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# Calcium Absorption on High and Low Calcium Intakes in Relation to Vitamin D Receptor Genotype*

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**ABSTRACT**

The finding that the link between polymorphism at the vitamin D receptor (VDR) gene and rates of bone loss from the femoral neck in postmenopausal women is enhanced at low calcium intakes suggests that intestinal calcium absorption is a site of differential action of the VDR alleles. 1,25-Dihydroxyvitamin D [1,25-$(OH)_2D$] and its receptor mediate active calcium transport, the major mechanism of calcium absorption at low calcium intakes. We compared fractional calcium absorption in healthy late postmenopausal women with (bb) and without (BB) the BSM-1 restriction site. In 60 women (26 BB and 34 bb), we measured calcium absorption and plasma 1,25-$(OH)_2D$ after 2 weeks on a high (1500 mg/day) and 2 weeks on a low (<300 mg/day) calcium intake. The mean $^{45}$Ca absorption indexes were similar in the two groups on the high calcium intake [19.01 ± 1.12% (±SEM)/L in BB and 20.45 ± 0.97%/L in bb; $P = 0.346$] and differed significantly on the low calcium intake (20.57 ± 1.10%/L *vs.* 23.66 ± 0.95%/L; $P = 0.044$). Calcium restriction induced similar percent increases in plasma 1,25-$(OH)_2D$, but the BB group had a smaller increase in the fractional $^{45}$Ca absorption index [7.8 ± 3.8% (±SEM) *vs.* 20.7 ± 3.3% in bb; $P = 0.016$; increments adjusted for initial absorption value].

In conclusion, compared to women with the bb variants, women with BB allelic variants of the VDR have reduced calcium absorption efficiency on low calcium intake, consistent with a functional defect in the intestinal VDR. The impact of this heritable difference is reduced at higher calcium intakes. (*J Clin Endocrinol Metab* **80:** 3657–3661, 1995)

BONE MASS is influenced by heredity (1–3) and calcium absorption efficiency (4–7), but to date, heredity and calcium absorption have not been firmly linked. Morrison *et al.* (8) reported that an allele defined by the BSM-1 polymorphism in the gene encoding the 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$] receptor (VDR) is associated with bone mineral density (BMD). Adults with the variant designated bb had higher spine and hip BMD than those with the BB variant. Several investigators (9–11) have confirmed this association, but others have not (12–15). Longitudinal studies have found greater rates of loss in BB than in bb subjects at the spine (16, 17), the hip (17), and the radius (17).

The mechanism(s) by which the VDR variants influence calcium homeostasis have not been determined, but they are likely to be related to established actions of 1,25-$(OH)_2D$. We recently found that calcium intake influences the VDR genotype/bone loss association (17). Postmenopausal women with the BB and bb alleles lost BMD from the femoral neck at similar rates when taking calcium supplements, but among women with low calcium intakes, rates of loss from the femoral neck were greater in the BB than in the bb group (17). These findings point to intestinal calcium absorption as a possible site of differential action of VDR variants.

Fractional calcium absorption increases as calcium intake declines (18–20). This partial adaptation to calcium restriction results from a 1,25-$(OH)_2D$-mediated increase in active calcium transport. Active transport is the major mechanism for calcium absorption at low calcium intakes; as calcium intake increases above about 500 mg/day, passive diffusion accounts for an increasing proportion of the calcium absorbed (21). Failure of the BB variants to conserve calcium at low calcium intakes (suggested by their failure to preserve bone mass) (17) may result from an altered gut VDR status and an associated inability to make the appropriate increase in fractional calcium absorption.

To test this, we recruited women with BB and bb allelic variants and measured their calcium absorption and calciotropic hormone levels on high and low calcium intakes. If the hypothesis that VDR status influences active intestinal calcium transport is correct, one would expect calcium absorption in postmenopausal women with the BB and bb alleles to be more similar on a high than on a low calcium intake, and after dietary calcium restriction, women with the BB alleles will have a smaller increment in absorption than women with the bb alleles.

## Subjects and Methods

### *Subjects*

Healthy postmenopausal women were enrolled and completed the 4-week study. Of 51 women previously determined to have the BB genotype, 26 were eligible and willing to participate. Of 106 women with the bb genotype, 34 were randomly selected to bring the total sample size to 60. Many of these women had previously participated in calcium and vitamin D supplement trials (22, 23) and in a retrospective study of VDR genotype and rates of bone loss (17).

Exclusion criteria for the study were hepatic or renal disease, mal-

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absorption, hyperparathyroidism, malignancy in the past 5 yr, and use in the past 3 months of estrogen, glucocorticoids, or other drugs known to influence calcium metabolism. The women agreed to take no calcium or vitamin D supplements except those provided during the study. The protocol was approved by the radiation safety and human investigation review committees at Tufts University, and written informed consent was obtained from each subject.

### *Study design*

Volunteers were counseled to avoid all dairy products and calcium-fortified foods throughout the 4-week study. Counselling took place before and at the midpoint of the study. For the first 2 weeks of the study, the women took 1200 mg/day calcium as the citrate (400 mg at breakfast, dinner, and bedtime). To reduce variation in vitamin D intake, each woman took a multivitamin containing 400 IU vitamin D daily throughout the study. The volunteers, staff who had contact with them, and staff who made calcium absorption and other measurements were blinded to genotype group throughout the study.

Measurements were made after 2 weeks (the high calcium intake period) and after 4 weeks (the low calcium intake period). On both occasions, subjects came to the Metabolic Research Unit at 0800 h after an 8-h fast. Each brought a 24-h urine collection. A blood sample was drawn by venipuncture at 0815 h. At 0830 h, subjects ingested the $^{45}$Ca tracer (for method, see below). Blood was analyzed for calcium (total), creatinine, phosphorus, PTH, 25-hydroxyvitamin D, and 1,25-$(OH)_2$D, and urine was analyzed for calcium and creatinine.

### *Intake assessment*

Self-selected intake of calcium and other nutrients was estimated at enrollment with use of the Fred Hutchinson Cancer Research Center Food Frequency Questionnaire (version 06.10.88, 1988, Fred Hutchinson Cancer Research Center, Seattle, WA). On the final visit, the questionnaire was readministered to evaluate calcium intake during the study.

### *Laboratory assays*

Plasma 25-hydroxyvitamin D was measured by the method of Preece *et al.* (24), with intra- and interassay coefficients of variation of 5.0% and 7.3%, respectively. Plasma 1,25-$(OH)_2$D was measured by the competitive protein binding method of Reinhardt *et al.* (25), with intra- and interassay coefficients of variation of 4.9% and 7.7%, respectively. Serum intact PTH was measured with Allegro immunoradiometric assay kits from Nichols Institute (San Juan Capistrano, CA), with intra- and interassay coefficients of variation of 5.6% and 6.6%, respectively. Serum calcium was measured with a Nova 7 analyzer (Nova Biomedical, Waltham, MA). Serum phosphorus and serum and urinary creatinine levels were assayed by colorimetry with a Cobas Fara centrifugal analyzer (Roche Instruments, Belleville, NJ). Urinary calcium was measured by direct current plasma emission spectroscopy with a Spectrospan 6 (Beckman Instruments, Palo Alto, CA). All samples for individual subjects were measured in a single assay.

### *Densitometry*

Lumbar spine (L2–L4) and femoral neck BMD were measured with a model DPX dual energy x-ray absorptiometer (Lunar Radiation Corp., Madison, WI), with coefficients of variation in our laboratory of 1% and 2%, respectively.

### *Calcium absorption*

We estimated fractional $^{45}$Ca absorption from the appearance of $^{45}$Ca in blood after the ingestion of 100 mL of an aqueous solution containing 3 $\mu$Ci $^{45}$Ca and 100 mg cold calcium as the chloride (26). This was followed by ingestion of 3 50-mL deionized water rinses. Before ingestion, duplicate 60-$\mu$L standards were removed from each test dose for counting. Exactly 3 h after ingestion of the tracer, 15 mL blood were drawn. Two 2-mL aliquots of serum and the standards were each added to 18 mL scintillation fluid, and $\beta$-emissions were counted in a scintillation counter (model LS3801, Beckman Instruments, Fullerton, CA). Counts were corrected for quenching. In 25 women, blood was drawn immediately before the second tracing and counted as described above to evaluate residual counts from the first tracer dose. Residual background counts represented a mean of 8.9% of the absorbed counts on the second measurement. Regression analysis revealed a positive linear association between background counts and the count rate in blood on the first measurement. All of the second absorption measurements, therefore, were corrected for residual background counts with use of the linear regression equation. Based on evidence compiled by Manery (27) and used by others (28), the fraction of $^{45}$Ca counts per L serum was corrected for appropriate pool size (fraction of dose in extracellular fluid) by multiplying by 15% of the body weight (in kilograms). The coefficient of variation of the method in our laboratory in 12 subjects measured twice each, 5 days apart, is 9.1%. Thus, the outcome variable in this study, the fractional $^{45}$Ca absorption index, is the fraction of the $^{45}$Ca counts in 1 L serum at 3 h, expressed as a percentage of the $^{45}$Ca counts ingested and multiplied by 15% of the body weight.

$^{45}$Ca was purchased from Amersham Corp. (Chicago, IL), and a spectral analysis was carried out on each batch before its use to ensure purity. Total radiation exposure from the study was 150 mrem to bone, the critical organ, and 15 mrem to the whole body.

### *VDR genotyping*

DNA was extracted from peripheral leukocytes and amplified using the polymerase chain reaction method (17). The enzyme BSM-1 endonuclease was used to define the VDR-gene allelic polymorphisms, with BB representing the absence and bb the presence of the restriction site on both alleles.

## Statistical methods

Baseline characteristics and laboratory values of the genotype groups were compared with standard two-sample *t* tests and $\chi^2$ tests. Within each genotype, paired *t* tests were used to compare laboratory values during the high and low calcium periods. Despite uniform intervals between each individual's first and second absorption tests (2 weeks) and the short duration of the overall absorption measurement period (17 weeks), there was a small time-dependent decline in changes in calcium absorption fraction over the study period. This decline was linear and similar in the two genotype groups. As a $\chi^2$ test of measurement month by VDR group was borderline significant ($P = 0.089$), we adjusted for time of measurement in all analysis of covariance models in which fractional absorption indexes and their changes were compared across VDR groups. All statistical tests were conducted at the 0.05 level, and except for the $\chi^2$ test, all were two-tailed. Analyses were performed in SPSS (29) and SAS (30).

## Results

Women in the BB and bb groups were similar in age and other clinical characteristics (Table 1). The femoral neck BMD was 5% lower in the BB than in the bb group, a difference that was not statistically significant. During the study, mean dietary calcium intake was 248.3 ± 57.0 (±SD) mg/day in the BB and 283.4 ± 137.9 mg in the bb group ($P = 0.187$). Plasma 25-hydroxyvitamin D levels were similar in the two groups during the study, *i.e.* at the midpoint [76.4 ± 18.4 (±SD) nmol/L *vs.* 75.2 ± 23.3 nmol/L]. On the high calcium intake, the fractional $^{45}$Ca absorption index was similar in the two groups [19.01 ± 1.12% (±SEM)/L in BB and 20.45 ± 0.97%/L in bb]. After calcium restriction (*i.e.* discontinuation of calcium supplements), both groups increased their fractional calcium absorption, but the increase in the BB group was less than that in the bb group. The mean unit changes were 1.37 ± 0.69%/L in BB and 3.35 ± 0.60%/L in bb ($P = 0.041$). The mean percent changes were also lower in the BB group (7.8

**TABLE 1.** Clinical characteristics at enrollment

| Measure | Genotype | | Significance of difference ($P$) |
|---|---|---|---|
| | BB | bb | |
| n | 26 | 34 | |
| Age (yr) | 67.1 ± 4.7 | 68.2 ± 6.0 | 0.407 |
| Time since menopause (yr)[a] | 19.6 ± 6.8 | 19.6 ± 7.4 | 0.971 |
| Ht (cm) | 160.6 ± 6.1 | 160.8 ± 7.2 | 0.940 |
| Wt (kg) | 68.5 ± 11.2 | 70.9 ± 14.3 | 0.462 |
| Calcium intake (mg/day) | 709.7 ± 545.5 | 594.7 ± 253.4 | 0.327 |
| Vitamin D intake (IU/day) | 215.3 ± 166.0 | 175.0 ± 85.0 | 0.266 |
| Current smokers (%) | 11.5 | 2.9 | 0.186 |
| Baseline BMD (g/cm$^2$) | | | |
| Femoral neck | 0.80 ± 0.11 | 0.84 ± 0.11 | 0.133 |
| Spine (L2–L4) | 1.02 ± 0.15 | 1.05 ± 0.16 | 0.452 |

Values are the mean ± SD.

[a] Age of menopause was assigned as 50 yr in the three BB and three bb women for whom exact age at menopause was unknown.

± 3.8 *vs.* 20.7 ± 3.3; $P = 0.016$; adjusted for initial values; Fig. 1). On the low calcium intake, the $^{45}$Ca absorption index was significantly lower in the BB than in the bb group (20.57 ± 1.10%/L *vs.* 23.66 ± 0.95%/L; $P = 0.044$). Intakes of phosphorus, magnesium, protein, and energy were similar in the two groups. Adjustment for a group difference in fiber intake [14.4 ± 4.5 (±SD) g/day in BB and 11.8 ± 4.0 g in bb; $P = 0.02$] did not alter any of these results.

On high and low calcium intakes, circulating levels of 1,25-(OH)$_2$D, calcium, and PTH and 24-h urine calcium excretion (as the calcium/creatinine ratio) did not differ significantly in the two groups (Table 2). With calcium restriction, plasma 1,25-(OH)$_2$D increases, adjusted for initial values, were similar in the two groups (Fig. 1). The diet change also induced similar increases in PTH and decreases in serum calcium and in the urinary calcium/creatinine ratio in the BB and bb groups (Table 2).

In response to calcium restriction, increments in 1,25-(OH)$_2$D and the $^{45}$Ca absorption index were significantly correlated in the bb group ($r = 0.41$; $P = 0.016$), but not in the BB group ($r = 0.23$; $P = 0.269$). Correlations between 1,25-(OH)$_2$D and the $^{45}$Ca absorption index on high and low calcium diets were not significant in either genotype group.

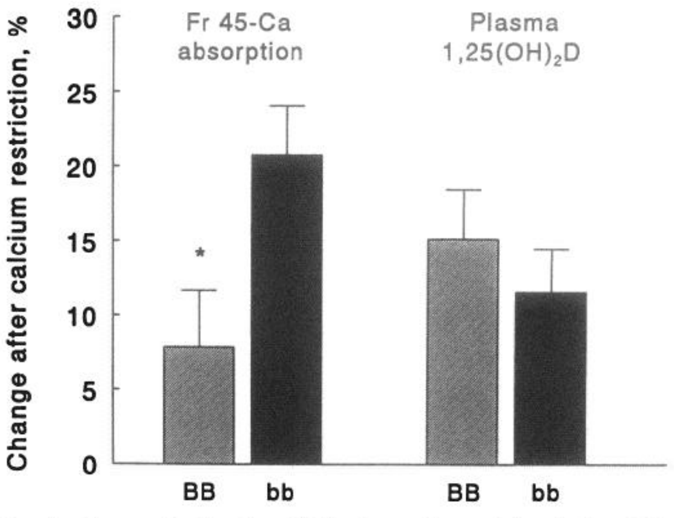

FIG. 1. Changes in fractional $^{45}$Ca absorption and circulating 1,25-(OH)$_2$D after calcium restriction, adjusted for differences in initial (high calcium intake) values and expressed as a percentage of the initial values. The *star* indicates a significant difference between the genotypes ($P = 0.016$).

## Discussion

This study links calcium absorption to heredity with the finding that women with the BB alleles absorb calcium less efficiently than those with the bb alleles under selected conditions. The BB homozygotes appear to have less efficient active calcium transport because their defect is exposed at a low calcium intake, the setting in which active transport is enhanced by higher circulating levels of 1,25-(OH)$_2$D, but not at high calcium intakes, at which absorption by 1,25-(OH)$_2$D-independent passive diffusion plays an increasing role (21). The observed genotype difference in absorption performance despite similar levels of 1,25-(OH)$_2$D points to the VDR as the element that is functionally different between the bb and BB alleles. Other studies of VDR genotype and calcium absorption are not yet available for comparison. Our findings may help explain the earlier observation that intersubject variability in calcium absorption is greater at low than at high calcium intakes (31). Although the differences were not statistically significant, the BB group had somewhat higher PTH and lower urinary calcium excretion levels than the bb group on both diets.

The calcium intake-dependent relationship between VDR genotype and calcium absorption that we observed in this study is consistent with our earlier finding that genotype differences in femoral neck bone loss depend on calcium intake level (17). Accelerated bone loss occurred in the BB women who had a low mean calcium intake (394 mg/day) but not in those who were supplemented and had an intake of 900 mg/day. In a longitudinal study in elderly men and women, Ferrari *et al.* (16) found that spinal loss was inversely correlated with calcium intake in the heterozygotes ($n = 37$), but not in the BB homozygotes ($n = 9$), perhaps because of limitations related to sample size. Estimates of calcium intake have not been reported in the cross-sectional studies of genotype and BMD (9–15). Population differences in past and current calcium intakes may account for some of the different results reported. In our study, femoral neck BMD was 5% lower in the BB than in the bb group, a difference that was not statistically significant, perhaps because of the sample size.

**TABLE 2.** Laboratory values in 26 BB and 34 bb women after 2 weeks on a high calcium intake and after 2 weeks on a low calcium intake

| Measure | BB genotype | | | bb genotype | | |
|---|---|---|---|---|---|---|
| | High calcium intake | Low calcium intake | Difference ($CI_{95}$) | High calcium intake | Low calcium intake | Difference ($CI_{95}$) |
| Plasma 1,25-$(OH)_2D$ (pmol/L) | 78.92 ± 3.16 | 88.80 ± 3.04 | −9.88 ± 1.85[a]<br>(−13.69, −6.07) | 75.39 ± 2.82 | 83.08 ± 3.14 | −7.69 ± 2.47[a]<br>(−12.72, −2.66) |
| Serum PTH (ng/L) | 36.62 ± 2.55 | 46.38 ± 3.49 | −9.77 ± 2.05[a]<br>(−13.99, −5.55) | 31.09 ± 2.12 | 42.09 ± 2.21 | −11.00 ± 1.51[a]<br>(−14.07, −7.93) |
| Serum calcium (mmol/L) | 2.28 ± 0.02 | 2.23 ± 0.01 | 0.05 ± 0.02[b]<br>(−0.00, 0.09) | 2.30 ± 0.01 | 2.26 ± 0.02 | 0.04 ± 0.02[c]<br>(−0.00, 0.08) |
| Serum phosphorus (mmol/L) | 1.13 ± 0.03 | 1.10 ± 0.02 | 0.03 ± 0.02[d]<br>(−0.00, 0.07) | 1.15 ± 0.02 | 1.10 ± 0.02 | 0.05 ± 0.02[b]<br>(0.00, 0.09) |
| 24-h urinary calcium (mmol/day) | 4.15 ± 0.42 | 2.56 ± 0.26 | 1.59 ± 0.27[a]<br>(1.03, 2.15) | 4.88 ± 0.30 | 2.83 ± 0.23 | 2.05 ± 0.22[a]<br>(1.60, 2.50) |
| 24 h urinary calcium/ creatinine (mmol/mol) | 533.8 ± 61.3 | 323.6 ± 36.0 | 210.2 ± 35.1[a]<br>(137.9, 282.5) | 634.6 ± 41.8 | 364.3 ± 32.0 | 270.3 ± 23.6[a]<br>(222.3, 318.3) |

Values are the mean ± SEM. $CI_{95}$, Ninety-five percent confidence interval.

[a] $P < 0.005$.

[b] $P < 0.02$.

[c] $P = 0.074$.

[d] $P = 0.060$.

Several considerations are important in interpreting the study results. The women were maintained on high and low calcium intakes for 2 weeks each to allow time for hormonal and gastrointestinal adaptation to the calcium intake change (32). The sequence of high, then low, calcium intake was used because it is the one under which the adaptation period was defined (32). The calcium intake estimates indicate that the two genotype groups achieved similar levels of calcium restriction. The absorption index used has excellent reproducibility and a strong linear correlation with values from the more elaborate double isotope and balance methods (28, 33). Our study should not imply that the only functional difference in VDR genotypes is their calcium absorption efficiency at low calcium intakes. Further study is required to test for differences in kidney and other organs with VDRs. Finally, this study did not include Bb heterozygotes. It will be important to define their absorption efficiency on different calcium intakes.

In conclusion, this study links calcium absorption and VDR genotype. Compared with bb homozygotes, women with the BB genotype have blunted calcium absorption during calcium restriction, consistent with reduced active calcium transport, a process that is dependent upon the 1,25-$(OH)_2D$ receptor. The BB genetic variant appears to have little impact at high calcium intake levels, but when intake is low or marginal, reduced calcium absorption may put these individuals at increased risk of developing osteoporosis.

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## References

1. **Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S.** 1987 Genetic determinants of bone mass in adults. J Clin Invest. 80:706–710.
2. **Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, Jerums G.** 1989 Reduced bone mass in daughters of women with osteoporosis. N Engl J Med. 320:554–558.
3. **Christian JC, Yu P-L, Slemenda CW, Johnston Jr CC.** 1989 Heritability of bone mass: a longitudinal study in aging male twins. Am J Hum Genet. 44:429–433.
4. **Avioli LV, McDonald JE, Lee SW.** 1965 The influence of age on the intestinal absorption of $^{47}Ca$ in women and its relation to $^{47}Ca$ absorption in postmenopausal osteoporosis. J Clin Invest. 44:1960–1967.
5. **Gallagher JC, Aaron J, Horsman A, Marshall DH, Wilkinson R, Nordin BEC.** 1973 The crush fracture syndrome in postmenopausal women. Clin Endocrinol Metab. 1:293–315.
6. **Morris HA, Need AG, Horowitz M, O'Loughlin PD, Nordin BEC.** 1991 Calcium absorption in normal and osteoporotic postmenopausal women. Calcif Tissue Int. 49:240–243.
7. **Krall E, Dawson-Hughes B.** 1991 Relation of fractional $^{47}Ca$ retention to season and rates of bone loss in healthy postmenopausal women. J Bone Miner Res. 6:1323–1329.
8. **Morrison NA, Qi JC, Tokita A, et al.** 1994 Prediction of bone density from vitamin D receptor alleles. Nature. 367:284–287.
9. **Spector TD, Keen RW, Arden NK, et al.** 1994 Vitamin D receptor gene (VDR) alleles and bone density in postmenopausal women: a UK twin study [Abstract]. J Bone Miner Res. 9(Suppl 1):91.
10. **Yamagata Z, Miyamura T, Iljima S, et al.** 1994 Vitamin D receptor gene polymorphism and bone mineral density in healthy Japanese women. Lancet. 344:1027.
11. **Tokita A, Watanabe T, Miura Y, et al.** 1995 Vitamin D receptor gene RFLP and bone mineral density in Japanese [Abstract]. Bone. 16(Suppl 1):17.
12. **Hustmyer FG, Peacock M, Hui S, Johnston Jr CC, Christian J.** 1994 Bone mineral density in relation to polymorphism at the vitamin D receptor gene locus. J Clin Invest. 94:2130–2134.
13. **Gallagher JC, Goldgar D, Kinyamu H, Fannon P.** 1994 Vitamin D receptor genotypes in type I osteoporosis [Abstract]. J Bone Miner Res. 9(Suppl 1):90.
14. **Looney J, Fischer M, Yoon H, et al.** 1994 Lack of evidence for an

increased prevalence of the BB vitamin D receptor genotype in severe osteoporosis [Abstract]. J Bone Miner Res. 9(Suppl 1):111.

15. **Melhus H, Kindmark A, Amér S, Wilén B, Lindh E, Ljunghall S.** 1994 Vitamin D receptor genotypes in osteoporosis. Lancet. 344: 949–950.
16. **Ferrari S, Rizzoli R, Chevalley T, Slosman D, Eisman JA, Bonjour J-P.** 1995 Vitamin D receptor gene polymorphisms and rate of change in lumbar spine bone mineral density in elderly men and women. Lancet. 10:423–426.
17. **Krall EA, Parry P, Lichter JB, Dawson-Hughes B.** 1995 Vitamin D receptor alleles and rates of bone loss: influences of years since menopause and calcium intake. J Bone Miner. Res. 10:978–984.
18. **Spencer H, Lewin I, Fowler J, Samachson J.** 1969 Influence of dietary calcium intake on $Ca^{47}$ absorption in man. Am J Med. 46: 197–205.
19. **Heaney RP, Saville PD, Recker RR.** 1975 Calcium absorption as a function of calcium intake. J Lab Clin Med. 85:881–890.
20. **Gallagher JC, Riggs BL, Eisman J, Hamstra D, Arnaud SD, Deluca HF.** 1979 Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients. Effects of age and dietary calcium. J Clin Invest. 64:729–736.
21. **Ireland P, Fordtran JS.** 1973 Effect of dietary calcium and age on jejunal calcium absorption in humans studied by intestinal perfusion. J Clin Invest. 52:2672–2681.
22. **Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sahyoun N, Tannenbaum S.** 1990 A placebo-controlled trial of calcium supplementation in postmenopausal women. N Engl J Med. 323:878–883.
23. **Dawson-Hughes B, Harris SS, Krall EA, Dallal G, Falconer G, Green C.** 1995 Rates of bone loss in postmenopausal women randomized to two dosages of vitamin D. Am J Clin Nutr. 61:1140–115.
24. **Preece MA, O'Riordan JL, Lawson DEM, Kodicek E.** 1974 A competitive protein-binding assay for 25-hydroxycholecalciferol and 25-hydroxyergocalciferol in serum. Clin Chim Acta. 54:235–242.
25. **Reinhardt TA, Horst RL, Orff JW, Hollis BW.** 1984 A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. J Clin Endocrinol Metab. 58:91–98.
26. **Bhandarkar SD, Bluhm MM, MacGregor J, Nordin BEC.** 1961 An isotope test of calcium absorption. Br Med J. 2:1539–1541.
27. **Manery JF.** 1954 Water and electrolyte metabolism. Physiol Rev. 34:334–417.
28. **Marshall DH, Nordin BEC.** 1981 A comparison of radioactive calcium absorption tests with net calcium absorption. Clin Sci. 61:477–481.
29. **SPSS Inc.** 1980 SPSS reference guide. SPSS: Chicago.
30. **1985 SAS user's guide: statistics, version 5.** Cary: SAS Institute.
31. **Heaney RP, Recker RR, Stegman MR, May AJ.** 1989 Calcium absorption in women: relationships to calcium intake, estrogen status, age. J Bone Miner Res. 4:469–475.
32. **Dawson-Hughes B, Harris S, Kramich C, Dallal G, Rasmussen HM.** 1993 Calcium retention and hormone levels in black and white women on high- and low-calcium diets. J Bone Miner Res. 8:779–787.
33. **Harrison JE, McNeill KG, Wilson DR, Oreopoulos DG, Krondl A, Finley JM.** 1973 An evaluation of isotopic calcium absorption tests. Clin Biochem. 6:237–245.