Aluminum equivalent densitometric study of the effect of a calcium supplement—UNICAL—a calcium citrate combined with IAM on bone loss in rats of adolescence

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Aluminum equivalent densitometric study of the effect of a calcium supplement—UNICAL—a calcium citrate combined with IAM on bone loss in rats of adolescence

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Abstract To explore the effects of the calcium citrate combined with IAM (Increase Absorption Materials), an oral calcium supplement made from sea shell, which is called UNICAL, on bone loss, we examined the tibias of adolescent Wistar male rats with the application of densitometric measurement by aluminum-equivalent image. The trabecular bone density of calcium-deficient UNICAL diet rats approached the level of the control group; although the value was a little lower than that of the control group and the difference between them was not significant, while the value was significantly higher than that of calcium-deficient-standard diet rats. The cortical bone density of calcium-deficient UNICAL diet rats showed improvement since the value was significantly higher than that of calcium-deficient-standard diet rats, but it had not yet reached the level of the control group. The results suggest that UNICAL may induce high trabecular bone density in rat models of bone loss resulted from dietary calcium deficiency. Further research is necessary to determine if UNICAL has an effect on cortical bone density.

Key words Adolescence, Bone loss, Calcium supplement, Microdensitometry

Introduction

With improving knowledge and advancing technology, physicians who care for the young are assuming an expanding role in the prevention of diseases that become evident during adulthood, but have their origins in childhood and adolescence. The prevention of osteoporosis, often deemed a geriatric disorder, may now be considered as the legitimate domain of pediatricians. Optimization of peak bone mass, about half of which is accumulated during the adolescent growth spurt\(^1\), is important to prevent osteoporosis and associated fractures in later life\(^2\). Dietary factors and physical activity can have positive effects though peak bone mass is mainly genetically determined\(^3\). It has been suggested\(^4\) that higher calcium intakes through adolescence promote greater peak bone mass and provide potential protection from age-related bone loss, whereas low calcium intakes have a nonreversible, deleterious effect on peak bone mass. Although it is generally recognized that optimum calcium intake is best obtained from food sources, calcium supplements are destined to become an important source of dietary calcium, considering that diet of modern humans is insufficient to cover daily requirements of this mineral\(^5\).

Various X-ray techniques have been employed to measure bone mineral content. Early approaches, such as single-photon absorptiometry and single-energy X-ray absorptiometry, were prone to systematic error, and have now been largely superseded by dual-energy techniques, principally dual-energy
X-ray absorptiometry\(^6\)-\(^7\), or by quantitative computed tomography. Quantitative ultrasound studies have been developed as an alternative radiation free method for the non-invasive assessment of the skeleton\(^8\)-\(^9\).

This investigation was undertaken to determine the effect of a calcium supplement, a calcium citrate combined with IAM (Increase Absorption Materials) made from sea shell that is called UNICAL, on the recovery of bone loss resulted from calcium deficiency in adolescent rats with the application of densitometric measurement by aluminum-equivalent image.

Materials and methods

1. Animals and treatment protocol

Thirty male Wistar strain rats, 8 weeks old at the start of the experiment, weighing about 240 g, were randomly divided into following 3 groups. In the control group, rats were fed on a standard diet, containing 0.449% calcium, 0.608% phosphorus, and 1000IU vitamin \(D_3\) per 100 g, with tap water for 6 weeks. In the experimental groups, rats were fed on a no-calcium-component diet with distilled water for the first 3 weeks; then they were allocated into two treatment groups and pair-fed on the standard diet (calcium-deficient-standard diet group) or the UNICAL diet (calcium-deficient-UNICAL diet group), which is a calcium citrate combined with IAM, containing 1.697% calcium, 0.612% phosphorus, and 1000IU vitamin \(D_3\) per 100 g, with tap water for a further 3 weeks. All the rats' food was made by Oriental Yeast (Tokyo, Japan). All the animals were housed individually in stainless steel cages under similar conditions free of ultraviolet light. These rats were sacrificed by exsanguination under pentobarbital sodium anesthesia and tibias were extracted immediately for investigation. The animals were handled by the principles for use of experimental animals outlined by Kyushu Dental College.

2. Microdensitometric measurement with aluminum equivalent image

To analyse the bone mineral density of the specimen, X-ray image was taken with Dixel (MCR-1000, Morita Co., Kyoto, Japan). Bone samples and aluminum wedge were exposed together with Dixel CCD sensor, at 60 kV, 10 mA, and 0.2 sec with focus-detector distance of 30 cm. The images were input to a personal computer-based image-analysis system. The pixel size was 48×48 micrometer. The data were converted to aluminum thickness equivalents to produce aluminum-equivalent images. The bone mineral content was calculated as the mean of the whole region of interest. The precision of the test for homogeneous sample of aluminum step is 0.17% and for measurement of pipe-form sample, stimulating bone sample, is 1.48%.

Bone linear scanning at the midshaft site and area measurement of tibia were performed, which were designated to measure the density of cortical bone and trabecular-abundant region respectively. The results were expressed as aluminum thickness equivalent in mm and the mean and standard deviation (SD) were used as descriptive statistics.

T-test was used to detect the significance of differences between the control and experimental groups.

Results

Table 1 shows the results of the densitometric assessment in control and experimental groups.

1. Area measurement (designated to measure the density of trabecular bone): The value of the calcium-deficient-UNICAL diet group was not significantly different to that of the control group; although the value was a little lower than that of the control group, while the value was significantly higher than that of calcium-deficient-standard diet group \((P<0.05)\).

<table>
<thead>
<tr>
<th></th>
<th>Area measurement</th>
<th>Linear scanning</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.42±0.04</td>
<td>2.01±0.04</td>
</tr>
<tr>
<td>Calcium deficient·Standard diet</td>
<td>1.28±0.02</td>
<td>1.82±0.03</td>
</tr>
<tr>
<td>Calcium deficient·UNICAL diet</td>
<td>1.41±0.01</td>
<td>1.93±0.12</td>
</tr>
</tbody>
</table>

Table 1 Aluminum Equivalent Densitometric Results (mm Al)
2. Linear scanning (designated to measure the density of cortical bone): The value of the calcium-deficient-UNICAL diet group was significantly lower than that of the control group, while it was significantly higher than that of the calcium-deficient-standard diet group ($P<0.05$).

**Discussion**

Prescription of calcium supplements is a frequent practice for the prophylaxis and therapy of osteoporosis, although their beneficial effect is not conclusively established\(^{(10-11)}\). Recent investigations have also focused on the roles for calcium of defending against cancer\(^{(12-13)}\) and lowering office, home and ambulatory blood pressure in hypertensive patients\(^{(14)}\). Oral calcium supplements is a very popular method of balancing inadequate dietary calcium mainly because of its convenient way of intake. Not only is the calcium content of a preparation significant for providing adequate calcium supplementation, but also its bioavailability is of essential importance\(^{15}\). Calcium carbonate is the major constituent in sea shell, and hydroxyapatite calcium is a preferred source of calcium supplements because of its high density of elemental calcium, about 40% by weight. The calcium carbonate preparations were only partially dissolved when administered, but the calcium absorption from these preparations was found to be no less than that from the completely soluble calcium lactogluconate preparations\(^{(16)}\). Calcium citrate preparations, having a lower calcium content of about 21% by weight, may provide a more optimum calcium bioavailability than calcium carbonate\(^{(17)}\), because they are considered much more soluble and lower risk of developing urinary stones than calcium carbonate\(^{(18)}\).

The calcium citrate combined with IAM (UNICAL) made from sea shell is a kind of oral calcium supplement. The results of this study indicated a positive effect of UNICAL on bone debilitation in adolescent animals. The trabecular bone density of calcium-deficient-UNICAL diet rats approached the level of the control group; although the value was a little lower than that of the control group and the difference between them was not significant, while the value was significantly higher than that of calcium-deficient-standard diet rats. The cortical bone density of calcium-deficient-UNICAL diet rats showed improvement since the value was significantly higher than that of calcium-deficient-standard diet rats, but it had not yet reached the level of the control group.

Insufficient dietary calcium during the critical growth and building period may result in failure to reach peak bone mass, leading to osteopenia, osteoporosis, and increased risk of fracture in later life\(^{(19)}\). In the present study, the experimental range was from adolescence to early adulthood of rats, thought to be the critical period for peak bone mass gain. High calcium intake has been reported to be associated with increased bone density in intervention and longitudinal studies on children and adults. Henderson et al.\(^{(19)}\) suggested that supplement of calcium intake have the potential to increase bone mineral density based on their study in a heterogeneous group of 139 children who had spastic cerebral palsy.

In this study, the trabecular bone density of UNICAL animals reached the level of control animals, but the cortical bone density of UNICAL animals did not. Reginster et al.\(^{(20)}\) reported that fluoride, a calcium supplement, is effective in increasing trabecular bone mineral density in the spine, but its effect on bone mineral density at cortical sites is controversial, so that we can not exclude the possibility that the calcium citrate combined with IAM has no effect on the cortical bone mineral density. On the other hand, the results suggested that the recovery of trabecular bone density may be faster than that of cortical bone density. In the intact living bone, bone marrow occupies between the trabeculae, causing that trabecular bone has faster bone metabolism. The appearance also suggested that a longer time of giving the calcium supplement might be required. So further research is necessary to determine if the calcium citrate combined with IAM has an effect on cortical bone density.

The present study with densitometric evaluation by aluminum equivalent image suggests that calcium supplementation induced high bone density in rat models of bone loss resulted from dietary calcium deficiency.

**References**


