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The Effect of Natural Formulated Functional Biscuits on Elderly Bone Health

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The purpose of this research is to evaluate the effect of the functional diet on bone health for elder women with osteoporosis and to elucidate its effect on bone turn-over. The diet is in the form of biscuits contain whey protein and soy bean. Fourteen osteoporotic elder women were recruited in this study and given 60 g biscuits daily for one month. Serum calcium and osteocalcin (OC), urinary deoxypyridoline (U-Dpd) and Cr were measured before and after ingestion of biscuits formula. Serum calcium level was changed after one month of ingestion the biscuits formula. Serum OC concentration had increased significantly after one month of ingestion, while U-Dpd excretion had decreased significantly after one month of ingestion. There was a positive significant correlation between serum OC and U-Dpd before and after ingestion the biscuits. The intake of vitamin D of elder women with osteoporosis was marked lower than that the Recommended Dietary Allowance (RDA). Also, there was a decline in the calcium intake and a higher intake of total protein, magnesium and phosphorus than RDA. A positive significant correlation was found between plant protein and phosphorus, magnesium, zinc. The results suggested that whey protein in combination with soy bean maintaining a balance of bone remodeling in elder women with osteoporosis. It might become a novel, natural and desirable nutritional formula of bone health.

Key words: Whey protein, bone health, bone formation, bone resorption, elder people

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INTRODUCTION

The phenomenon of progressive loss of both the organic and inorganic components of bone mass is one of the major aspects of bone metabolism in elder people. The bone mass of many women after menopause decreases and the risk of fracture increases rapidly (Kanis *et al*., 1991; Seeman *et al*., 1988). There is a enormous interest in research to prevent menopause-related bone loss and fracture. It is important for the prevention of bone loss and fracture for people assimilate sufficient dietary nutrients, especially calcium. Dietary calcium is essential when considering calcium requirements of the elderly, since the bioavailability of calcium declines with age. In addition, there is also a need to develop food components that stimulate bone formation.

Milk is well known as a good source of bioavailabe calcium compared with other food sources. Recent *in vitro* and *in vivo* studies had shown that milk whey protein, especially its basic protein fraction, contains several compounds capable of both promoting bone formation and inhibiting bone resorption and further had demonstrated that milk whey protein plays a functional role in bone remodeling (Seiichiro *et al*., 2005; Takada *et al*., 1996, 1997a). 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, Takada *et al*. (1997b, c) showed that milk whey protein and its fractionated increased femoral bone strength in young ovariectomized rats. Also, MBP prevented bone loss, increasing calcaneal Bone Mineral Density (BMD) and affected metabolism in aged and young ovariectomized rats as a suitable model of osteoporosis (Aoe *et al*., 2001). MBP suppressed the osteoclast-mediated bone resorption by clearly reduced the urinary excretion level of deoxypyridinoline (a biochemical marker of bone resorption) in the animal study (Toba *et al*., 2000).

Phytochemicals are a broad group of plant-derived compounds of nonesteradiol structure that can behave as estrogen mimics. The major classes of phytoestrogens of current interest from a nutritional and health prospective are the lignans and isoflavons (Setchel, 1985). A conspicuous feature of the chemical structure of phytoestrogens is the presence of a phenolic ring that, with few exceptions is a prerequisite for binding to the estrogen receptor (Leclerq and Heuson, 1979). For this reason, phytoestrogen act as estrogen agonists or antagonists (Makla *et al*., 1994, 1995). The incidence of osteoporosis and the risk of hip fracture were significantly lower in postmenopausal Japanese women than in postmenopausal Western women (Cooper *et al*., 1992; WHO Study Group Report, 1994). The lower bone density observed one mo after overiectomy of 95-d-old rats was not seen in animals that were maintained on either estradiol or soy protein, suggesting that soy protein had the potential to protect against the bone loss associated with ovarian estrogen deficiency (Arjmandi *et al*., 1996).

The purpose of the present study is to evaluate the effect of the functional diet on bone health for elder women with osteoporosis and to elucidate its effect on bone turn-over. The diet is in the form of biscuits contain whey protein and soy bean.

MATERIALS AND METHODS

Subjects: Twenty six osteoporotic elder women, with an age range 60-70 years were recruited through direct mailing and attending presentations about this study in National Research Center. The protocol was approved by the ethical Committee of the participating institution. Written informed consent was obtained from such subjects. Bone Mineral Density (BMD) of those subjects was measured using dual-energy X-ray absorpiometery (DPX-L; lunar, Madisom, WE). They had osteoporosis, defined as low bone mineral density (30% below the mean for younger adults) (WHO Study Group Report, 1994).

The subjects similar in socio-economic (upper-mild class), they were living in their house with their families and they were free from any critical illness or medical problems. The subjects were advised not to take any vitamins or minerals specially vitamin D or calcium supplementation.

Preparation of biscuits: The ingredients of the functional biscuits (Whey protein and soy bean- brewer's yeast-germinated wheat- carrots and skimmed milk) were supplied from Agriculture Research Institute and local markets. The ingredients were dried, mixed together and formed in biscuits formula (Table 1).

Table 1: The composition of biscuits

Ingredients	%
Whey protein	20
Soy protein	15
Germinated wheat	32
Carrots	5
Skimmed milk	6
Brewer's yeast	4
Sugar	6
Fat (corn oil)	10
Flavor	2

Study design: Fourteen osteoporotic elder women were completed the study course, who received 60 g biscuits daily to complete their daily requirements for one month and were advised to maintain their diets. The biscuits also contained sweetener and flavor to provide a pleasant taste for the volunteers (Table 1).

The dietary recall was assessed three days weekly and analyzed using food composition table, managed through a computer program (World Food 2.0, 1996).

Venous blood samples were withdrawn after an overnight fasting; serum was separated after clotting and centrifugation and then frozen at -20°C until analysis. The second morning of urine samples were collected and frozen at -20°C until analysis.

Serum calcium, OC, U-Dpd, urinary Cr were measured before and after ingestion of biscuit

Analytical methods: Serum OC was measured by a competitive enzyme linked immuno- sorbet assay (ELISA) method according to the manufacture's instructions (BioSource host ELISA kit, BioSource Europe A.S., Rue de l·Indestrie, 8, B-1400 Nivelles, Betgium). U-Dpd was estimated by a competitive enzyme linked immuno- sorbet assay (ELISA) method according to the manufacture's instructions and the results were corrected for urinary creatinine concentration (Pyrilinks_D, Metra Biosystem, Polo AILO, USA). Urinary creatinine was quantities kinetic according to the method of Houot (1985) as cited in Biosystem. Serum calcium was estimated calorimetrically by Faulker and Meites (1982) as cited in Linear chemicals.

Statistical analysis: The data was expressed as means±SE. The significance differences were determined by t-test student's for paired data to examine the difference before and after one month of ingesting the biscuits formula. Pearson correlation was done. Differences were considered significant at $p<0.05$.

RESULTS

Baseline characteristics of elder women: As shown in this Table 2, T-score (BMD) of the elder women was -2.41, this indicates that the elder women which enrolled in this study were characterized as established osteoporotic.

Biochemical indices in serum and urine: As shown in Fig. 1, serum calcium level was changed after one month of ingestion the biscuits formula ($p<0.02$). Serum OC concentration had increased significantly after one month of ingestion (Fig. 2, $p<0.05$), while urinary deoxypyridinoline U-Dpd excretion had

Table 2: Age, time of menopause and T-score values of osteoporotic elder women

Parameters	
Age (years)	59.8±1.56
Time of menopause (years)	7.6±2.13
T-score	-2.41±0.11

Table 3: Food consumption pattern daily intake (mean±SE) of osteoporotic elder women.

	Groups	
Parameters	Elderly women	RDA FAO/WHO (women)
Total calories	2290.65±110.33	1800
Proteins (g)		
Animals	45.28±4.05	
Plant	46.68±3.92	
Total	89.72±4.51	63
Fats		
Animals	41.43±5.73	
Plant	29.84±5.08	
Total	70.80±6.19	80
Carbohydrate (g)	289.67±22.62	357
Fiber (g)	20.61±1.95	
Cholesterol (mg)	227.12±56.71	
Vitamin D (mg)	2.04±0.69	10
Calcium (mg)	842.59±71.57	1200
Phosphorus (mg)	1495.27±130.50	700
Zinc (mg)	13.20±1.12	15
Magnesium (mg)	429.26±36.84	420

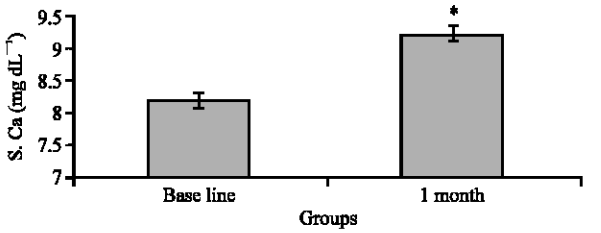

Fig. 1: Serum Calcium (S. Ca) of osteoporotic elder women, one month received biscuits formula

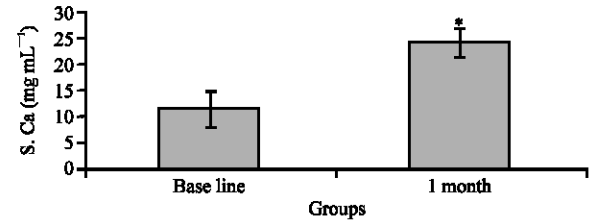

Fig. 2: Serum osteocalcin (S. OC) of osteoporotic elder women, one month received biscuits formula

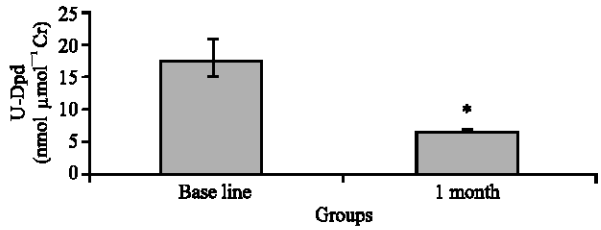

Fig. 3: Urinary deoxypyridinoline (U-Dpd) of osteoporotic elder women, one month received biscuits formula

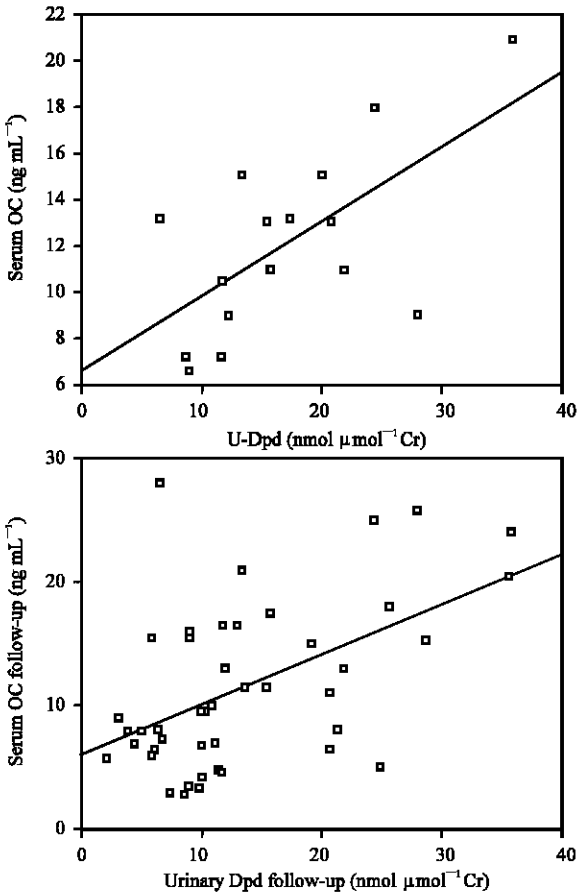

Fig. 4: The correlation coefficient between serum OC and U-Dpd before and after ingestion

decreased significantly after one month of ingestion (Fig. 3, $p<0.001$). An increased serum OC concentration being found in nine (64.3%) of 14 elder women and decreased U-Dpd excretion being found in eleven (78.6%) of 14 elder women. A positive significant correlation was found between serum OC and U-Dpd before ingestion the biscuits ($p<0.05$, Fig. 4) and a marked significant correlation after ingestion ($p<0.01$, Fig. 4).

The composition and nutritional intake of biscuits formula: Table 3 shows the values of food consumption pattern of elder women with osteoporosis. The intake of vitamin D of elder women with osteoporosis was marked lower than recommended dietary allowance of FAO/WHO and there was a decline in the calcium intake than FAO/WHO. There were higher intakes of total protein and phosphorus than FAO/WHO. The elder women with osteoporosis was consumed higher amounts of fiber. The consumption of other nutrients was more or less the recommended dietary allowance. There were a positive significant correlation between plant protein and phosphorus, magnesium, zinc (Fig. 5).

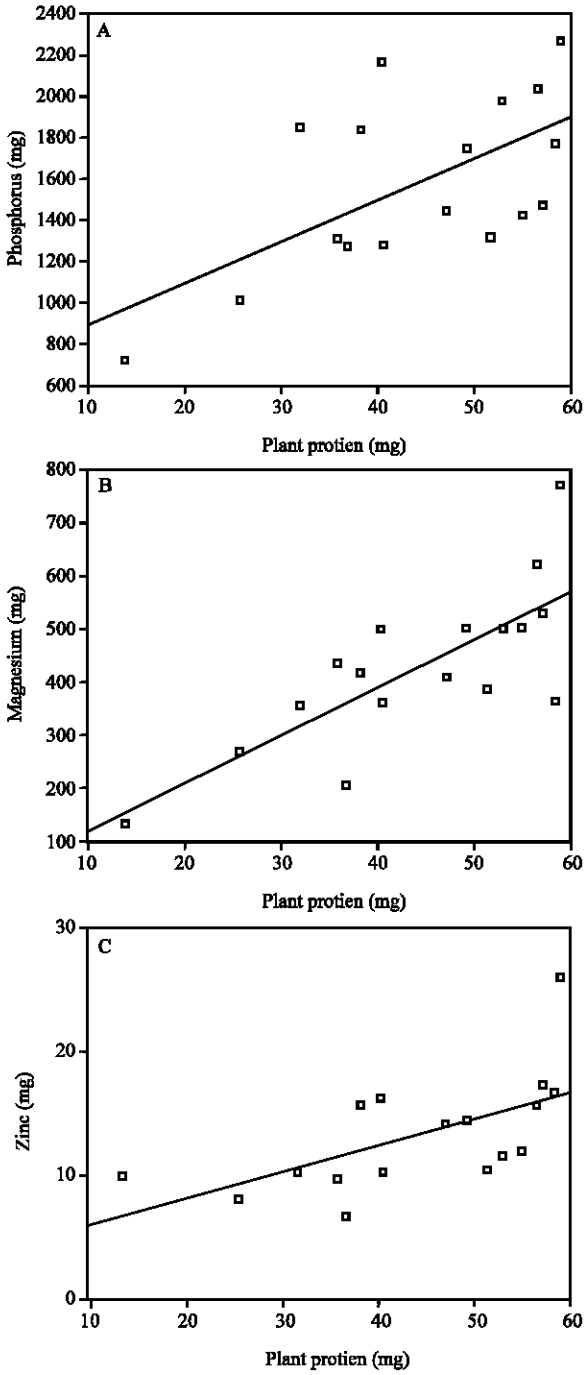

Fig. 5: The correlation coefficient between plant protein and phosphorus, magnesium, zinc

DISCUSSION

As age-related, the balance between bone formation and bone resorption shifts to favor bone resorption which can result in debilitating diseases such as osteoporosis. To prevent bone diseases, it might be questionable to strongly block bone resorption because

this will unbalance bone remodeling, it is important to investigate whether whey protein and soy protein actually, causes a loss in the balance of bone remodeling because it has a suppressive on bone resorption.

In the present study, the results provided evidence of a direct effect of whey and soy proteins on bone metabolism in elder women with osteoporosis. We found that whey protein could be one of the nutritional components that increase peak bone mass and reduce the future risk of osteoporosis.

A biochemical marker of bone turnover that reflects bone changes faster than BMD is available for measuring serum and urine. We measured serum OC as biochemical marker of bone formation, because proteins released from osteoblasts, including osteocalcin can be used to assess bone formation. The product from collagen breakdown, including collagen cross-links, can be used to assess bone resorption. We measured urinary U-Dpd as biochemical marker of bone resorption, because U-Dpd was more sensitive to a change in bone metabolism.

In the present study, serum OC was significantly increased in the elder women with osteoporosis who received biscuits formula compared to the baseline after one month (Fig. 1). Two components that had growth-promoting activity for bone formation, one was a high mobility group like-protein (Yamamura *et al*., 1999) and the other was a kininogen fragment 1,2 (Yamamura *et al*., 2000) in whey protein stimulated the cell proliferation and collagen synthesis of osteoblasts. Also, The positive effect of soy on bone may be in part due to enhanced intestinal calcium absorption by stimulated duodenal calcium transport. However, the calcium absorption promoting effects of soy did not appear to be related to vitamin D. These data might be confirmed by a study by Sheiber *et al.* (1999) involving 50 postmenopausal women who received 60-70 mg of isoflavone in the form of soymilk for 12 weeks demonstrated an increase in serum osteocalcin.

In our results, the U-Dpd excretion was not found to be related to serum OC concentration before ingestion of biscuit (Fig. 2 and 3), but it was found to be related to serum OC after one month of ingestion. This phenomenon suggested that, while whey protein in the biscuit formula suppressed bone resorption, it did not block bone resorption by bone remodeling. We speculate that whey protein promoted bone formation and suppressed bone resorption while maintaining the balance of bone remodeling. Bone was desorbed by the following mechanisms: osteoclasts on the bone surface secreted cathepsin K (Drake *et al*., 1996; Inaoka *et al*., 1995) to digest collagen in the bone matrix, they also secreted protons that dissolved calcium from the bone. Since, whey protein suppressed bone resorption, we speculate that a possible cathepsin inhibitor in MBP from whey protein inhibited bone resorption by osteoclasts. In the pervious study, it reported that one of the active components related to bone resorption in the whey protein was milk cystatin. Also, it demonstrated that the active responsible for suppression of bone resorption retained their biological activity after gastro-intestinal digestion and showed by the reverted gut-sac method that they were absorbed through the intestine (Takada *et al*., 1997a). Thus, the active components of the partially digested MBP in whey protein might be absorbed through the intestine and inhibit bone resorption directly be possible physiological process. This mean that whey protein suppressed bone resorption and led to a relatively predominant status of bone formation against bone resorption in the bone remodeling process *in vivo* (Aoe *et al*., 2001). On the other hand, Sheiber *et al.* (1999) demonstrated a significant reduction in urinary NTx after 12 weeks received 60-70 mg of isoflavone in the form of soymilk of postmenopausal women. Unfortunately, Wangen *et al.* (2000) concluded that the effects of soy isoflavones on the marker of bone turn-over were of a small magnitude and unlikely to be clinically relevant. Thus, the biscuit formula which rich in whey protein and soy bean might be a beneficial effect on bone health.

Present results revealed that serum calcium was statistically higher significantly after ingestion of biscuit formula. These finding was not consistent with the pervious *in vivo* study, it showed that whey protein did not affect Ca balance in rats and no significant correlation between gains of BMD and directly intake of any minerals and vitamins was detected. Therefore, the promoting of bone health by the whey protein and soy bean in biscuit formula was independent on dietary intake of minerals and vitamins (Takada *et al*., 1997b,c).

The dietary intake of elder women included in this study show that the protein intake of those individuals was markedly increased compared to DRA of FAO/WHO (Table 3). Munges *et al.* (1999) found that high intake of dietary protein especially from animals sources, may be associated with reduced incidence of hip fracture in postmenopausal women. The daily intake of vitamin D of elder women was markedly low than FAO/WHO (Table 3), suggesting a contributing role of vitamin D deficiency in the osteoporotic state among those women.

The calcium intake of elderly women was low the recommended daily allowance (1200 mg d^{-1}) (National Institute Consensus, 1994). This shows that those women did not obtain adequate quantity of calcium. This situation becomes more serious case of postmenopausal women where calcium requirement have to be increased to compensate for this active state of bone turn-over under such condition.

In conclusion, present results suggested that biscuit formula promoted bone formation and suppresses bone resorption in elder women with osteoporosis and that it affected bone metabolism while maintaining a balance of bone remodeling. Whey protein in combination with soy might been become a novel, natural and desirable functional food formula of bone health.

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