pH Level
27-Jun	5.5
28-Jun	5.5
29-Jun	5.5
30-Jun	5.5
1-Jul	5.5
2-Jul	5.5
3-Jul	5.5
4-Jul	5.5
5-Jul	5.5
6-Jul	5.5
7-Jul	5.5
8-Jul	5.5
9-Jul	5.5
10-Jul	5.5
11-Jul	5.5
12-Jul	5.8
13-Jul	5.8
14-Jul	5.5
15-Jul	5.5
16-Jul	5.5
17-Jul	5.8
18-Jul	5.8
19-Jul	5.8
20-Jul	5.8
21-Jul	5.8
22-Jul	5.8
23-Jul	5.8
24-Jul	5.5
25-Jul	5.8

STATISTICAL ANALYSIS

Statistical Analysis of Impact of Dpd of AAACa versus Calcium Citrate

Conducted by Dr. Jerilynn Prior and Dr. Chris Hitchcock
University of British Columbia

DPD analysis:
Paired t-tests (standard or modified for unequal variances)

Choice of time periods:
Version 1. Compare Control (average of Screening & Baseline) to end of study (average of Weeks 3 & 4)
Version 2. Compare Baseline to Week 4
Version 3. Compare Control (average of Screening & Baseline) to Week 4

Sample modification
Sample A. All people (n = 5 in Ca Citrate group, n = 6 in AAACa group)
Sample B. Exclude first AAACa participant because of her high baseline value

Chronology:

	Version 1	Version 2	Version 3
Sample A	#1		#4
Sample B		#2	#3

#1 was my original choice for analysis.
#2 was performed by request
#3 and #4 are my responses to Oct 4's email requests. The numbers in the email suggest that #4 is intended.

Reanalysis using 5 women in each group (exclude the woman with a large value at baseline). Some analyses used the baseline value only, and some used the average of screening and baseline values (which I have called "control").

Analysis 1.
Q. Is there a significant difference between baseline and final DPD levels?
Answer: [Sample B, Regression using {Baseline, Week 1- Week 4}]
DPD figure - no screen.ppt contains the graph of data and fitted lines. Again, it seems that there is a clear drop across the 4 weeks in both groups and very similar patterns. DPD levels dropped significantly across the study (-1.11 ± 0.292 units per week, P = 0.0004 for a two-tailed t-test against the hypothesis that the slope is 0). There was no significant difference between the two groups (P = 0.228) and no significant interaction between group and time interval (P = 0.3315).

Again, from the fitted lines, and from the analysis, it seems clear that AAACa is equivalent to Ca Citrate in effectiveness, but is no better.

A second approach was to compare the change from initial values (baseline) to final values (week 4) within individuals in a paired t-test, asking whether the difference between final values and initial values differed significantly between the two treatments.
Results: [Version 2, Sample B]

```
Printout from S-Plus 2000 analysis:
For data with unequal variances in the two groups:
       Welch Modified Two-Sample t-Test

data:  x: Week4.change with Baseline.Group = CaCi , and y: Week4.change with
Baseline.Group = AAACa
t = 0.2256, df = 5.897, p-value = 0.8291
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.176581  2.616581
sample estimates:
 mean of x mean of y
     -3.64     -3.86

Assuming equal variances in the two groups:
       Standard Two-Sample t-Test

data:  x: Week4.change with Baseline.Group = CaCi , and y: Week4.change with
Baseline.Group = AAACa
t = 0.2256, df = 8, p-value = 0.8272
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.029034  2.469034
sample estimates:
 mean of x mean of y
     -3.64     -3.86
```

Translation: [Version 2, Sample B]

Analysis using a t-test assuming equal variances gave essentially the same result.

The average change for Ca Citrate was -3.64, while the average change for AAACa was -3.86. (in units of original DPD measures). The 95% CI (confidence interval) for the difference between these two changes was (-2.177, 2.617). There was no significant difference (Welch modified two sample t-test for groups with unequal variances, t = 0.226, df = 5.9, P = 0.829) between the two groups in the degree of change.

While the direction of difference favors AAACa, the power to detect a change of this magnitude with this sample size is very small.

There was an overall decline in DPD, but no difference between the two groups in how much of a decline there was.

[Version 3, Sample B]
COMPARISON OF CHANGE IN DPD FROM CONTROL (average of Screening & Baseline values) to WEEK4. DOES THE CHANGE (drop) IN DPD DURING THE STUDY DIFFER BETWEEN THE TWO TREATMENT GROUPS? [Using n=5 in each group, that is, deleting the woman with the high baseline value]

```
S-Plus 2000 printouts:
       Standard Two-Sample t-Test

data:  x: Control.to.Week4 with Baseline.Group = CaCi , and y: Control.to.Week4
with Baseline.Group = AAACa
t = -0.0918, df = 8, p-value = 0.9291
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3.654898  3.374898
sample estimates:
 mean of x mean of y
     -3.52     -3.38


       Welch Modified Two-Sample t-Test

data:  x: Control.to.Week4 with Baseline.Group = CaCi , and y: Control.to.Week4
with Baseline.Group = AAACa
t = -0.0918, df = 4.607, p-value = 0.9307
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -4.160972  3.880972
sample estimates:
 mean of x mean of y
     -3.52     -3.38
```

TRANSLATION: [Version 3, Sample B]
No, there is no evidence that the treatments differ in the change in DPD during the study. For the Welch modified two-sample t-test (which assumes that the variance within the two groups may differ), t=-0.0918, df = 4.607, p-value = 0.9307. The standard t-test (which assumes variances are the same) gives the same result, t = -0.0918, df = 8, p-value = 0.9291.

[Version 3, Sample A]
This is our preferred selection of the data for statistical analysis. This is the organization of the data in the form of statistical data used in the report.

COMPARISON OF CHANGE IN DPD FROM CONTROL (average of Screening & Baseline values) to WEEK4. DOES THE CHANGE (drop) IN DPD DURING THE STUDY DIFFER BETWEEN THE TWO TREATMENT GROUPS? [Using n=5 in CaCitrate group and n=6 in AAACa group]

Again, no statistically significant differences:
For unequal variances test (Welch Modified Two-Sample t-Test)

```
t = 0.4702, df = 7.509, p-value = 0.6515
95 percent confidence interval:(-3.353096, 5.046430)
change for Ca Citrate: -3.52; change for AAACa: -4.366667

       Welch Modified Two-Sample t-Test

data:  x: Week4.change.from.control with Group = CaCitrate , and y:
Week4.change.from.control with Group = AAACa
t = 0.4702, df = 7.509, p-value = 0.6515
alternative hypothesis: true difference in means is not equal to 0
```

```
95 percent confidence interval:
 -3.353096  5.046430
sample estimates:
 mean of x mean of y
     -3.52 -4.366667

       Standard Two-Sample t-Test

data:  x: Week4.change.from.control with Group = CaCitrate , and y:
Week4.change.from.control with Group = AAACa
t = 0.4822, df = 9, p-value = 0.6412
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3.125276  4.818609
sample estimates:
 mean of x mean of y
     -3.52 -4.366667
```

To support this analysis, I also performed some initial tests:

1.Was there a difference in Control values (average of Screening & baseline values) between the two groups?
No: (t = 0.2078, df = 8, p-value = 0.8406)

```
       Standard Two-Sample t-Test

data:  x: Baseline.DPD with Baseline.Group = CaCi , and y: Baseline.DPD with
Baseline.Group = AAACa
t = 0.2078, df = 8, p-value = 0.8406
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3.231546  3.871546
sample estimates:
 mean of x mean of y
      9.48      9.16
```

2. Was there a difference in Final values (average of weeks 3 & 4) between the two groups?
No. (t = 0.6322, df = 8, p-value = 0.5449)

```
       Standard Two-Sample t-Test

data:  x: Week.4.DPD with Baseline.Group = CaCi , and y: Week.4.DPD with
Baseline.Group = AAACa
t = 0.6322, df = 8, p-value = 0.5449
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.429711  2.509711
sample estimates:
 mean of x mean of y
      5.84       5.3
```

3. Was there a difference in week 4 values between the two groups?

```
       Standard Two-Sample t-Test

data:  x: Week.4.DPD with Baseline.Group = CaCi , and y: Week.4.DPD with
Baseline.Group = AAACa
t = 0.6322, df = 8, p-value = 0.5449
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.429711  2.509711
sample estimates:
 mean of x mean of y
      5.84       5.3
```

ASIDE:
The difference between the Standard t-test and the Welch's modified t-test is in the assumptions they make about variances. An assumption of the standard t-test is that the variance of the two groups is about the same. Welch's modified t-test is an adjustment of the standard t-test that does not require equal variances. From what I can see, this doesn't make much difference to the results, so probably the equality of variances assumption is fine in this case.

Statistical Analysis of iPTH data from AAACa versus Ca Citrate

Conducted by Dr. Jerilynn Prior and Dr. Chris Hitchcock
University of British Columbia

Confirm that there were no significant differences between the groups at baseline:

Statistical output:

Standard Two-Sample t-Test

data: x: pTH.baseline with Group = AAACa , and y: pTH.baseline with Group = CaCi
t = 0.745, df = 9, p-value = 0.4753
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-13.34570 26.45236
sample estimates:
mean of x mean of y
30.93333 24.38

TRANSLATION: There were no significant differences between the two groups at baseline (2-tailed t-test for differences between two groups. mean for AAACa = 30.93 and mean for CaCi = 24.38; t = 0.745; df = 9; p = 0.47).

Was there a statistically reliable difference in iPTH scores between the beginning and the end of the study (overall)?
NO. There was no significant difference between baseline and final levels of iPTH (two-tailed paired t-test, t = 0.632; df = 10; p = 0.542). I also did a nonparametric test, and it too was non-significant. (Exact Wilcoxon signed rank test, V = 25, n = 11, p = 0.520).

Paired t-Test

data: x: pTH.baseline in pTH , and y: pTH.final in pTH
t = -0.6316, df = 10, p-value = 0.5418
alternative hypothesis: true mean of differences is not equal to 0
95 percent confidence interval:
-9.467389 5.285571
sample estimates:
mean of x - y
-2.090909

Exact Wilcoxon signed-rank test

data: x: pTH.baseline in pTH , and y: pTH.final in pTH
signed-rank statistic V = 25, n = 11, p-value = 0.5195
alternative hypothesis: true mu is not equal to 0

So, there was no reliable difference, including the two groups as a whole, but there still might have been some difference between the two groups:

CHANGE OVER TIME: COMPARISON BETWEEN GROUPS

Here is the output of a two-tailed, two-group t-test asking the question "Was there a statistically reliable difference between the change in iPTH values over the study?".

Welch Modified Two-Sample t-Test

data: x: pTH.change with Group = AAACa , and y: pTH.change with Group = CaCi
t = -0.9394, df = 7.786, p-value = 0.3758
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-22.174575 9.381241
sample estimates:
mean of x mean of y
-0.8166667 5.58

TRANSLATION: There was no difference (Welch modified two-sample, 2-tailed, t-test with unequal variances, t = -0.939; df = 7.8; p = 0.376) between the groups. However, the confidence interval for the difference between groups is very large (95% CI = (-22.17, 9.28)), reflecting the large variability among people.

Some statisticians would do a non-parametric test under these circumstances. Because the sample sizes are very small, it is impossible to verify that the assumptions of a t-test have been met. Here are the results of an Exact Wilcoxon rank-sum test:

```
data:  x: pTH.change with Group = AAACa , and y: pTH.change with Group = CaCi
rank-sum statistic W = 31, n = 6, m = 5, p-value = 0.4286
alternative hypothesis: true mu is not equal to 0
```

In other words, there is no evidence that the two medications differed in their effects on iPTH. (Wilcoxon rank sum W = 31, n = 6, m = 5, p = 0.429).

Recall that a failure to find a difference is not evidence that two things are the same. In this case, the sample sizes are so small and the variability is so large that we would have trouble finding a difference unless it were very dramatic.

Statistical Analysis of AAACa versus Ca Citrate: Effects on pH

Conducted by Dr. Jerilynn Prior and Dr. Chris Hitchcock
University of British Columbia

About my approach:
I prefer, when averaging data, to compare averages of similar number of days. Because of this, I chose the first 7 and the last 7 days of the study to contrast.
I also computed a regression analysis for each individual. Note, ph.6 is the individual with the high baseline DPD value. You may want to censor her results for consistency with what you report for the DPD results.

Two group comparison:
Beginning (days 1-7) versus end (days 23-29). There were no statistically significant differences between the two groups at beginning or end of the study, and no significant difference in the amount of change in pH during the study between the two groups.

	CaCitrate	AAACa	t-test (equal variances)	t-test (unequal variances)
Sample size (with both Beginning and End values)	N = 4	N = 4		
Beginning	6.32	6.21	t = −0.258 df = 6 p-value = 0.805	t = −0.258 df = 5.108 p-value = 0.8065
End	6.70	6.46	t = −0.6172 df = 6 p-value = 0.5598	t = −0.6172 df = 3.474 p-value = 0.5752
Change	0.38	0.25	t = −0.4215 df = 6 p-value = 0.6881	t = −0.4215 df = 5.326 p-value = 0.6898

Table: Slope of the Regression of pH against day for those participants with enough data to allow this calculation. There is no overall pattern, most slopes are not significantly different from zero (ie, no change over time). The 4 statistically significant slopes are all small in magnitude and are found in both treatment groups.

Group	ID	Slope	SE	P	
AAACa	ph.10	0.0056	0.0082	0.500	
AAACa	ph.11	-0.0230	0.0107	0.049	*
AAACa	ph.6	0.0160	0.0158	0.3228	
AAACa	ph.7	0.0299	0.0105	0.011	*
AAACa	ph.8	-0.0089	0.0101	0.390	
AAACa	ph.9	0.0145	0.0137	0.305	
CaCi	ph.1	0.0118	0.0024	0.000	*
CaCI	ph.2	-0.0012	0.0097	0.903	

CaCi	ph.4	0.0101	0.0121	0.413	
CaCi	ph.5	0.0452	0.0175	0.015	*