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The Effect of Chicory Fructan Fiber on Calcium Absorption and Bone Metabolism in Korean Postmenopausal Women*

Yun-Young Kim, Ki-Hyo Jang[1], Eun-Young Lee, Yunhi Cho, Soon Ah Kang[2], Woel-Kyu Ha[3] and Ryowon Choue[4,§]

Department of Medical Nutrition, Graduate School of East-West Medical Science, Kyung Hee University, Seoul 130-701, Korea
[1]Department of Food and Nutrition, Samcheok National University, Gangwon 245-711, Korea,
[2]Department of Molecular Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea,
[3]Pasteur Milk Co., Ltd., Hoengsung-Gun, Gangwon 225-823, Korea,
[4]Research Institute of Clinical Nutrition, Kyung Hee University, Seoul 130-701, Korea

The aim of this study was to investigate the effects of chicory fructan fiber supplementation on bone mineral density, apparent absorption of minerals and serum parameters related to bone turnover in postmenopausal women. Twenty-six healthy Korean postmenopausal women participated in the study. The participants were randomly divided into two groups in a double-blind parallel design and took one of the supplements for 3 months; either a placebo of 8 g maltodextrins /sucrose mixture (control group) or 8 g chicory fructan fiber (fructan group). During the 3-month experimental period no differences were found in bone mineral density (BMD) between the two groups. Apparent calcium absorption significantly increased by 42% in the fructan group, while that of the control group decreased by 29% as compared to the values at baseline. Urinary calcium excretion was not significantly different between the groups. After 3 months, the level of serum alkaline phosphatase (ALP) was significantly lower in the fructan group than in the control group and deoxypyridinolin showed a trend toward a slight reduction. In conclusion, intake of chicory fructan fiber with a regular diet increases apparent calcium absorption in postmenopausal women.

Keywords: Chicory fiber, Fructan, Calcium absorption, Bone mineral density, Postmenopausal women

INTRODUCTION

Osteoporosis is a major public health problem in elderly women around the world. Recent estimates suggest that 30% of Caucasian postmenopausal women in the United States and 23% of women over 50 years of age in European countries have osteoporosis.[1)] Therefore, many treatments including drugs and exercise therapy have been developed to prevent and treat osteoporosis. In exercise therapy, the mechanical stress of exercise stimulates bone formation;[2-4)] however, this therapy brings about some fracture risk in the elderly. Another approach is the supplementation of nutrients such as calcium and vitamin D. These nutrients are required for normal bone metabolism and insufficient intake may be a cause of osteopenia.[5)] Although the causes of osteopenia are not well understood, a common phenomenon of the osteopenic process may be due to a negative calcium balance. Thus, minimizing bone resorption in the elderly is accomplished through adequate intake of calcium in addition to regular exercise. In postmenopausal women, true intestinal calcium absorption rates between 28% and 32% have been reported.[6)] In Europe, Asia and the United States, current dietary calcium intake is far below Recommended Daily Allowance (RDA) levels. In South Korea, average calcium consumption is 500-560 mg/day for adults and only 400 mg/day for people older than 65 years, whereas the Korean RDA is 700 mg/day.[7)]

One of the physiologic changes characterizing aging and menopause is reduced intestinal absorption of calcium.[8)] This may be due to decreased renal formation of 1,25-dihydroxyvitamin D,[9)] combined with reduced intestinal 1,25-dihydroxyvitamin D receptors[10-12)] and receptor responsiveness.[13)] Thus, increasing not only the dietary intake of calcium but also its bioavailability might help postmenopausal women to avoid osteoporosis and bone fractures.[14)]

Inulin is a soluble fructan fiber that is naturally present in a wide range of plants such as chicory, artichoke, salsify, leek, onion, asparagus, wheat, barley, rye, garlic and bananas. For use as a natural food ingredient, inulin is extracted from the chicory root. Chicory inulin is composed of a mixture of β-(2,1) linked fructose chains[15)] mostly ending on a glucose unit with a degree of polymerization (DP) ranging from 2 to 60. Oligofructose can be obtained after partial hydrolysis of

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§ To whom correspondence should be adressed.

chicory inulin or enzymatic synthesis starting from sucrose. Dietary intake of chicory fructans (inulin or oligofructose) has well-established benefits with regard to intestinal conditions. These benefits include a more balanced intestinal microflora with selective stimulation of bifidobacteria, an increased production of short-chain fatty acids in the intestinal lumen and relief of constipation.

The stimulation of absorption of minerals (Ca, Mg, and Fe) by inulin and oligofructose ingestion has been repeatedly demonstrated in animal feeding studies.[16-26] Inulin and oligofructose were even found to increase bone density in rats.[23,27-30] A positive effect of chicory inulin and oligofructose on calcium absorption was also confirmed in human subjects.[31-33] Positive effects of calcium absorption have been shown in adults and adolescents but no results have been published yet for the elderly, especially for postmenopausal women. In the present study, we investigated the effects of supplementation of chicory fructan fiber on apparent absorption of minerals, bone turnover and bone mineral density in Korean postmenopausal women who were not receiving any kind of hormonal replacement therapy.

SUBJECTS AND METHODS

1. Subjects

Twenty-six postmenopausal women were recruited. It was verified that the subjects were not on hormone replacement therapy, Ca supplements or any kind of medication that might interfere with bone metabolism. The subjects had no vertebral compression fractures on lateral spine radiographs and no history of trauma, smoking or alcohol abuse. The women each gave written, informed consent to take part in the study, which was approved by the Human Ethics Committee of Kyung Hee University, Seoul, Korea.

2. Treatments

The commercial chicory fructan fiber was obtained from Cosucra (Belgium) and has an average degree of polymerization of about 10. Using a parallel, randomized, double blind design, the subjects were randomly assigned to either a control group (n=13) or a fructan group (n=13). All women received 2 doses (at breakfast and dinner) of 4 g of chicory fructan fiber or a placebo (maltodextrins/sucrose mixture) with 200 mL of tap water for 3 months. All women were instructed to maintain their usual physical activity and to report any changes in supplements taken during the study.

3. Anthropometry

Height, weight, body fat (% body weight) and lean body mass (LBM) were measured using a Body Fat Analyzer (TBF-202, Japan). Body mass index (BMI) was calculated using the formula of body weight (kg)/height (m^2). Subjects were dressed in light clothing with no shoes on at the time the measurements were taken. Measurements were recorded to the nearest 0.01 cm or 0.01 kg. The circumferences of the upper arm, waist and hips were measured using a non-stretch measuring tape. Triceps were measured using a Skinfold Caliper. Dietary data were collected through the use of a 3-day food record and were analyzed using the Nutritional Analysis Program (CAN pro, The Korean Nutrition Society, Korea, 2000). The dietary patterns of the subjects were assessed by face-to-face interview. Gastrointestinal symptoms were recorded after ingestion of supplements for 3 days and scored as previously reported by Rumessen et al.[34] The questionnaire included the occurrence of pain, diarrhea, borborygmia, distension, flatulence and nausea. All subjective symptoms were rated by the study subjects (none, 0; mild, 1; moderate, 2; or severe, 3) after ingestion of diets for 3 days and total symptom scores were calculated.

4. Sample Preparation

Samples of blood, urine and feces were collected on the day before the experimental period began and on the last day of the experimental period. Venous blood samples were taken after fasting for at least 12 hours, allowed to clot for 30 minutes at room temperature and centrifuged for 10 minutes at 5,000 rpm. Serum samples were removed and stored at -80 ℃. Feces (g/day) were weighed and urine volumes (mL/day) were measured and then stored at -80 ℃ before assay.

5. Bone Mineral Density(BMD)

BMD at the femoral neck and lumber spine were measured using standardized protocols with dual-energy X-ray absorptiometry (Lunar Prodizy, USA) for uniform subject positioning, scan mode and scan analysis. Standardized procedures for patient positioning and use of the DXA software were carried out. In the case of the lumbar spine, BMD was measured from four different places (L1-L4) and the lowest value was recorded. T scores were calculated using the mean of total femur BMD (g/cm^2) for a 20-29-year-old female reference population using data from the third National Health and Nutrition Examination Survey (NHANES Ⅲ). We used the World Health Organization criteria to define women with a T score at the lumbar spine between -1.0 and -2.5 as being osteopenia and below -2.5 as being osteoporotic.

6. Minerals Absorption

Calcium was measured according to the OCPC method

using an Asan 701-622 kit (Asan Pharmaceutical, Seoul, Korea), spectrophotometrically at 575 nm (DU530, BECKMEN, Coulter, Inc. USA). The method used to measure urine calcium was based on the cresolphthalein complexone (CPS) method of Moorehead and Briggs.[35] For the analysis of minerals in the feces, about 0.1 g of feces samples were dissolved in 10 mL of nitric acid and 2.5 mL of perchloric acid mixture and then heated at 100 ℃ for 12 hours. The concentration of minerals (Ca, P, Fe, and Zn) from the feces samples was measured using an ICP (Inductively Coupled Argon Plasma) Emission Spectrometer (Thermo Jarrell Ash ARIS-AP, USA). These procedures were carried out at Korea Basic Science Institute (Seoul, Korea). Apparent absorptions of minerals were calculated using the following equation: Apparent absorption = [{daily mineral intake (mg/day) − daily mineral fecal excretion (mg/day)}/ daily mineral intake (mg/day)] × 100.

7. Chemical Analyses of Bone Turnover Markers

Urinary creatinine was measured using Jaffe's method on an Asan 701-431 (Asan, Korea).[36] Serum osteocalcin level was analyzed in accordance with the IRMA radio-immunoassay method on KAP 1381 (BioSource Europe, Nivelles, Belgium).[37] Total alkaline phosphatase (ALP) activity was measured in accordance with the Kind-King phenylphosphate method on an Asan AM105 S-K, at 500 nm.[38] Urinary free deoxypyridinoline (DPD) was measured using chemiluminescence with an immunoassay kit using the METRA DPD EIA kit (Quidel Corporation, San Diego, CA, USA).

8. Statistical Analysis

Statistical calculations were performed using the Statistical Analysis System (SAS) program (SAS Institute, Cary NC) version 6.12. Results were expressed as mean±SD. All statistical analyses were performed by one-way analysis of variance and the differences between the figures before and after supplementation were tested using a paired t-test. $P<0.05$ was considered statistically significant. Pearson's correlation coefficients were calculated to determine whether the BMD measurements at various sites were related to any of the variables of age, height, weight, years since menopause and number of children.

RESULTS

1. Characteristics of the Study Subjects

The physical characteristics of the subjects are shown in Table 1. The average ages were 60.58±6.74 years for the control group and 60.15±6.99 years for the fructan group. No significant changes were found in body weight, W/H ratio, body fat, LBM, BMI or triceps during the supplementation period. Complicatious abdominal pain and nausea were not found in the subjects. At the beginning of the experiment, a few subjects complained about mild gastrointestinal symptoms (mainly flatulence), but the symptoms diminished such that none of the subjects gave up the experiment.

Table 1. Ages, anthropometric measurements, years since menopause, and number of children of the subjects

Characteristic[2]		Control (n=13)	Fructan (n=13)
Age (years)		60.58±6.74[1]	60.15±6.99
Height (cm)	Before	155.00±4.68	153.07±6.11
	After	154.59±4.63	153.72±6.29
Weight (kg)	Before	59.62±7.21	55.78±6.64
	After	59.23±6.46	57.76±6.94
Waist:hip ratio	Before	0.86±0.05	0.86±0.06
	After	0.86±0.05	0.85±0.05
Body Fat (%)	Before	30.85±5.73	31.36±5.77
	After	30.85±4.52	30.64±3.73
LBM (kg)	Before	40.55±3.78	38.58±3.54
	After	40.80±3.87	40.07±5.26
BMI (kg/m^2)	Before	24.79±2.71	23.82±2.68
	After	24.83±2.36	24.39±1.91
Triceps	Before	26.62±8.15	23.23±6.42
	After	26.25±5.70	22.72±5.23
YSM (years)		11.4±7.7	12.5±9.0
Number of children		3.0±1.1	3.1±1.5

1) Values are mean±SD. N=13 in each group.
2) Abbreviations: LBM, lean body mass; BMI, body mass index; YSM, years since menopause.

2. Nutrient Intakes of the Subjects

Fig. 1 shows the nutrient intakes of the control and the fructan groups compared with the Korean RDA for elderly women (50-64 years). There was no statistical difference between the two groups for any of these

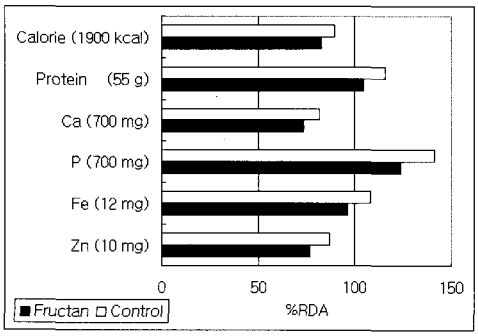

Fig. 1. Comparison of daily nutrients intake with Korean RDA for elderly women (50-64 years).
Numbers in parenthesis represent Korean RDA for each nutrient for elderly women (50-64 years). N=13 in each group.

nutrients. Most of the nutrient consumption was similar to the Korean RDA. The daily intake of calcium and zinc were very low in both groups, about 20% lower than the Korean RDA.

3. Bone Mineral Density (BMD)

BMD of the lumbar spine and femoral neck are shown in Table 2. All of the participants in the control and the fructan groups suffered from either osteopenia (-1.0 > Tscore > -2.5) or osteoporosis (-2.5 > T score). There were no differences between the experimental groups either before or after supplementation. BMD levels at the lumbar spine negatively correlated with age and years since menopause (YSM) (r=-0.445 and p=0.000, r=-0.536 and p=0.000) and with number of children (r=-0.366 and p=0.014) (Table 3). There was no correlation between BMD and height (r=0.117 and p=0.423) and weight (r=0.166 and p=0.275). Similar results were also seen for the femoral neck (Table 3).

Table 2. Bone mineral density of lumbar spine and femoral neck before and after supplementation

		Control	Fructan
Lumbar spine (g/cm^2)	Before	0.82±0.17[1)]	0.79±0.11
	After	0.81±0.18	0.79±0.12
T-score	Before	-2.36±1.47	-2.57±0.87
	After	-2.41±1.53	-2.52±1.01
Femoral neck (g/cm^2)	Before	0.86±0.12	0.80±0.12
	After	0.85±0.11	0.80±0.12
T-score	Before	-0.64±0.98	-1.09±1.03
	After	-0.66±0.94	-1.09±1.00

1) Values are the mean±SD. N=13 in each group.

Table 3. Correlation of bone mineral density with age, height, weight, number of years since menopause, and number of children

Variables	Lumbar spine (g/cm^2)		Femoral neck (g/cm^2)	
	r[1)]	p	r	p
Age (years)	-0.445	0.000	-0.488	0.000
Height (cm)	0.117	0.423	0.002	0.981
Weight (kg)	0.166	0.275	0.111	0.471
YSM (years)[2)]	-0.536	0.000	-0.520	0.000
Number of children	-0.366	0.014	-0.273	0.063

1) r: Pearson's correlation coefficient. N=26.
2) YSM: number of years since menopause.

4. Apparent Mineral Absorption

Serum levels of calcium did not differ between the 2 groups after 3 months, confirming that the serum calcium level was strictly controlled. The apparent absorption results for calcium, phosphorous, iron and zinc are summarized in Table 4. There were no significant differences between the experimental groups for apparent absorption of phosphorous and zinc. However, there was a significant effect of fructan supplementation on apparent calcium absorption ($P<0.05$). At baseline, there was no significant difference between the two groups, but after 3 months apparent calcium absorption in the fructan group was significantly higher (79%) than in the control group. In fact, chicory fructan fiber supplementation significantly increased apparent calcium absorption, from 38.6±7.3 at baseline to 54.9±7.2%($P<0.05$). Apparent iron absorption also increased significantly after fructan supplementation, from 46.8±4.7% at baseline to 70.1±6.2%($P<0.05$).

5. Urinary Excretion of Minerals

The urinary calcium concentrations for the two groups were evaluated (Table 5). Urinary calcium level slightly increased in the control group during the experimental period (9.79±4.82 at baseline, 13.85±4.91 at 3 months). However, urinary calcium concentration in the fructan group decreased slightly without reaching a statistically significant level.

Table 4. Daily intake, fecal excretion, and apparent absorption of mineral before and after consumption of 3 months supplementation

		Ca		P		Fe		Zn	
		Control	Fructan	Control	Fructan	Control	Fructan	Control	Fructan
Intake (mg/day)		560.9±149.5[1)]	514.8±114.1	973.2±113.1	865.7±137.7	17.3±2.8	15.4±1.9	8.7±1.2	7.6±1.5
Excretion in feces (mg/day)	Before	318.1±133.7	315.6±134.0	254.9±91.9	234.3±87.9	8.0±2.6	8.2±3.1	7.1±3.2	6.2±2.7
	After	388.2±159.3	231.8±63.1	352.2±113.7	258.5±103.8	6.4±2.6	4.6±2.0**	6.9±2.6	5.9±2.0
Apparent absorption (%)[2)]	Before	43.2±9.3	38.6±7.3	73.7±14.2	72.9±11.1	53.7±5.1	46.8±4.7	18.4±2.1	18.4±3.3
	After	30.7±6.4*	54.9±7.2+,**	63.8±8.8	70.1±10.0	63.0±7.4*	70.1±6.2+,***	20.7±2.5	22.3±2.6

1) Values are mean±SD. N=13 in each group.
2) Apparent absorption=[{daily mineral intake(mg/day) - daily mineral fecal excretion (mg/day)}/daily mineral intake(mg/day)]×100.
+ : Within each row, a+indicates significant differences ($P<0.05$) between control and inulinfructan groups.
*, **, *** : mean statistical difference(*$P<0.05$; **$P<0.01$; ***$P<0.001$) between before and after supplements.

Table 5. The urinary concentration of calcium corrected or not for the urinary concentration

		Control	Fructan
Ca (mg/dL)	Before	9.79±4.82[1)]	14.89±5.73[+]
	After	13.85±4.91[*]	13.81±5.92
Ca/creatinine (mg/mg)	Before	0.36±0.08	0.50±0.11[+]
	After	0.51±0.10[*]	0.46±0.13

1) Values are mean±SD. N=13 in each.
+ : Within each row, a + indicates significant differences (P<0.05) between control and fructan groups.
* : mean statistical difference (P<0.05) between before and after supplements.

6. Bone Turnover Markers

Although differences in serum ALP levels between baseline and 3 months were not significant for either group, percentage of mean changes between baseline and 3 months in the activity of ALP increased by 0.9% in the control group, but decreased by 19% in the fructan group (Table 6). After 3 months, the serum ALP level was significantly lower in the fructan group than in the control group (P<0.05). No significant differences in serum osteocalcin levels were detected.

Urinary deoxypyridinoline (nM/mM of creatinine in urine) for the two groups is shown in Table 6. Though differences did not reach the level of statistical significance, fructan supplementation tended to reduce urinary deoxypyridinoline concentration (nM/mM of creatinine in urine) while the placebo tended to increase this parameter.

Table 6. Concentration of serum ALP, osteocalcin, and urinary deoxypyrinoline before and after supplementation

		Control	Fructan
ALP (K-A Unit)	Before	9.16±2.52[1)]	7.74±1.70
	After	9.24±3.85	6.27±2.90[+]
Osteocalcin (ng/mlmL)	Before	11.23±2.62	13.45±5.61
	After	10.24±4.01	11.66±6.13
Deoxypyridinoline (nM/mM creatinine)	Before	6.73±1.39	7.08±1.40
	After	6.82±1.51	6.54±2.07

1) Values are mean±SD. N=13 in each.
+ : Within each row, a + indicates significant differences (P<0.05) between control and fructan groups.

DISCUSSION

In the present study, it was confirmed that consumption of calcium by Korean postmenopausal women was far below the RDA for calcium for elderly Korean women (700 mg/day)[39)] and that many Korean postmenopausal women suffered from either osteopenia or osteoporosis. The present results provide further evidence of a positive association between chicory fructan fiber consumption and calcium absorption in postmenopausal women. With regard to the effects of fructan compared to the control group, fructan supplementation significantly increased the apparent absorption of calcium. At low consumption of Ca (70% of Korean RDA), supplementation of 8 g of chicory fructan fiber daily for 3 months significantly increased apparent calcium absorption by 42%, while the placebo treatment decreased apparent calcium absorption by 29%. Whereas initial levels of apparent calcium absorption were not significantly different between the groups, at the end of the 3-month treatment period, apparent calcium absorption was 79% higher in the fructan group (54.9%) than in the control group (30.7%) as shown in Table 4. The current data demonstrate that although differences did not reach the level of statistical significance, fructan supplementation reduced urinary calcium concentration, corrected for the urinary creatinine level from 0.50±0.11 (before) to 0.46± 0.13 (after), while this parameter increased from 0.36± 0.08 (before) to 0.51±0.10 (after) in the control group.

This result was associated with a tendency toward reduced fecal excretion of calcium. The current trial assessed urinary concentration of calcium, corrected for urinary creatinine concentration, as an indicator of urinary calcium loss. Creatinine excretion over 24 hours is relatively stable and is commonly used as a correction factor to take into account variations in urine collection volumes.[40)] Results in Table 5 show no significant differences in urinary Ca loss (Ca/creatinine) between the control group and the fructan group, indicating that the increased apparent Ca absorption as a result of chicory fructan fiber consumption was not offset by increased urinary Ca loss. The methodology used for the measurement of mineral bioavailability in the present study is based on balance studies rather than on oral administration of isotopes. Although oral administration of isotopes is the most reliable method, its application in the present study was not easy due to the permission issue and, therefore, further analyses using isotopes might be warranted.

Several hypotheses about the mechanisms of the effects of chicory fructan fiber on calcium absorption could be proposed. First, the fermentation of fructan in the intestine resulting in the stimulation of mineral absorption, including calcium, in rats and humans has been reported.[19)] The stimulating effects of fructan on calcium absorption are hypothesized to take place in the large intestine. Chicory fructan fiber is fermented to produce short chain fatty acids, mainly acetate, propionate and butyrate.[19)] The resultant acidification of the large intestine has been proposed to stimulate calcium absorption by either H^+/Ca^{++} exchange or by increasing the soluble portion of the calcium pool. Second, fructan fermentation in the animal cecum is accompanied by cecum enlargement, which may increase the intestinal surface area for absorption.[41)] Third,

fructan-stimulated absorption of calcium may be mediated by stimulated expression of calcium binding proteins such as calbindin-D9k.[42]

It is important to measure the bone density to be sure of the effects of treatment on osteoporosis patients, but it would take at least 12 months to certify the differences between before and after treatment.[43] However, differences in bone turnover parameters should become obvious after 3 to 6 months of treatment.[44-45] Our data showed that the supplementation of chicory fructan fiber for 3 months did not change the BMD but did alter the levels of bone turnover markers, specifically ALP.

In the current study, two bone formation markers, osteocalcin and ALP, and a bone resorption marker, deoxypyridinoline, were investigated. Overall, chicory fructan fiber intake reduced the activity of ALP. However, the reduction in deoxypyridinoline did not show significance in statistical analyses and, therefore, further analyses are warranted. It is interesting to note that osteoporotic subjects had significantly higher osteocalcin concentrations than young control subjects.[46-48] The results suggest that a fructan diet might affect the bone metabolism in postmenopausal women by slowing both bone formation and bone resorption. As a positive effect on bone density of fructan supplementation was not confirmed by measuring BMD, longer study periods will be required for further investigation. In conclusion, intake of fructan with a regular diet increases apparent calcium absorption in postmenopausal women without increasing urinary calcium loss, which may lead to improved bone health.

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