

Biosci. Biotechnol. Biochem., 66 (3), 702-704, 2002

**Milk Basic Protein (MBP) Increases Radial Bone Mineral Density
in Healthy Adult Women**

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Preliminary Communication

Milk Basic Protein (MBP) Increases Radial Bone Mineral Density in Healthy Adult Women

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Received September 26, 2001; Accepted December 4, 2001

We studied the effects of daily intake of milk basic protein (MBP) on radial bone mineral density (BMD) in healthy adult women. Thirty-three healthy women were randomly assigned to a 6-month trial with either placebo or MBP (40 mg per day). The radial BMD of each volunteer was measured at the beginning of and at six months after the trial. The mean BMD value at the 6th month in the MBP group increased significantly at both 1/6 and 1/10 portion from the distal end of the radius, whereas that in the control group did not. The BMD gain of each volunteer in the MBP group was significantly higher than that in the placebo group. Thus a daily MBP supplementation of 40 mg in healthy adult women can significantly increase radial BMD.

Key words: milk basic protein; MBP; bone mineral density; bone metabolism

Milk is well known as a good source of bioavailable calcium compared with other food sources. Recent *in vitro* and *in vivo* studies have shown that milk whey protein, especially its basic protein fraction, contains several components capable of both promoting bone formation and inhibiting bone resorption and further have demonstrated that milk whey protein plays a functional role in bone remodeling.^{1,2} In these reports, the active components responsible for promotion of bone formation and suppression of bone resorption were characterized as its basic protein fraction (milk basic protein, MBP). *In vivo* studies in our laboratory showed that the milk whey protein and fractionated whey protein increased femoral bone strength in young ovariectomized rats.^{3,4} We also showed that MBP prevented bone loss in aged and young ovariectomized rats, the former being a suitable model of osteoporosis in hu-

mans.^{5,6} Because MBP reduced the urinary excretion level of deoxypyridinoline (a biochemical marker of bone resorption) clearly in the animal study, we presumed that MBP suppressed the osteoclast-mediated bone resorption.³⁾

A previous report of ours showed that MBP increased calcaneal BMD and affected bone metabolism.⁷⁾ In this study, we examined the effects of MBP on radial BMD in healthy adult women, because the radius is known to be less affected by body weight stress.

Thirty-three healthy women (mean \pm SD age, 28.8 \pm 8.7) were recruited through direct mailings and attending presentations about this study in our institute. A written informed consent was obtained from each subject. The level of physical activity of all volunteers was moderate. In this 6-month, double-blind, placebo-controlled study, the volunteers were randomly assigned to either the placebo or the MBP group with stratification according to body weight, height, body mass index, and BMD. Seventeen volunteers took a 50-ml experimental beverage containing 40 mg of MBP and the other 16 women received a matching placebo beverage. Both beverages contained lactic acid, sugar, and a flavoring as masking ingredients in 50 ml of water. The MBP was prepared from milk as described previously.⁸⁾ The volunteers in each group were instructed to drink one bottle (50 ml) of the beverage daily at any time. They were also advised to maintain their usual diets and to avoid taking supplemental minerals and vitamins throughout the study.

The radial BMD was measured by dual-energy X-ray absorptiometry by use of a DX 600 EX scanner (Aloka, Tokyo, Japan). The validation of the machine was less than 1.0%, and the coefficient of

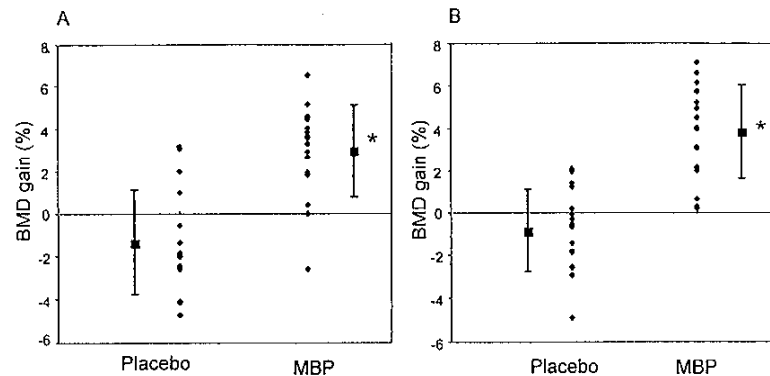


Fig. 1. The Radial BMD Gain in the Control and MBP Groups.

The individual BMD gain at the end of a 6-month study at the 1/6 (panel A) and 1/10 (panel B) portion from the distal end of the radius at the end of the 6 months is plotted, and the mean values and SD of these groups are shown by squares and bars. The BMD gain was significantly higher in the MBP group (*) than in the placebo group in both 1/6 and 1/10 portion ($p < 0.05$).

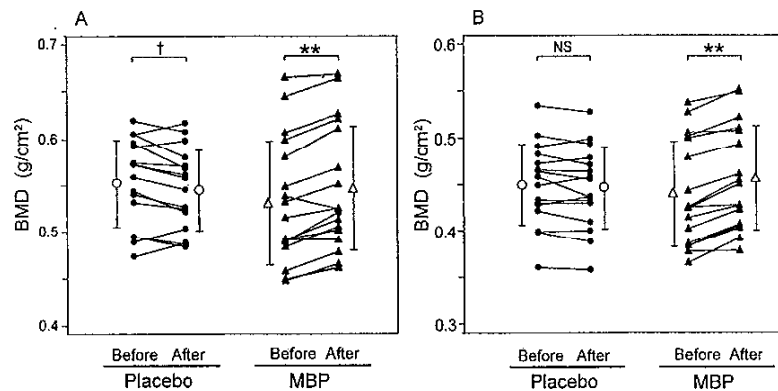


Fig. 2. Individual Radial BMD in the Measurements before and after 6-Month, Double-blind, Placebo-controlled Study.

The individual BMD changes before and after a 6-month study at the 1/6 (panel A) and 1/10 (panel B) portion from the distal end of the radius at the end of the 6 months are plotted and lined. The mean values and SD are shown. The radial BMD changes were examined by using Student's *t*-test for paired data. † represents a significant decrease ($p < 0.05$), and ** represents a significant increase ($p < 0.0001$) after the study.

variation for the measurements was 0.86%. The radial BMD at the end of the study was compared between the study groups by use of the two-sample *t*-test. All calculations were done by the GLM procedure in the SAS statistical analysis package.⁸⁾ All tests were two-tailed. Personal radial BMD were analyzed by Student's *t*-test for paired data to examine the difference between before- and after-study values.

There was no significant difference between the MBP and placebo groups in any of the parameters, *i.e.*, age, weight, height, body mass index, and BMD. During the 6-month study period, no bloating, diarrhea or allergy was observed in either group, and no one withdraw from the study. All volunteers completed the study according to the protocol based on the Helsinki Declaration.

The initial mean values of radial BMD were similar in the 2 groups. The mean of the individual BMD gain at the 1/6 portion from the distal end of the radial bone was significantly higher in the MBP

group than in the placebo group (Fig. 1A). Likewise, that at 1/10 portion from the distal end was significantly higher in the MBP group (Fig. 1B).

Using Student's *t*-test for paired data to examine the difference between measurements before and after 6 months, we found that the individual radial BMD gain in the MBP group had significantly increased during the study, but that in the placebo group had not (Figs. 2A, 2B). The reduction of BMD was observed only at the 1/6 portion in the placebo group. The radius is less affected by body weight stress, and its age-related BMD reduction might have begun.

An earlier report by us showed that there was no significant correlation between the BMD gain and the intake of any dietary minerals or vitamins in either group,⁷⁾ suggesting that the significant increase in BMD in the MBP group was independent of dietary intake of minerals (Ca, P, Mg) and vitamins (vitamin D, K, C). An MBP supplement (40 mg of MBP/day

for 6 months) given to healthy adult women increased calcaneal BMD and suppressed the urinary excretion of cross-linked N-telopeptides of type-I collagen (NTx; a biochemical marker for bone resorption).⁷⁾ These findings were consistent with our current knowledge of responses of osteoclasts *in vitro*.^{2,3)} We also found that cystatin, purified from MBP, suppressed osteoclast-mediated bone resorption (unpublished data). It was earlier reported that recombinant cystatin C inhibited bone resorption *in vitro*.^{9,10)} Thus, we speculate that one of the active components responsible for suppressing bone resorption in MBP is milk cystatin. We also demonstrated that the active components suppressing bone resorption retained their biological activity after gastrointestinal digestion and showed by the everted gut-sac method that they were absorbed through the intestine.²⁾ Thus, the active components in the MBP or partially digested MBP could be absorbed through the intestine and possibly inhibit bone resorption by acting directly on osteoclasts. MBP also contains active components that promote proliferation of and collagen synthesis of osteoblasts.¹⁾ Two components having growth-promoting activity in the MBP were earlier characterized. One was a high mobility group-like protein,¹¹⁾ and the other was a kininogen fragment.¹²⁾

In conclusion, we have shown that a MBP supplementation increased BMD in healthy adult women. From this study, it appears that 40 mg per day of MBP supplementation is effective for promoting bone metabolism and an increase in BMD, at least in their radius. MBP can thus be considered a nutritional component capable of increasing peak bone mass and reducing the future risk of osteoporosis in premenopausal women.

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