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Influence of vitamin D and estrogen receptor gene polymorphisms on calcium absorption: *BsmI* predicts a greater decrease during energy restriction

Chang B¹, Schlüssel Y¹, Sukumar D², Schneider SH³, and Shapses SA¹

¹Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ

² Drexel University, Philadelphia, PA

³ Department of Medicine, Rutgers-Robert Wood Johnson University Hospital

Abstract

Low calcium absorption is associated with low bone mass and fracture. In this study, we use gold standard methods of fractional calcium absorption (FCA) to determine whether polymorphisms of intestinal receptors, vitamin D receptor (*VDR*) and estrogen receptor α (*ESRI*), influence the response to energy restriction. Fractional calcium absorption was measured using dual stable isotopes (⁴²Ca and ⁴³Ca) in women given adequate calcium and vitamin D and examined at baseline and after 6 weeks of energy restriction or no intervention. After genotyping, the relationship between *VDR* and *ESRI* genotypes/haplotypes and FCA response was assessed using several genetic models. One-hundred and sixty-eight women (53 \pm 11 years of age) were included in this analysis. The *ESRI* polymorphisms, *PvuII* and *XbaI* and *VDR* polymorphisms (*TaqI*, *ApaI*) did not significantly influence FCA. The *BB* genotype of the *VDR* polymorphism, *BsmI*, was associated with a greater decrease in FCA than the *Bb/bb* genotype. Multiple linear regression showed that the *BsmI* polymorphism or the *VDR* haplotype, *BAt*, in addition to changes in weight and vitamin D intake explained ~16% of the variation in changes in FCA. In conclusion, the reduction in calcium absorption due to energy restriction is greatest for those with the *BB* genotype. Previous candidate gene studies show that *VDR* polymorphisms are associated with higher risk for osteoporosis, and the current study supports the notion that the *BsmI* polymorphism in intestinal *VDR* may be contributing to alterations in bone health.

Keywords

calcium absorption; estrogen receptor (*ESRI*); gene polymorphism; vitamin D receptor (*VDR*)

Corresponding Author: Sue A. Shapses, PhD Rutgers University, 96 Lipman Drive, New Brunswick, N.J. 08901-8525 848-932-9403 (office); 848-932-6837 (fax); shapses@aesop.rutgers.edu.

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Introduction

The pathogenesis of osteoporosis is influenced by a wide variety of environmental factors such as age, nutrition, and race. Calcium absorption is an important determinant of bone mineral density (BMD) and osteoporotic fracture risk [1]. Dietary and hormonal factors do not completely explain the variation in calcium absorption. Up to 79% of the variation in calcium absorption is unexplained and may be due to genetic variability [2-7].

Vitamin D is a major regulator of the saturable component of calcium absorption in the small intestine and bone remodeling, and its effects are largely mediated by the vitamin D nuclear receptor (VDR). The *VDR* gene has been postulated to be a major locus for the genetic effects on calcium absorption, but there are limited studies [8, 9]. In a cross-over design study using 2 levels of calcium intake (1500 mg/d for 2 weeks and <300 mg/d for 2 weeks) in sixty postmenopausal women, the *VDR* polymorphism, *BsmI*, was found to be associated with calcium absorption, but only during very low calcium intake [8]. Also, in a small cross-sectional study in premenopausal women consuming the recommended calcium intake, it was found that a *VDR* haplotype (*BsmI*, *ApaI*, *TaqI* polymorphisms) is associated with calcium absorption [9]. The *ESR1* gene is another strong candidate for the genetic regulation of calcium absorption because of the presence of estrogen receptor- α in the intestine and the well-established role of estrogen in intestinal calcium absorption [10, 11]. However, the association between the commonly studied *ESR1* polymorphisms, *XbaI* and *PvuII*, and calcium absorption has not been extensively examined [12]. It has previously been shown that calcium absorption is attenuated with energy restriction in older women [13]. Therefore, the goal in this investigation was to determine whether the known effects of energy restriction to reduce calcium absorption are further influenced by several well-studied *VDR* and *ESR1* polymorphisms.

Materials and Methods

Subjects

One hundred and ninety-nine overweight or obese women who participated in previous calcium absorption trials in the laboratory from 2001-2012 were eligible to be included in this study [13-15]. Of these, one hundred and sixty-eight women (ages 24-75 yrs) had whole blood drawn and were included in this retrospective analysis. These samples are part of the Osteoporosis, Weight Loss and Endocrine database (trials supported by AG12161). All participants were free from any disease state or medications known to influence bone metabolism, and none of them were on hormone replacement therapy. The subjects signed an informed consent approved by the Institutional Review Board at Rutgers University and Saint Peters University Hospital, New Brunswick, NJ, USA.

Study Design

Prior to the calcium absorption measurement, all women were provided with 1-1.2g/day of calcium (diet plus supplement) for a month to stabilize calcium intake (Citracal, Bayer Inc., Morristown, NJ, USA). Similarly, all women were given 400 IU of vitamin D in the form of a multivitamin tablet (NatureMade, Mission Hill, CA, USA). After the 1 month stabilization

period, women were assigned to groups that either lost weight (WL, n = 104) or maintained weight (WM, n=64). Women in the WM group were asked to maintain their weight during the 6 week intervention. In the WL group, subjects were asked to reduce energy intake by 500-600 kcal/day using a standard nutrition education and behavior modification weight-reduction program that was offered weekly under the supervision of a dietitian (8-10/group).

Calcium absorption was measured at baseline and after 6 weeks of energy restriction or no intervention. A subset of 14 WL and 13 WM women were randomly assigned in a double blind manner to a higher intake of vitamin D₃ (2500 IU/d) over 6 weeks, as described previously [14]. Because these women were equally distributed between the WL and WM groups using an identical protocol and showed similar FCA trends, they were also included in this analysis.

Bone and body composition

A total body scan using dual-energy x-ray absorptiometry (Lunar Prodigy Advanced; GE-Lunar, Madison, WI; enCORE software, v. 8.10.027) was performed to characterize the population for baseline body composition of soft tissues and bone (CV < 1%).

Biochemical Analysis

Serum 25-hydroxyvitamin D [25(OH)D], 1,25 dihydroxyvitamin D [1,25(OH)₂D₃], and ultra-sensitive estradiol (E₂) were measured by radioimmunoassay (RIA; DiaSorin, Stillwater, MI, USA; CV <12.5% for vitamin D; DSL, Webster, TX, USA; CV < 8.9% for E₂) using internal standards on all samples and external standards on a subset (vitamin D external quality-assessment scheme). Intact parathyroid hormone (PTH) was determined by immunoradioassay (Scantibodies, Santee, CA, USA, CV <6.8%; DSL, Webster, TX, USA; CV, < 5.2%).

True fractional calcium absorption (FCA)

The stable isotopes, ⁴²Ca (96% enrichment) and ⁴³Ca (90% enrichment), were prepared for human use by the Pharmacy Department at St. Peters University Hospital, New Brunswick, NJ and purchased from Trace Sciences International (Delaware, USA). After mixtures were prepared, samples were tested for sterility and pyrogenicity. The dose was individualized (0.012mg/kg Ca⁴² and 0.017mg/kg Ca⁴³) and prepared in syringes prior to the test. Briefly, the Ca absorption test required women to be admitted to the clinic between 7 and 9 AM after an overnight fast. After a fasting blood and urine sample were taken, a standard breakfast consisting of milk, toast, fruit and orange juice was served. The total Ca load of this meal was 200 mg and included ⁴³Ca mixed with 120 ml skim milk (153 mg Ca). The milk was consumed under supervision, and the cup was rinsed with deionized water 3 times and all of this water was also consumed by volunteers. Immediately after breakfast, ⁴²Ca was injected intravenously. A precision balance scale was used to weigh the syringes used containing the isotopes before and after the test. Instructions were given how to collect a pooled 24-hour urine sample. Volunteers were monitored during the day and remained at the University Inn overnight, and the 24-hour urine collection was collected by the researchers the following morning. Calcium was precipitated in urine samples as the oxalate salt and ashed to assess mineral content. Ratios of each isotope to ⁴⁴Ca in 24 hour urine samples were determined by

high-resolution, inductively coupled plasma mass spectrometry (Element-1; Finnigan MAT) (1% precision and accuracy). The day-to-day CV was 1.2%, as measured twice in 6 women [13]. Samples were measured before and six weeks after the dietary intervention.

Genotype and Haplotype Analysis

Genotypes for the *VDR* gene polymorphisms (*BsmI*, *TaqI*, *ApaI*) and *ESR1* gene polymorphisms (*XbaI* and *PvuII*) were determined through polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis. Using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA), genomic DNA was extracted from whole blood samples that had been collected at baseline from each study subject. PCR amplification using a MJ Mini thermal cycler (Bio-Rad, Hercules, CA, USA) for each of the polymorphisms was then carried out as described previously [16, 17]. Forward and reverse primers for PCR for each individual *VDR* and *ESR1* polymorphism were purchased from Sigma-Aldrich, St. Louis, MO, USA. The PCR products were then digested by their respective endonucleases (New England Biolabs, Ipswich, MA, USA) and run on a 1.75% agarose gel stained with ethidium bromide for visualization. Alleles with and without restriction sites for the polymorphism are denoted by lower and upper case letters, respectively (e.g. *BsmI*, “*b*” = presence of restriction site and “*B*” = absence of restriction site). Subsequent haplotype reconstruction was performed using PHASEv2.1.1 [18, 19].

Statistical Analysis

The genotype frequencies for each polymorphism were tested against Hardy-Weinberg ratios and larger study populations by the χ^2 test. Linkage disequilibrium (LD), which is the non-random association of alleles at different positions on a gene, of the 3'UTR *VDR* polymorphisms, *BsmI*, *ApaI*, and *TaqI* and the *ESR1* polymorphisms located in intron 1, *XbaI* and *PvuII*, was determined using Haploview v4.2 (Broad Institute, Cambridge, MA, USA) [20].

Additive, dominant, and recessive models were defined and tested for each of the *VDR* and *ESR1* polymorphisms. The additive model treats all three genotypes (e.g. YY, Yy, yy) differently, whereas the dominant model assumes that only one copy of the recessive allele is sufficient to result in a phenotype, and the recessive model assumes that two copies of the recessive allele are required to result in a specific phenotype. One-way analysis of covariance (ANCOVA), including age, BMI, and estradiol as covariates with Tukey's post-hoc analyses was used to determine how the individual *VDR* and *ESR1* polymorphisms and haplotypes predict variations in FCA at baseline. Two-way ANOVA, using diet and the additive, dominant, or recessive genetic models as the two independent factors was conducted to determine the genetic influence on changes in FCA. Estradiol and age were included as covariates in subsequent analyses. Tukey's post-hoc analysis was performed when the *F* statistic for the model was below the specified α value of 0.05. Kruskal-Wallis non-parametric ANOVA was also used due to the different group sizes.

Multiple linear regression models were used to quantify the amount of variability in the FCA response that is attributable to the gene polymorphisms. The additive, dominant, and recessive genetic models, as well as the percent change in fat intake, vitamin D intake, body

weight, serum PTH, 25OHD, 1,25(OH)₂D, and estradiol were used as independent predictors of change in FCA. The magnitude of the FCA difference between genotype groups was estimated by effect size using the standardized difference between two means.

All variables were tested for normality by comparing the mean and median and creating normal probability plots of the individual variables. If the variables were not normally distributed we reported the median and interquartile range, and conducted log transformations. Statistical analyses were carried out using SAS v9.2 software (SAS Institute, Cary, North Carolina). A P-value < 0.05 was considered to be statistically significant.

Results

Baseline characteristics of body composition, FCA, serum concentrations of hormones, and nutrient intake are included in Table 1 for 168 women. The population is largely Caucasian (86%). Characteristics in the WL and WM groups did not differ significantly at baseline (Table 1).

Genotype Frequencies

The genotype frequencies for the *VDR* and *ESR1* polymorphisms in the entire population were found to be in Hardy-Weinberg equilibrium. The χ^2 analysis revealed that frequencies of the *ApaI*, *FokI*, and *PvuII* genotypes are comparable to a larger population (Table 2) [21]. The *BsmI*, *ApaI*, and *TaqI* polymorphisms on the *VDR* gene were in strong linkage disequilibrium (LD) with one another ($D' > 0.88$), where $D'=1.0$ represents perfect LD. The *XbaI* and *PvuII* polymorphisms on the *ESR1* gene were also in strong LD with one another ($D' > 0.79$). This strong LD among the polymorphisms justified the determination of the *VDR* and *ESR1* haplotypes. The frequencies for the most common haplotypes in our population are presented in Table 3. Chi square (χ^2) analysis showed that the frequencies of the *ESR1* haplotypes did not differ significantly from a larger reference population [22,23].

Weight, hormone, and calcium absorption changes due to six week intervention

Women in the WL group lost $4.0 \pm 1.5\%$ of initial body weight, which was significantly more than women who maintained weight ($0.02 \pm 1.5\%$) ($p < 0.0001$). Serum concentrations of 25(OH)D increased significantly more in the WL (3.4 ± 6.2 ng/mL) than WM group (0.8 ± 4.6 ng/mL) ($p < 0.01$). However, the greater decrease in FCA for the WL group ($-9.0 \pm 21.6\%$) than the WM group ($-4.0 \pm 19.1\%$) did not reach significance ($p = 0.13$). In addition, there were no significant differences for changes in serum 1,25(OH)₂D (WL: 0.6 ± 15 ; WM: 0.9 ± 16.6 pg/mL), PTH (WL: -3.5 ± 12.1 ; WM: -1.7 ± 13.8 pg/mL), and estradiol (WL: 1.7 ± 33.1 ; WM: 0.9 ± 22.8 pg/mL) due to energy restriction over the 6 week study. There were also no significant differences in weight changes with the *BB* genotype compared to *Bb* or *bb* genotypes.

VDR polymorphisms and calcium absorption

Baseline FCA was not significantly influenced by *VDR* polymorphisms ($p > 0.10$), *BsmI* (*BB*: $25.5 \pm 1.6\%$, *Bb*: $24.4 \pm 0.8\%$, *bb*: $25.3 \pm 1.0\%$), *ApaI* (*AA*: $23.7 \pm 1.1\%$, *Aa*: $25.6 \pm$

0.8%, *aa*: $25.0 \pm 1.4\%$), *TaqI* (*TT*: $24.8 \pm 1.0\%$, *Tr*: $24.7 \pm 0.8\%$, *tt*: $26.2 \pm 1.7\%$), and *FokI* (*FF*: $24.5 \pm 0.9\%$, *Ff*: $25.6 \pm 0.9\%$, *ff*: $24.2 \pm 1.7\%$). Using the additive model in the 2-way ANOVA, the *BB* genotype resulted in an attenuated FCA response compared to *Bb* or *bb* ($p < 0.01$). Tukey's post-hoc analysis indicated that for women who lost weight, those with the *BB* genotype experienced a greater decrease in FCA ($-24.1 \pm 5.4\%$) compared to women with either the *bb* ($-7.3 \pm 3.3\%$) or the *Bb* genotypes ($-7.6 \pm 3.1\%$) ($p < 0.05$). Meanwhile, among women who maintained weight, those with the *BB* genotype ($-12.8 \pm 3.6\%$) experienced a greater decrease in FCA compared to women with the *Bb* genotype ($0.1 \pm 3.3\%$) ($p < 0.05$), but not those with the *bb* genotype ($-3.6 \pm 5.2\%$) (**Figure 1**). Because there were no significant differences between the *Bb* and *bb* genotypes and the additive model is not always adequate when the minor allele frequency is not close to 50%, we also tested recessive and dominant models for the individual polymorphisms [24]. Using the recessive model for *BsmI*, we also found that women with the *BB* genotype experienced a greater percentage decrease in FCA, compared to women in the combined group of *bb/Bb* genotypes (WL: $p < 0.05$; WM: $p < 0.06$). There was a moderate to large effect size of the *BsmI* genotypes of 0.67 and 0.86 in the WM and WL groups, respectively.

The additive model for the *BAt* haplotype showed that in the WL group, women with 2 copies ($-23.7 \pm 6.0\%$) experienced a significantly greater decrease in FCA ($p < 0.05$) compared to women with 0 copies ($-7.4 \pm 2.9\%$), but not those with 1 copy ($-8.1 \pm 3.4\%$). In the weight maintenance group there was a trend ($p < 0.10$) for women with 2 copies of the *BAt* allele ($-11.9 \pm 4.5\%$) to experience a greater decrease than women with 1 copy of the allele ($0.5 \pm 3.3\%$), but not those with 0 copies (-5.9 ± 4.5) (**Figure 1**). Using the recessive model for the *BAt* haplotype showed that having 2 copies compared to 0 or 1 copy resulted in a 16% greater decrease in FCA in the WL group ($p < 0.05$); whereas having 2 copies of the *BAt* allele resulted in a 10% greater decrease in FCA in the WM group ($p > 0.10$). Similar, to the *BB* genotype, the *BAt* haplotype had a large effect size of 0.81 in the WL group. When we included age and baseline estradiol (log-transformed) in the two-way ANOVA analysis, the *BsmI* polymorphism remained a significant predictor of change in FCA.

There were more women who have 0/1 copies of the *BAt* allele than women who have 2 copies, and more women with the *Bb* and *bb* genotypes than the *BB* genotype, as expected and described in Tables 2-3. Therefore, we also used non-parametric analysis, which revealed that the relationship between the *BsmI* and *BAt* recessive models and change in FCA remained significant ($p < 0.05$).

Multiple linear regression analysis including percent change in FCA as the dependent variable, revealed that the recessive *BsmI* model and percent change in weight and vitamin D intake (dietary + supplement) were significant predictors of change in FCA over a 6 week period ($p < 0.05$) and explained about 16% of the variation in FCA response (**Table 4**). Having the *BB* genotype led to a 0.23 standard deviation decrease in FCA. The regression model which replaced the *BsmI* model with the *BAt* haplotype predicted about 15% of the variation. In this model having two copies of the *BAt* allele resulted in a 0.21 standard deviation decrease in FCA (**Table 4**). When the changes in hormones were included in the multiple linear regression analysis, they were not found to be significant predictors of

change in FCA. Because estradiol is not normally distributed in our population, we also repeated these statistical tests using log-transformed change in estradiol, which revealed the same findings. Also, understanding that both vitamin D intake and change in 25(OH)D are strongly correlated, we included change in 25(OH)D and vitamin D intake in separate models, and vitamin D intake was the only significant predictor.

ESR1 polymorphisms and calcium absorption

The *ESR1* polymorphisms, *PvuII* and *XbaI*, did not have any effect on baseline FCA or the response to energy restriction. In addition, these genotypes and haplotypes did not explain any of the variation in calcium absorption response.

Discussion

Calcium absorption is an important predictor of fracture risk, yet the large majority of calcium absorption variability cannot be explained by diet and hormones alone [1-6]. The association between *VDR* and *ESR1* gene polymorphisms and fracture risk has been examined extensively [25-29]. In the current study, we examined common polymorphisms of the *VDR* and *ESR1* genes, to determine their influence on fractional calcium absorption. We found that the *VDR BsmI BB* genotype and the *BA_t* haplotype predicted a greater decrease in calcium absorption during short term energy restriction.

One mechanism believed to regulate the decline in bone mass during weight reduction is a reduction in calcium absorption during energy restriction [13]. It is believed that decreases in estradiol due to loss of fat mass, as well as increases in cortisol due to the presence of a low-energy state, may attribute to these observed decreases [13,14]. Lower fat intake during energy restriction may also contribute to the reduced calcium absorption [2,4]. It may also be related to the extent of weight loss, but this has not been confirmed in a clinical trial [30].

The current findings indicate that during energy restriction, genetic differences (e.g., *BB* genotype and *BA_t* haplotype) exacerbate the expected decrease in calcium absorption. Previous studies have also found that there is less efficient absorption associated with the *BB* genotype [8,9]. An examination of late postmenopausal women revealed that the *BB* genotype compared to the *bb* genotype is associated with reduced calcium absorption in women with low (300 mg/d) calcium intake, but not when intake was higher [8]. Also, in a cross-sectional study, premenopausal women with the *BA_t* haplotype had significantly lower levels of calcium absorption than women with the *ba_T* haplotype [9]. In addition, in a separate pilot analysis of twenty-seven women who were randomly assigned in a double blind manner to 2500 IU/d or 400 IU/d supplemental vitamin D₃ intake, the FCA response to high vitamin D intake was significantly attenuated in women with the *BB* compared to *bb* genotype [31]. These data indicate that *VDR* polymorphisms exhibit a modest effect on calcium absorption even under stable conditions, suggesting that weight loss is not the only contributing factor to calcium absorption. Nevertheless, the *BB* phenotype is more strongly expressed under more stressful physiological conditions, such as energy and calcium intake restriction, known to alter calcium demands [8, 9, 16].

With greater decreases in calcium absorption due to the *BB* genotype and the *BAt* haplotype, both would be expected to negatively affect bone. In fact, previous meta-analyses have suggested that the *BB* genotype is associated with lower BMD and greater fracture risk than *bb* [25, 26]. In a meta-analysis of 39 studies it was shown that postmenopausal women with the *BB* genotype had significantly lower BMD at the spine than women with the *bb* genotype [25]. Similarly, in a more recent meta-analysis of 17 studies, there was a higher frequency of the *BB* genotype in fracture cases that included hip, spine and other sites [26]. GWAS studies have not been able to replicate this association, but these findings may differ due to the use of heterogeneous populations [32-35]. Our results, in addition to the findings in meta-analyses, suggest that the negative influence of *VDR* polymorphisms on bone health [25, 26] acts, at least in part, through a classical nutrition mechanism (intestinal absorption) and extends the speculation of others [36]. Understanding that lower calcium absorption might contribute to bone loss associated with weight reduction, it is important to consider that women with the *BB* genotype may be at an increased risk for detrimental bone outcomes.

Despite these findings, the mechanism through which the *BsmI* site and the *BAt* haplotype predict changes in calcium absorption is unknown. The reduction of calcium absorption in the WL women even though there is an increase in serum 25(OH)D suggests there may be a weight loss-induced vitamin D resistance that is exacerbated in the presence of the *BB* genotype. The *BsmI* polymorphism displays cell-type specific effects on mRNA stability [37], and studies report that the *BsmI* polymorphism, in conjunction with other 3' UTR polymorphisms alters the transcription and stability of *VDR* mRNA [37,38]. Additionally, it has been found that a genetic polymorphism in the *VDR* promoter leads to lower *VDR* expression in the duodenum [39]. Therefore, it may be that the *BsmI* polymorphism modulates FCA in a similar manner by affecting the transcription and stability of intestinal *VDR* mRNA. It is also possible that the *B* allele and the *BAt* haplotype are in linkage disequilibrium with alleles of a functional polymorphic site that alters the transcript levels of the *VDR* gene or the stability of the resultant protein, thus affecting the *VDR* dependent intestinal calcium absorption [37]. A study examining the impact of the *BsmI* polymorphism and *BsmI-ApaI-TaqI* haplotypes on stability efficiency of *VDR* mRNA in intestinal cells may help elucidate the mechanism through which these polymorphisms influence FCA.

Our candidate gene study has strengths and limitations. Compared to other studies that have examined the relationship between candidate genes and bone health outcomes, our sample size is small [25,26]. Yet, in comparison to other calcium absorption studies that have examined the association with *VDR* and *ESR1* gene polymorphisms [8,9], our sample size is larger providing more definitive evidence that the *BB* genotype is a risk factor for low absorption. Because of our small sample size, we were also able to reduce the heterogeneity of the population by controlling for Ca and vitamin D intake, weight, race, and sunlight exposure. However, using a primarily Caucasian and overweight/obese population may have skewed the frequencies of the *BsmI* polymorphism and the *VDR* haplotypes compared to the greater racial diversity in other studies [21], reducing the generalizability of the findings. For instance, there is a greater frequency of the *B* allele in the Caucasian population compared to both Asian and African populations [37]. Furthermore, in a recent study including 402 obese

and 489 non-obese women, the *B* allele was found to be significantly more frequent in obese individuals [40]. Moreover, we purposefully included individuals of all ages to maximize the sample size for the genetic polymorphisms, with the assumption that the genotype would exacerbate the effect of diet on FCA, if there was indeed a gene-nutrient interaction.

Another advantage of small studies is that they allow for the use of more valid outcome measurements (e.g., FCA using dual isotopes) on a limited sample, which would be cost-prohibitive or too invasive to impose on a larger sample. Thus, a strength of this study is that we used the dual stable-isotope method that is considered a gold standard to calculate calcium absorption that has not been used previously to examine the influence of genotypes [8,9,12].

This study is also unique because we analyzed the associations between the polymorphisms and the outcome variables using the additive, dominant, and recessive models. Many genetic studies only employ the additive model, which considers the genotypes, *YY*, *Yy*, and *yy*, to be different; however, this model is not always sufficient [24]. Furthermore, because our data remained significant after including hormones such as estradiol in two-way ANOVA and multiple linear regression analyses, we are more confident that the gene effect on calcium absorption was due to the polymorphisms and not due to other confounding variables. Nevertheless, the absence of these findings may be a limitation of sample size.

Recognizing that there are a multitude of polymorphisms on both the *ESR1* and *VDR* candidate genes, we posit that genotyping subjects for additional polymorphisms within the *VDR* and *ESR1* candidate genes will provide a more complete picture of the genetic basis for the variation in calcium absorption. Additionally, studying the relationship between other candidate genes such as *GC*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *CYP27A1*, and *DHCR7*, all of which are important in vitamin D metabolism [41-43], and calcium absorption, may help explain the variation in absorption in response to diet or disease conditions. Overall, this work underscores the importance of both the genomic and/or metabolic profile, and the need to focus attention on treatment for those who need it most.

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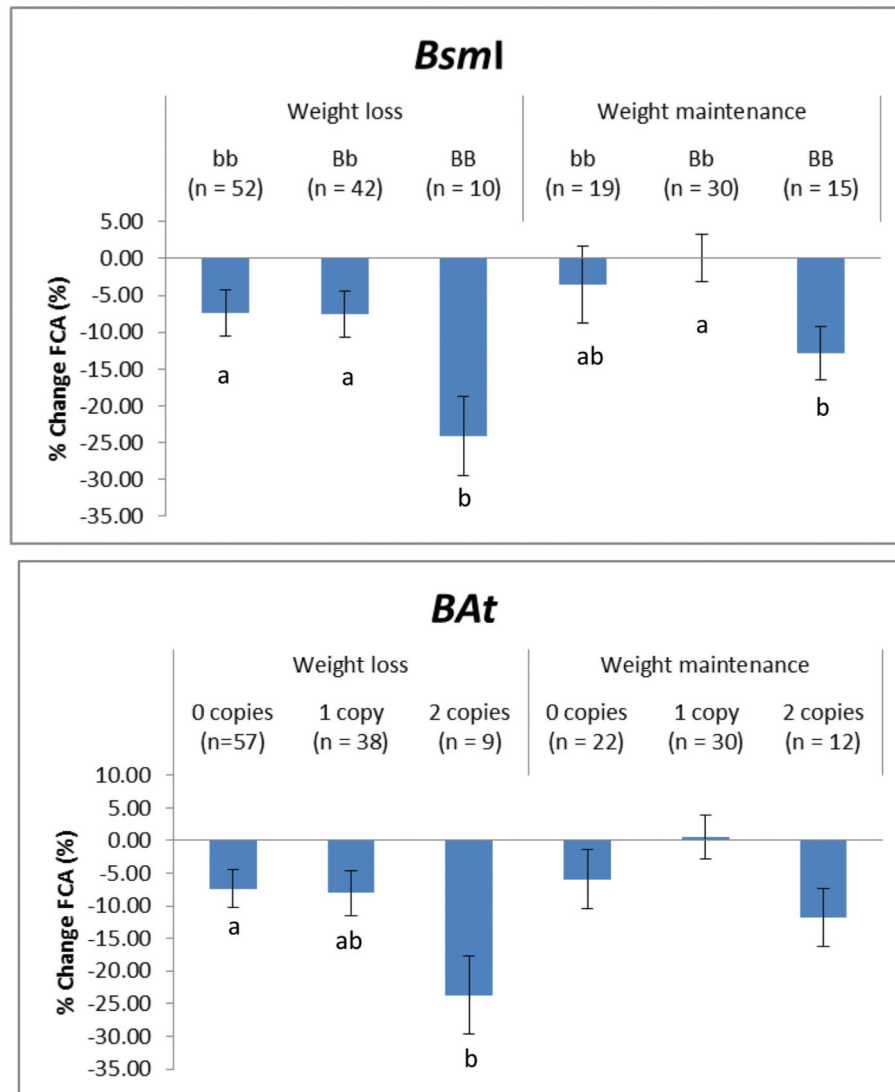
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Highlights

Low calcium absorption is associated with low bone mass and fracture risk, and caloric restriction reduces calcium absorption.

The influence of vitamin D receptor and estrogen receptor- α gene polymorphisms on fractional calcium absorption was examined during caloric restriction.

The *BB* genotype of the vitamin D receptor polymorphism, *BsmI*, was associated with the greatest reduction in calcium absorption.



¹ Data (Mean \pm SEM)

² True fractional calcium absorption (FCA) in 168 women; two-way ANOVA F statistic was statistically significant for the model, *BsmI*, and *BA1* haplotype, $p < 0.05$.

Tukeys post-hoc analysis showed that the *BB* women losing weight (WL) experienced a greater decrease in FCA compared to either *Bb/bb* women; *BB* women who maintained weight (WM) experienced a greater decrease in FCA compared to *Bb* women; women with 2 copies of the *BA1* allele experienced a greater decrease compared to women with 0 copies of the allele.

Figure 1.

BsmI and *BA1* recessive models predict greater decrease in FCA during weight loss^{1,2}

Table 1

Baseline characteristics of women¹

Variable	All (n=168)	Weight loss (n=104)	Weight maintenance (n=64)	P value for WL/WM groups ²
Age	53.0 ± 11.4	53.1 ± 11.5	52.7 ± 11.3	0.799
Weight (kg)	75.8 ± 10.2	76.2 ± 10.8	75.0 ± 9.1	0.422
BMI (kg/m ²)	28.6 ± 3.2	28.5 ± 3.3	28.7 ± 3.0	0.657
Lean mass (kg) ³	40.1 ± 4.8	40.2 ± 4.9	40.0 ± 4.7	0.848
Fat mass (kg) ³	31.6 ± 6.9	31.9 ± 7.2	31.2 ± 6.3	0.493
BMC (g) ⁴	2442 ± 336	2452 ± 330	2424 ± 348	0.601
FCA (%)	24.9 ± 7.6	24.6 ± 7.0	25.5 ± 8.5	0.471
Hormones				
Estradiol (pg/ml) ⁶	22.7 ± 21.5	23.3 ± 22.2	21.9 ± 20.5	0.690
PTH (pg/ml)	40.2 ± 21.7	40.9 ± 22.6	39.0 ± 20.4	0.398
25(OH)D (ng/ml)	29.6 ± 8.6	28.7 ± 8.1	31.0 ± 9.4	0.093
1,25(OH) ₂ D (pg/ml)	50.6 ± 16.6	51.3 ± 16.6	49.4 ± 16.8	0.488
Nutrient Intake⁷				
Calcium (mg/day)	1193 ± 401	1181 ± 417	1212 ± 375	0.628
Vitamin D (µg/day) ⁵	11.9 ± 2.2	11.9 ± 2.5	11.8 ± 1.9	0.776

BMI body mass index, *FCA* true fractional calcium absorption, *BMC* total body bone mineral content, *1,25(OH)₂D* 1,25-dihydroxyvitamin D₃, *25OHD* 25-hydroxyvitamin D (multiply by 2.5 to convert to nmol/L), *PTH* parathyroid hormone

¹Data (mean ± SD)

²Analysis was performed using a one-way ANOVA.

³Missing subjects, ^c(n=1), ^d(n=2); ^e(n=4)

⁴Missing subjects, ^c(n=1), ^d(n=2); ^e(n=4)

⁵Missing subjects, ^c(n=1), ^d(n=2); ^e(n=4)

⁶Estradiol was non-normally distributed, therefore we calculated the median and IQR for each group: All, 16.1 (3.6-133.5); WL, 17.5 (4.9-133.5); WM, 15.2 (3.6-125.0). The log transformation of estradiol was normally distributed.

⁷Nutrient intake includes the amount from diet plus multivitamin/mineral with 10 µg (400 IU) of vitamin D and 200 mg Ca plus individualized Ca supplement.

Table 2

VDR and *ESR1* genotype frequencies in the current study compared to a larger population [21]

Gene	Polymorphism ^I	Current study n=168	1000 Genomes Project n=1092
<i>VDR</i>	<i>BsmI</i> [*]		
	<i>BB</i>	14.9%	7.0%
	<i>Bb</i>	42.9%	39.0%
	<i>bb</i>	42.3%	54.0%
	<i>ApaI</i>		
	<i>AA</i>	29.2%	25.4%
	<i>Aa</i>	51.2%	50.0%
	<i>aa</i>	19.6%	24.6%
	<i>TaqI</i> [*]		
	<i>TT</i>	41.1%	54.2%
	<i>Tt</i>	46.4%	38.9%
	<i>tt</i>	12.5%	7.0%
	<i>FokI</i> [*]		
	<i>FF</i>	44.1%	42.6%
	<i>Ff</i>	42.9%	45.3%
<i>Ff</i>	13.1%	12.0%	
<i>ESR1</i>	<i>PvuII</i>		
	<i>PP</i>	23.8%	19.0%
	<i>Pp</i>	44.1%	49.2%
	<i>Pp</i>	32.1%	31.8%
	<i>XbaI</i> [*]		
	<i>XX</i>	13.1%	6.6%
	<i>Xx</i>	43.5%	38.1%
	<i>xx</i>	43.5%	55.4%

VDR vitamin D receptor gene, *ESR1* estrogen receptor- α

^IReference SNP (rs) numbers for “common” genotypes: *BsmI* – rs1544410; *ApaI* – rs7975232; *TaqI* – rs731236; *FokI* – rs2228570; *PvuII* – rs2234693; *XbaI* – rs9340799

* Differed significantly compared to reference population [21]

Table 3Haplotypes frequencies in current study compared to larger populations¹ [22,23]

<i>VDR</i>	Current study	Uitterlinden et al.	<i>ESR1</i>	Current study	Bagger et al.
<i>BAt</i> *	32.7%	37.9%	<i>PX</i>	29.9%	33.6%
<i>bAT</i> *	17.8%	11.6%	<i>Px</i>	15.3%	13.3%
<i>baT</i> *	42.5%	43.4%	<i>px</i>	50.0%	53.1%

VDR vitamin D receptor gene, *ESR1* estrogen receptor- α ¹ Current study n=168; Uitterlinden et al. n =1782 [22]; Bagger et al. n=454 [23]

* Differed significantly from reference population [22]

Table 4

VDR polymorphisms predict variation in FCA change^{1,2}

Variable	β coefficient	R ²	P value
<i>BsmI</i>	-0.229	4.3%	0.006
Weight	0.155	2.4%	0.041
Vitamin D intake	0.302	9.5%	<0.001
Model R ² = 16.2%			
<i>BAI</i>	-0.207	3.3%	0.004
Weight	0.158	2.4%	0.012
Vitamin D intake	0.294	9.5%	<0.001
Model R ² = 15.2%			

¹ *BsmI* genotype or *BAI* haplotype recessive models include independent predictors (Fat intake, vitamin D intake, body weight, serum PTH, 25OHD, 1,25D, and estradiol). Only significant predictors are included in the table. Abbreviations: VDR vitamin D receptor gene, FCA true fractional calcium absorption

² A separate analysis was conducted for the WL and WM groups. In both the WL and WM group the *BsmI* recessive model remained a significant predictor of calcium absorption change ($\beta = -0.229, -0.255$ respectively, $p < 0.05$). The *BAI* recessive model was only a significant predictor of calcium absorption change in the WL group ($\beta = -0.210, p < 0.05$).