Original Article

Change of BMD after Weaning or Resumption of Menstruation in Chinese Women with Different *Fok*I VDR-genotypes: A Randomized, Placebo-controlled, Calcium Supplementation Trial*

YU Bo[1], WU HongYing[2], LI Fang[3], GONG Jie[3], ZHOU DunJin[3], ZHANG ZhiFeng[3], YANG XiaoGuang[1], and HUANG ZhenWu[1,#]

1.National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing 100021, China; 2.Department of Radiography，Union Hospital, Huazhong University of Science & Technology, Wuhan 430022, Hubei, China; 3.Wuhan Regional Center for Disease Control and Prevention, Wuhan 430024, Hubei, China

Abstract

Objective To investigate the effect of calcium supplementation on bone mineral density (BMD) in Chinese women with different FokI vitamin D receptor (VDR) genotypes (FF, Ff, and ff) after weaning or resumption of menstruation during lactation.

Methods A total of 40 subjects with the same FokI VDR genotype were randomly divided into two groups: one received calcium tablet (600 mg once daily as $CaCO_3$) and the other placebo tablet once daily for 1 year. At baseline, BMD was measured by dual-energy X-ray absorptiometry at lumbar spine (L2-L4) and at left hip whereas serum PICP, serum OC, and urinary CTX, serum 25(OH)VitD_3, and serum estradiol were measured at weaning and 1 year thereafter.

Results After the intervention, BMD at lumbar spine and at left hip increased significantly in all these women with a trend among different FokI VDR genotypes such as FF > Ff > ff (P<0.05, <0.01, and <0.001, respectively). BMD at lumbar spine in women with FF VDR genotype increased much more rapidly than in those with ff VDR genotype (P<0.05). Compared with the control group women with the FF genotype regained more BMD after calcium supplementation (P<0.05).

Conclusion Daily calcium 600 mg supplementation has beneficial effect on the bone health of women with FF VDR genotype.

Key words: FokI VDR genotype; Calcium supplementation; Bone mineral accretion after weaning; Chinese women; Low dietary calcium intake.

Biomed Environ Sci, 2011; 24(3): 243-248 doi: 10.3967/0895-3988.2011.03.006 ISSN: 0895-3988

www.besjournal.com(full text) CN: 11-2816/Q

INTRODUCTION

According to the Chinese national advisory body latest recommendations published in 2002, the daily recommended intake (DRI) of calcium during pregnancy and lactation is 1 200 mg/d, which is very different from the new DRI for Japanese women[1] but are similar to that recommended in USA and Canada[2], where dietary calcium intakes often exceed 1 000 mg/d[3].

*This work was supported by the Ministry of Health and Ministry of Science and Technology (2008BAI58B02) and National Nature Science Foundation (30571573).

#Correspondence should be addressed to HUANG ZhenWu. Tel: 86-10-83132906. Fax: 86-10-83132021. E-mail: zhenwuhuang@sina.com

Biographical note of the first author: YU Bo, born in 1971, associate professor, majoring in nutrition and food safety. E-mail: yuboshi@263.com

Received: February 25, 2011; Accepted: April 21, 2011

Pregnancy, postpartum amenorrhoea, and lactation are known to alter the hormonal and physiological status of women and influence peak bone mass[4]. However, this decrease of bone mineral density (BMD) especially at lumbar spine is usually transient and shows complete recovery on cessation of lactation or resumption of menstruation during lactation in developed countries where women normally have sufficient daily calcium intake[5-7].

In addition to these physiologic factors, some environmental factors, including insufficient calcium supply during pregnancy and lactation and insufficient sunlight due to a traditional 1-month bed-rest after delivery in China, can lead to more maternal bone loss and incomplete recovery in Chinese women. Therefore they are likely to have a lower peak bone mass in adulthood and will be at higher risk of developing osteoporosis in later life.

Limited studies conducted in African women with low habitual dietary calcium intake showed that the pattern and magnitude of bone response during lactation or after weaning were independent of their dietary calcium intake or calcium supplementation[8-10].

On the other hand, the importance of genetic interactions on bone metabolism has been recognized and polymorphisms of vitamin D receptor (VDR) genes have been known associated with BMD since 1994. However, there have been few studies of associations between VDR gene polymorphisms and BMD in pregnant or lactating women[11-14], and no study focusing on the effect of calcium supplementation.

In this randomized, placebo-controlled, calcium supplementation trial we examined relations between FokI polymorphisms of the VDR gene and BMD changes in Chinese women with a low habitual dietary calcium intake who were given daily calcium 600 mg supplement after weaning or resumption of menstruation during lactation.

SUBJECTS AND METHODS

Genotyping, Grouping, and Calcium Supplementation

Blood samples of pregnant women aged 18-40 y living in Dongxihu district, Wuhan, China were collected and genotyped. A 265-bp fragment of genomic DNA, which includes the site of a C to T polymorphism at the start codon, was amplified by polymerase chain reaction (PCR) then digested by the FokI restriction enzyme as described by Ames et al. (1999). The alleles are designated "f" (FokI site present) or "F" (FokI site absent).

Within 1 week after the "bed-rest" month, 120 lactating mothers (40 for each FokI VDR genotype FF, Ff, and ff) were enrolled and randomly and evenly assigned to receive either calcium 600 mg tablet once daily or once-daily placebo tablet for 1 y.

Based on estimations of daily calcium intake from common diets in China, calcium supplementation as $CaCO_3$ chewable tablet (Century Jinde Ltd, Beijing, China) provided the subjects approximately 1 200 mg calcium intake daily whereas placebo tablet (VitC chewable tablet with a different shape; Yangshengtang Ltd, Bejing, China). Subjects and investigators were blinded to the identity of tablets.

Inclusion criterion was women who were still breastfeeding after the "bed-rest" month. Subjects with history of bone disease or taking medications known to affect bone metabolism were excluded.

Approval for the study was obtained from the Ethical Committee of the National Institute for Nutrition and Food Safety, China CDC. Written informed consent was from each subject before the trial.

BMD Measurement

BMD at lumbar spine (L2-L4) and at left hip was measured by dual-energy X-ray absorptiometry (DEXA, A NORLAND XR36, USA) on cessation of lactation or confirmed resumption of menstruation and 1 year thereafter. Quality control was performed every day during the study period according to the manufacturer's instructions. The coefficient of variation (CV) % was 0.68-0.92.

Biochemical Assays

Bone turnover biomarkers such as serum osteocalcin (OC), urinary type 1 collagen telopeptide (CTX), and serum carboxyterminal propeptide of type I procollagen (PICP) were detected by ELISA using antibodies supplied by Immunodiagnostic Systems Limited(IDS Ltd), UK, whereas serum 25(OH)VitD_3 (DioSorin Co., USA), a marker for vitamin D status, and serum estradiol (Beifang Co., China) were measured by radioimmunoassay.

Calcium Intake from Diet

Calcium intake from diet was estimated by food-frequency questionnaire (FFQ) that probed for recent consumption of calcium-rich food sources in the Chinese diet, particularly dairy products and calcium-enriched flour.

Statistical Analyses

The effect of FokI polymorphisms of the VDR gene on BMD and bone turnover markers was evaluated by ANCOVA. Mean absolute and percent changes from baseline to endpoint in the two groups were compared by independent *t*-test; paired samples *t*-test was used to compare mean absolute and percentage changes in subjects of the same group. Values of $P<0.05$ were considered significant. All statistical analyses were performed using SPSS 11.5 software. Values are reported as $\overline{x} \pm s$.

RESULTS

Characteristics of Subjects

Baseline characteristics of subjects are shown in Table 1. Average age of lactating mothers was 27.8±4.1 years; body weight was 60.7±8.9 kg, height 158.1±5.1 cm, and BMI 24.2±3.4 kg/m^2. No significant intergroup difference was found in these parameters. BMI was similar in women with different genotypes as well as in the calcium supplementation group and control group within each genotype (*P*=NS).

Table 1. Selected Characteristics of Chinese Women at Baseline

Characteristic	Genotype					
	FF		Ff		ff	
	Calcium	Control	Calcium	Control	Calcium	Control
n	18	15	19	18	17	15
Age (y)	27.1±5.7	28.2±5.4	28.7±3.7	27.8±3.9	28.1±2.5	26.7±2.8
Height (cm)	160±4.8	156±4.3	159±5.6	158±5.3	158±4.2	159±5.9
Weight (kg)	62.2±6.5	58.5±9.0	60.9±11.1	62.2±10.0	60.8±8.0	59.2±7.8
BMI (kg/m^2)	24.3±2.3	23.6±4.4	24.1±3.6	25.0±3.4	24.3±3.5	23.5±2.8
Calcium intake (mg/d)	503±220	482±145	578±239	608±184	626±262	533±160
Parity	1.1±0.3	1.2±0.4	1.1±0.3	1.3±0.5	1.1±0.3	1.1±0.3
No. previous pregnancies	1.7±1.0	1.9±1.8	1.4±0.7	1.6±0.8	1.9±1.6	1.8±1.3

Habitual calcium intake of these Chinese women [559±209 (range, 124–1205) mg/d] was only 45% of DRI, but similar among women with different genotypes and between the calcium supplementation group and control group within each genotype (*P*=NS; Table 1). Rice, wheat flour, fruits, and vegetables were the primary sources of calcium intake, contributing >80% of total daily intake in these Chinese women who were short of dairy products.

The times of parity and pregnancy were similar among women with different genotypes and between the calcium supplementation group and control group (*P*=NS; Table 1).

Duration of Lactation and BMD Measurements

The duration of lactation was on average 100±46 days; no significant difference was found among different genotypes and between the calcium supplementation group and control group within each genotype (*P*=NS; Table 2).

At baseline, there was no significant difference in BMD at lumbar spine and at left hip among different FokI VDR genotypes and between the calcium supplementation group and control group within the same FokI VDR genotypes (*P*=NS; Table 2).

At nearly 1 year versus baseline, BMD at lumbar spine and left hip in Chinese women with FF, Ff, and ff VDR genotypes increased significantly ($P<0.05$, <0.01, and <0.001, respectively; Table 2) both in the calcium supplementation group and placebo control group.

During 1 year after weaning, among different FokI VDR genotypes percent change of BMD (ΔBMD%) at lumbar spine and left hip was in the order of magnitude: FF>Ff>ff. In lumbar spine in women with calcium supplementation a significant difference of ΔBMD% was found between the FF and ff genotype ($P<0.05$; Table 2). Furthermore, women with FF genotype in the calcium supplementation group had higher ΔBMD% than those with the same FF genotype on placebo ($P<0.05$; Table 2).

Biochemical Parameters

As shown in Table 3, there was no significant change in 25(OH)VitD_3 and estradiol in women with any genotype or significant difference between the calcium supplementation group and placebo control group during $\leqslant$1 year after weaning (*P*=NS).

Table 2. Duration of Lactation, BMD, and Change Rate of BMD（ΔBMD%）

Variable	Genotype					
	FF		Ff		ff	
	Calcium	Control	Calcium	Control	Calcium	Control
n	18	15	19	18	17	15
Duration of lactation (d)	91±46	116±48	101±46	106±68	90±34	91±47
BMD at Lumbar spine						
Baseline (g/cm^2)	0.954±0.078	0.950±0.112	1.002±0.090	0.991±0.122	0.963±0.097	0.962±0.100
1 year (g/cm^2)	1.119±0.091^{a3}	1.049±0.093^{a3}	1.123±0.111^{a3}	1.089±0.126^{a3}	1.050±0.092^{a3}	1.036±0.099^{a3}
ΔBMD%	17.40±5.80	10.75±4.88^{b1}	12.29±9.07	10.25±7.13	9.48±7.66^{c1}	7.88±5.16
BMD atfemoral neck						
Baseline g/cm^2)	0.771±0.082	0.769±0.091	0.774±0.087	0.763±0.086	0.791±0.109	0.811±0.127
1 year (g/cm^2)	0.884±0.113^{a3}	0.839±0.068^{a3}	0.856±0.113^{a3}	0.819±0.114^{a2}	0.852±0.112^{a3}	0.842±0.108^{a1}
ΔBMD%	14.68±8.77	9.70±7.93	10.73±6.81	7.19±7.93	8.09±6.62	4.39±6.26
BMD at Ward's triangle						
Baseline g/cm^2)	0.696±0.082	0.696±0.107	0.715±0.114	0.701±0.112	0.744±0.153	0.765±0.162
1 year (g/cm^2)	0.779±0.084^{a3}	0.767±0.112^{a3}	0.780±0.147^{a2}	0.735±0.102	0.795±0.179^{a1}	0.799±0.156^{a1}
ΔBMD%	12.13±7.20	10.56±6.48	9.04±9.78	5.76±12.20	7.29±12.00	4.93±7.24
BMD at trochanter						
Baseline g/cm^2)	0.618±0.076	0.600±0.096	0.629±0.085	0.625±0.108	0.634±0.117	0.630±0.166
1 year (g/cm^2)	0.694±0.077^{a3}	0.657±0.123^{a3}	0.688±0.093^{a3}	0.665±0.104	0.688±0.130^{a3}	0.661±0.178^{a1}
ΔBMD%	12.80±8.93	9.34±5.20	9.47±5.84	6.69±6.82	8.56±6.38	5.00±7.50
BMD at left hip						
Baseline (g/cm^2)	0.852±0.088	0.829±0.104	0.856±0.118	0.861±0.116	0.857±0.110	0.843±0.134
1 year (g/cm^2)	0.904±0.088^{a3}	0.863±0.127^{a1}	0.891±0.117^{a2}	0.886±0.112^{a2}	0.883±0.102^{a2}	0.864±0.125^{a1}
ΔBMD%	6.25±4.97	4.04±7.24	4.27±4.93	2.98±3.50	3.20±4.59	2.67±3.91

Note. $^{a1}P<0.05$; $^{a2}P<0.01$; $^{a3}P<0.001$ *vs.* baseline; $^{b1}P<0.05$ *vs.* calcium group in women with FF VDR genotype; $^{c1}P<0.05$ in women with FF *vs.* ff VDR genotype.

Table 3. Change of Serum 25(OH)vitD$_3$, Serum Estradiol, and Bone Turnover Markers

Variable		Genotype					
		FF		Ff		ff	
		Calcium	Control	Calcium	Control	Calcium	Control
n		18	15	19	18	17	15
25(OH)vitD$_3$ (ng/mL)	Baseline	25.89±14.61	30.21±17.74	26.84±15.09	26.35±15.88	23.76±14.81	26.85±18.40
	1 year	27.16±19.61	27.61±11.88	25.27±11.91	27.07±14.90	21.56±10.75	27.67±13.06
Estradiol (nmol/L)	Baseline	0.221±0.067	0.244±0.097	0.284±0.156	0.208±0.052	0.289±0.135	0.304±0.114
	1 year	0.186±0.055	0.190±0.077	0.220±0.110	0.212±0.101	0.233±0.157	0.225±0.133
Urinary CTX (μg/L)	Baseline	6.93±12.92	8.13±9.73	5.38±6.88	4.43±6.19	4.76±5.64	3.61±4.33
	1 year	8.85±7.97	6.78±7.05	5.16±4.49	3.47±3.64	4.01±3.72	3.09±3.46
Serum OC(μg/L)	Baseline	17.65±10.76	20.28±10.40	13.80±8.17	15.19±8.46	14.32±9.70	15.01±9.77
	1 year	13.45±7.71	14.75±5.44	9.80±6.17	12.33±9.27	10.12±8.36	12.51±5.28
Serum PICP (μg/L)	Baseline	83.3±50.6	107.7±51.7	90.3±57.1	93.0±51.1	113.8±62.0	108.2±52.2
	1 year	136.6±51.4^{a2}	149.2±91.3^{a1}	129.4±67.9^{a1}	125.6±53.0^{a2}	148.2±57.0^{a1}	137.8±56.4

Note. $^{a1}P<0.05$; $^{a2}P<0.01$ *vs.* baseline.

Urinary CTX slightly decreased during the same period in all subjects except women with FF genotype in the calcium supplementation group. Serum OC decreased slightly (*P*=NS) while serum PICP significantly increased in all subjects except women with ff genotype in the placebo control group (Table 3).

DISCUSSION

The gene for VDR has many polymorphisms in the 3'-end region (as recognized by the restriction enzymes BsmI, ApaI, and TaqI) and VDR start codon (as determined by the enzyme FokI). Since 1994, an association has been known to exist between polymorphisms of VDR gene and BMD in children[15-19] and premenopausal women[20-21].

To date, a few studies have been conducted to examine relations between VDR alleles and changes of BMD during lactation or after weaning[11-14], in women with and without sufficient dietary calcium intake. Significant differences in change of BMD postpartum were observed among calcium-insufficient Brazilian adolescent mothers with different ApaI or TaqI VDR genotypes, and also a significant difference in breast milk calcium among those different FokI VDR genotypes[13], but not in Japanese women with low calcium intake[14].

To our knowledge, this is the first study to observe different effects of calcium supplementation on BMD recovery after weaning or resumption of menstruation among FokI VDR genotypes in women with low dietary calcium intake.

During ⩽1 year after weaning or resumption of menstruation, a trend of magnitude of change of BMD at all sites considered in Chinese women with different VDR genotypes was found such as: FF>Ff or ff, similar to results from Matsushita's research group[14].

When Chinese women were supplemented with calcium 600 mg/day, a significant difference in the change rate of BMD at lumbar spine after weaning was found between the FF genotype and ff genotype. This implies that FokI polymorphism of the VDR gene is a candidate determinant that can affect bone mineral status in Chinese women after weaning, as was found among VDR genotypes (recognized by the ApaI or TaqI restriction enzymes) in lactating Brazilian adolescent mothers[13].

This association can be partly explained by VDR genotype-related differences in calcium metabolism[22-27]. In children, polymorphisms of the VDR gene (recognized by FokI, ApaI, or TaqI) were significantly related to calcium absorption and urinary calcium excretion; children who were FF homozygotes had mean calcium absorption 41.5% and 17% greater than those who were ff homozygotes and Ff heterozygotes, respectively. In young Chinese women (students aged 18-23 years), this FokI VDR genotype-related difference in calcium absorption was also found[27]. That is to say, the FokI polymorphism of the VDR receptor seems directly to affect bone mineral accretion in women after weaning or resumption of menstruation through an effect on calcium absorption, which is as same as that in children during pubertal growth.

Previous studies conducted in Gambian women with low calcium intake showed that daily calcium supplementation during pregnancy or early lactation may disrupt metabolic adaptation and thereby not benefit maternal bone health[8,10]. Another study of lactating Japanese women showed that calcium supplementation from dairy products could benefit their bone health[28]. In the present study, a significant difference of BMD recovery between the calcium supplement and control group was found in Chinese women with FF genotype but not in those with Ff and ff genotype.

Three biomarkers for bone turnover were measured in this study. We observed that CTX, a biomarker of bone resorption, together with serum OC, a biomarker of bone formation, decreased in all subjects during ⩽1 year of follow-up, although not significantly. However, another biomarker of bone formation, PICP, increased significantly during ⩽1 year, unlike in another study[29]. Likely, active bone formation occurred in Chinese women during ⩽1 year after weaning.

In conclusion, calcium supplementation benefited bone status in Chinese women with FF VDR genotype, suggesting that FokI VDR gene polymorphisms have significant effects on bone mineral accretion after weaning in Chinese women independent of calcium supplementation. Further studies are required to explore the wisdom of advising women to increase their calcium intake during lactation, such as whether calcium supplementation served in dairy products exerts significant benefit on BMD recovery.

ACKNOWLEDGEMENTS

The authors are grateful to local Centers for Disease Control and Prevention in Wuhan, China, for their co-operation in collection of blood to screen women for this study.

REFERENCES

1. Sasaki S. Dietary Reference Intakes (DRIs) in Japan. Asia Pac J Clin Nutr, 2008; 17(S2), 420-44.
2. Bryant RJ, Cadogan, J, and Weaver CM. The new dietary reference intakes for calcium: implications for Osteoporosis. Journal of the American College of Nutrition, 1999; 18(5), 406s-412.
3. Prentice A. Maternal calcium requirements during pregnancy and lactation. Am J Clin Nutr. 1994; 59 (suppl), 477s-83s.
4. Prentice A. Maternal calcium metabolism and bone mineral status. Am J Clin Nutr, 1994; 71(suppl), 1312s-6s.
5. Lopez J M, Gonazalez G, Reyes V, Campino C et al. Bone turnover and density in healthy women during breastfeeding and after weaning. Osteoporos Int, 1996; 6, 153-9.
6. Karlsson C, Obrant KJ, and Karlsson M. Pregnancy and lactation confer reversible bone loss in humans. Osteoporos Int, 2001; 12, 828-34.
7. Paton LM, Alexander JL, Nowson C, et al. Pregnancy and lactation have no long-term deterious effect on measures of bone miner in healthy women: a twin study. Am J Clin Nutr, 2003; 77, 707-14.
8. Prentice A, Jarjou LM, Cole TJ, et al. Calcium requirements of lactating Gambian mothers: effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. Am J Clin Nutr, 1995; 62(1), 58-67.
9. Prentice A, Jarjou LM, Stirling DM, et al. Biochemical Markers of Calcium and Bone Metabolism during 18 Months of Lactation in Gambian Women Accustomed to a Low Calcium Intake and in Those Consuming a Calcium Supplement. J Clin Endocrinol Metab, 1998; 83(4),1059-66.
10. Jarjou LM, Laskey MA, Sawo Y, et al. Effect of calcium supplementation in pregnancy on maternal bone outcomes in women with a low calcium intake. Am J Clin Nutr, 2010; 92(2), 450-7.
11. Holmberg-Marttila D, Sievänen H, Järvinen TL, et al. Vitamin D and estrogen receptor polymorphisms and bone mineral changes in postpartum women. Calcif Tissue Int, 2000; 66(3), 184-9.
12. Laskey MA, Prentice A, Hanratty LA, et al. Bone changes after 3 mo of lactation: influence of calcium intake, breast-milk output, and vitamin D-receptor genotype. Am J Clin Nutr, 1998; 67(4), 685-92.
13. Bezerra FF, Cabello GM, Mendonça LM, et al. Bone mass and breast milk calcium concentration are associated with vitamin D receptor gene polymorphisms in adolescent mothers. J Nutr, 2008; 138(2), 277-81.
14. Matsushita H, Kurabayashi T, Tomita M et al. Effects of vitamin D and estrogen receptor gene polymorphisms on the changes in lumbar bone mineral density with multiple pregnancies in Japanese women. Human Reproduction, 2004; 19(1), 59-64.
15. Davis JH, Evans BAJ, and Gregory JW. Bone mass acquisition in healthy children. Arch Dis Child, 2005; 90, 373-8.
16. Zhang C, Wang C, Liang J, et al. The vitamin D receptor Fok1 polymorphism and bone mineral density in Chinese children. Clin Chim Acta, 2008; 395(1-2), 111-4.
17. Strandberg S, Nordström P, Lorentzon R, et al. Vitamin D receptor start codon polymorphism (FokI) is related to bone mineral density in healthy adolescent boys. J Bone Miner Metab, 2003; 21(2), 109-13.
18. Yu XD, Shen XM, Xue MB, et al. Vitamin D receptor gene polymorphism and bone mineral density in 0-6-year-old Han children. J Bone Miner Metab, 2010; 11. in press.
19. Diogenes ME, Bezerra FF, Cabello GM, et al. Vitamin D receptor gene FokI polymorphisms influence bone mass in adolescent football (soccer) players. Eur J Appl Physiol, 2010; 108(1), 31-8.
20. Harris SS, Eccleshall TR, Gross C, et al. The vitamin D receptor start codon polymorphism (FokI) and bone mineral density in premenopausal American black and white women. J Bone Miner Res, 1997; 12(7), 1043-8.
21. Cheng WC, Tsai KS. The vitamin D receptor start codon polymorphism (Fok1) and bone mineral density in premenopausal women in Taiwan. Osteoporos Int, 1999; 9(6), 545-9.
22. Dawson-Hughes B, Harris SS, Finneran S. Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. J Clin Endocrinol Metab, 1995; 80(12):3657-61.
23. Wishart JM, Horowitz M, Need AG, et al. Relations between calcium intake, calcitriol, polymorphisms of the vitamin D receptor gene, and calcium absorption in premenopausal women. Am J Clin Nutr, 1997; 65(3), 798-802.
24. Ongphiphadhanakul B, Rajatanavin R, Chanprasertyothin S, et al. Vitamin D receptor gene polymorphism is associated with urinary calcium excretion but not with bone mineral density in postmenopausal women. J Endocrinol Invest, 1997; 20(10), 592-6.
25. Abrams SA, Griffin IJ, Hawthorne KM, et al. Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. J Bone Miner Res, 2005; 20(6), 945-53.
26. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. J Bone Miner Res, 1999; 14, 740-6.
27. Huang ZW, Dong J, Piao JH, et al. Relationship between the absorption of dietary calcium and the Fok I polymorphism of VDR gene in young women. Zhonghua Yu Fang Yi Xue Za Zhi, 2006; 40(2), 75-8.
28. Yoneyama K, Ikeda J. The effects of increased dietary calcium intake on bone mineral density in long-term lactating women, and recovery of bone loss caused by long-term lactation with low calcium diet. Nippon Koshu Eisei Zasshi, 2004; 51(12), 1008-17.
29. Kalwarf HJ, Specker BL, and Ho M. Effects of calcium supplementation on calcium homestasis and bone turnover in lactating women. J Clin Endocrinol Metab, 1999; 84(2), 467-71.