

## Peripheral computed tomography (pQCT) detected short-term effect of AAACa (heated oyster shell with heated algal ingredient HAI): A double-blind comparison with CaCO<sub>3</sub> and placebo

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**Abstract:** Trabecular bone density at the distal radius and cortical bone density at the midradius were measured in four randomized groups of women before and after 4 months administration of AAACa, oyster shell heated under reduced pressure with addition of heated algal ingredient (HAI) (group A); AACa, the same preparation without HAI (group B); CaCO<sub>3</sub> (group C); and placebo (group D) in a double-blind system using peripheral quantitative computed tomography (pQCT) with lumbar spine density measurement by dual-energy X-ray absorptiometry (DXA). Groups A, B, and C received 900mg/day elemental calcium and D received none. In subjects of group A, but not B, C, and D, radial trabecular bone density increased significantly, to 106.2% ± 2.1% of the initial value (mean ± SEM). The increase of trabecular bone density was significantly different from the placebo group (D) only in AAACa (group A) and not in AACa (group B) and the calcium carbonate (group C). Cortical bone density increase was also greater in group A (but not in B and C) than in D. Lumbar spine density did not change significantly. AAACa was apparently more effective, increasing trabecular bone density more than AACa and CaCO<sub>3</sub> containing the same amount of elemental calcium.

**Key words:** AAACa (heated oyster shell with heated algal ingredient), HAI (heated algal ingredient), pQCT (peripheral computed tomography), radial trabecular bone density, radial cortical bone density

### Introduction

Active absorbable calcium (AACa) produced by heating oyster shell to 800°C at reduced oxygen pressure with characteristic crystalline shape distinguished from CaO and CaCO<sub>3</sub> exhibits a high electric conductivity in aqueous solution and excellent absorba-

bility from the intestine [1], increasing bone mineral density more efficiently than CaCO<sub>3</sub> [2]. Active absorbable algal calcium (AAACa, Adva Cal) produced by adding similarly treated seaweed *Cystophyllum fusiforme* (HAI) was even better absorbed than AACa [3] and increased bone mineral density as measured by dual-energy X-ray absorptiometry (DXA) in a double-blind study over a period of 2 years in a group of women of advanced age [4,5]. Peripheral computed tomography (pQCT), which measures the true volumetric density of trabecular and cortical bone separately, may be capable of best detecting changes in bone density in response to metabolic and pharmacological intervention [6,7]. A double-blind, randomized clinical trial was therefore conducted in a group of women on the relative effects of AAACa, AACa, CaCO<sub>3</sub>, and placebo on bone mineral density measured by pQCT.

### Materials and methods

This study was conducted on 34 pre- and postmenopausal women ranging in age between 26 and 91 years who randomly divided into four groups. Osteoporosis was found in 69.2% and osteopenia (71%–80% of years after menopause [YAM]) in 30.8%. Osteopenia (71%–80% of young adult mean of anteroposterior spine bone mineral density (BMD) corresponding to –1 to –2 SD) and osteoporosis (less than 70%, corresponding to less than –2 SD) was defined according to the criteria of the Japanese Society of Bone and Mineral Metabolism. Four kinds of test supplements were used: A, 900mg calcium as AAACa; B, 900mg calcium as AACa; C, 900mg calcium as CaCO<sub>3</sub>; and D, placebo containing no calcium in a double-blind system. These supplements, placed in capsules each containing 150mg calcium (A, B, C) or placebo (D) and indistinguishable in appearance, were prepared by Fujix (Tokyo, Japan).

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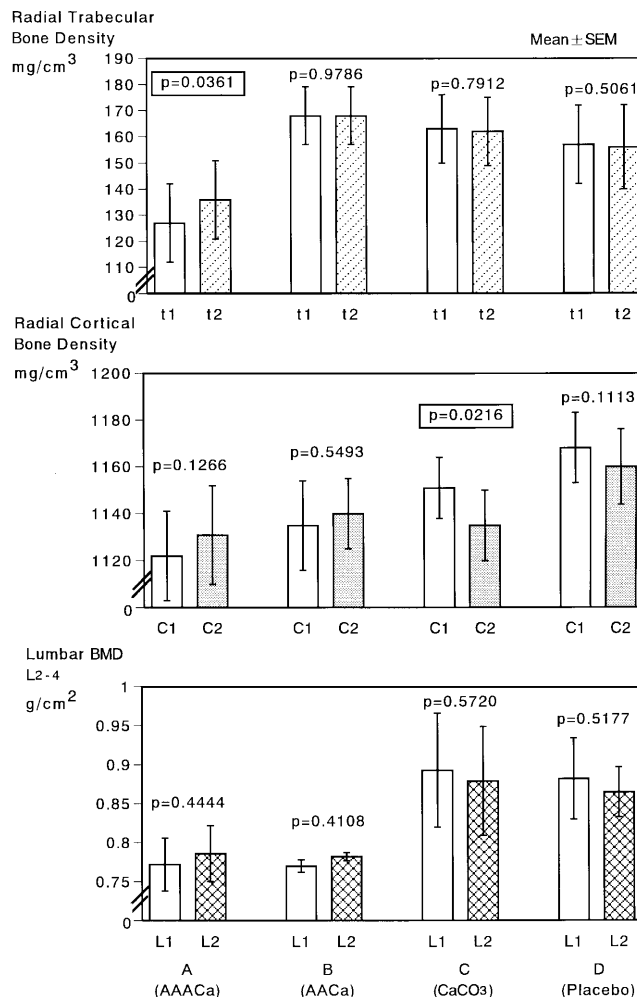
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Trabecular bone density was measured at the distal radius and cortical bone density at midradius by XCT-960 (Stratec-Norland), and lumbar spine bone mineral density in the anteroposterior direction in dual-energy X-ray absorptiometry using XR-26 (Norland). The coefficient of variation of trabecular bone density was 1.19%, of cortical bone density 0.76%, of relative cortical volume 1.75%, and of lumbar BMD 1.21%. The results were analyzed by analysis of variance followed by multiple comparison by Fisher's PLSD and paired *t*-test in the Statview 5.0 system. The Institutional Review Board for Clinical Research of the National Sanatorium Hyogo Chuo Hospital approved the study. Dr. Shigeki Ohgitani (Division of Laboratory and Research) acted as the controller of the study and kept the sealed code of the supplements until the end of the study.

**Results**

The background factors and results of pretest bone measurement are summarized in Table 1. No significant difference was detected among the four groups as to age, radial trabecular bone density, radial cortical bone density and lumbar bone mineral density at the beginning of the test.

As shown in Fig. 1, paired comparison between the pretest and 4-month value revealed a significant increase of radial trabecular bone in group A given AAACa, and significant decrease of radial cortical bone in group C given CaCO<sub>3</sub>. In groups B and D, given AACa and placebo, respectively, none of the bone density data showed a significant change. Intergroup comparison of the rates of changes from the initial to 4-month value expressed in percent (Fig. 2) revealed a significantly more pronounced increase in trabecular bone in group A than in D (*P* = 0.0435), but B and C showed no significant difference from D. A significantly higher increase of BMD was also found in group A than



**Fig. 1.** Paired comparison of pretest (1) (open bars) and 4-month (2) (shaded bars) values of bone density values of radial trabecular bone density, radial cortical bone density, and lumbar bone density in the four groups given active absorbable algal calcium (AAACa), active absorbable calcium (AACa), CaCO<sub>3</sub>, and placebo, respectively. A significant increase was noted in radial trabecular bone density in group A and a significant decrease in radial cortical bone density in group C by paired comparison

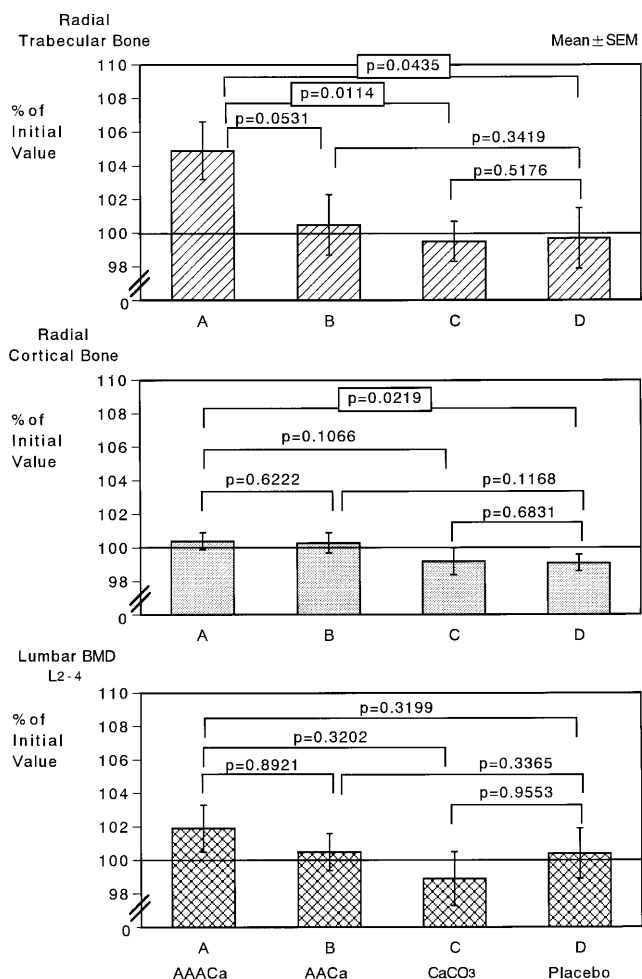
**Table 1.** Background factors of subjects in test groups

Group	Number of subjects	Age in years	Pretest trabecular density (mg/cm <sup>3</sup> )	Pretest cortical density (mg/cm <sup>3</sup> )	Pretest lumbar density (g/cm <sup>2</sup> )
A (AAACa)	10	60 ± 4 (0.1742) <sup>a</sup>	127 ± 15 (0.4432) <sup>a</sup>	1122 ± 19 (0.1877) <sup>a</sup>	0.772 ± 0.034 (0.1268) <sup>a</sup>
B (AACa)	11	55 ± 2 (0.3411)	168 ± 11 (0.3419)	1135 ± 19 (0.3454)	0.770 ± 0.008 (0.1030)
C (CaCO <sub>3</sub> )	11	53 ± 2 (0.5494)	163 ± 13 (0.5176)	1151 ± 13 (0.6425)	0.893 ± 0.073 (0.9090)
D (Placebo)	6	50 ± 5	147 ± 19	1161 ± 16	0.882 ± 0.052 (0.7969)

AAACa, active absorbable algal calcium; AACa, active absorbable calcium

Data are mean ± SEM

<sup>a</sup>Numbers (in parentheses) are *P*-values for difference from D



**Fig. 2.** Changes of radial trabecular (*upper panel*) and cortical bone density (*middle panel*) measured by pQCT and of lumbar bone mineral density by DXA (*lower panel*) in four randomized groups of women. Group A, 900 mg Ca was given as AAACa; group B, 900 mg Ca as AAACa; group C, 900 mg Ca as CaCO<sub>3</sub>; group D, placebo. Bone mineral density values are expressed as 4-month value/pretest value  $\times 100\%$ . Increase of radial trabecular bone density in group A was significantly greater than C. No difference was noted in the increase rates of radial cortical density and lumbar bone mineral density

in C; A also tended to be higher than B, although this was not statistically significant ( $P = 0.0531$ ). Increase of radial cortical bone density was also significantly higher in group A than in D ( $P = 0.0219$ ) but no significant difference was noted between groups D and B, D and C, A and B, and A and C. Increase of lumbar BMD was not significantly different among the four groups.

## Discussion

The present study was prompted by an open prospective study on the effect of AAACa, alfacalcidol,

elcatonin, and placebo [8]. After 6 and 36 months of oral supplementation of 900 mg Ca as AAACa, lumbar bone mineral density rose by  $2.1\% \pm 2.6\%$  (SD;  $P = 0.006$ ) by paired comparison and  $4.5\% \pm 2.7\%$  (SD;  $P = 0.0293$ ), respectively, over the corresponding group given placebo,  $-2.7\% \pm 4.2\%$  and  $-3.5\% \pm 0.8\%$ , respectively. These increases were compatible with those obtained by  $1 \mu\text{g}/\text{day}$  alfacalcidol,  $2.0\% \pm 3.2\%$  ( $P = 0.002$ ) and  $3.7 \pm 5.5$  ( $P = 0.0195$ ), at corresponding times. In a 2-year double-blind study on elderly women comparing AAACa with CaCO<sub>3</sub> and placebo [4], lumbar BMD at 3 and 6 months was 100.4% and 101.1% of the baseline in AAACa group, compared to 98.5% and 97.1% in the CaCO<sub>3</sub> group and 98.4% and 97.2% in the placebo group, suggesting that lumbar BMD starts to increase between 3 and 6 months after the beginning of treatment.

Because pQCT was reported to be capable of detecting minute changes of trabecular bone over the peri- and postmenopausal period [9], a 4-month double-blind prospective study was planned. In the present study, radial trabecular bone measured by pQCT significantly increased in response to 4-month supplementation of calcium as AAACa, but radial cortical bone density and lumbar bone density measured in the anteroposterior direction by dual-energy X-ray absorptiometry did not change significantly, although some tendency of increase was noted. In groups given the same amount of calcium as AAACa or CaCO<sub>3</sub> and the group given placebo containing no calcium, the bone measurement values changed very little, and cortical bone actually decreased in the CaCO<sub>3</sub> group.

Slight initial decrease of lumbar BMD on supplementation with CaCO<sub>3</sub> was also noted in another prospective double-blind study. Although this phenomenon is still unexplained, possibilities of gastric irritation causing loss of appetite leading to a decrease of dietary calcium intake may play a part. The rather small number of test subjects could have made the randomization process somewhat ineffective, however, having placed subjects with poor response to a calcium supplement in the CaCO<sub>3</sub> group as a remotely possible explanation. Because a projectional density measurement like DXA always measures cortical bone several times as dense as trabecular bone together with trabecular bone, true trabecular bone density is always elusive even when the covering cortical bone layer is relatively thin. Measurement of pure trabecular bone without interference by cortical bone may therefore be useful to detect small changes in the trabecular bone. Trabecular bone density was reported to be proportional to the length of the bone exposure to estrogen [9], decreasing preferentially after menopause [10]. Trabecular bone measurement by three-dimensional

pQCT was reported to be useful to detect rapid bone losers among postmenopausal women [11].

By separate measurement of trabecular and cortical bone density, body mass index, grip strength, serum IGF-I, 25(OH) vitamin D, and parathyroid hormone (PTH) 1-84 were found to predict total bone density. Grip strength, serum IGF-I, and PTH 1-84 were significantly related to cortical bone but not to trabecular bone [12]. Postmenopausal loss and estrogen-induced gain mainly involves trabecular bone [13]. The precision of pQCT is comparable to that of DXA, and the correlation between bone mineral density measured by DXA and that measured by pQCT reported to be rather low in one report [14] was made higher by technical improvement in another one [15]. The apparently greater ability of AAACa to increase bone mineral density compared to AACa may be explained by the action of HAI, which augments intestinal calcium absorption [16,17]. AAACa seems to be more useful to prevent osteoporosis than AACa or CaCO<sub>3</sub>.

Limitation of the present study may be related to a rather small number of subjects with a variety of age, including pre- and postmenopausal women, but all showed either osteopenia or osteoporosis after exclusion of subjects with normal bone density. Attempts were made to analyze all data, aiming at minimizing such variation by calculating the percent change of the measured bone measurement values in each subject. Bone markers were not measured in the present study, but AAACa was shown to decrease urinary collagen degradation products (Crosslaps), parathyroid hormone, and serum alkaline phosphatase more efficiently than the same dose of calcium provided by CaCO<sub>3</sub> [4,18,19].

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