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Vitamin D-mediated calcium absorption in patients with clinically stable Crohn's disease: A pilot study

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Abstract

Vitamin D is the critical hormone for intestinal absorption of calcium. Optimal calcium absorption is important for proper mineralization of bone in the prevention of osteoporosis and osteoporotic fractures, among other important functions. Diseases associated with gut inflammation, such as Crohn's disease (CD), may impair calcium absorption. This pilot study evaluated vitamin D-dependent calcium absorption in subjects with CD. Male subjects with CD ($n = 4$) and healthy age-matched controls ($n = 5$) were studied. All subjects had fractional calcium absorption (FCA; by the dual calcium isotope method), serum 25-hydroxyvitamin D, serum calcium and 24 h urinary calcium excretion measurements at baseline. The FCA in response to vitamin D therapy was re-assessed following administration of oral calcitriol 0.25 mcg twice daily for 1 wk, followed by oral calcitriol 0.50 mcg twice daily for 1 wk. Serum calcium and 24 h urinary calcium determinations were re-assessed after each increasing dose of calcitriol as safety measures. There was no significant difference in calcium FCA at baseline or after increasing doses of calcitriol between the CD and controls. FCA in the control and CD group was approximately 35% at baseline, which increased to 60% after calcitriol therapy. No subject developed hypercalcemia or hypercalciuria. Our results suggest that CD patients have a normal response to vitamin D in enhancing the efficacy of calcium absorption. This suggests that stable CD patients can follow calcium and vitamin D guidelines of non-CD adults. Other factors independent of vitamin D status may impair intestinal calcium absorption in CD, including the degree and location of inflammation, presence of surgical resection and/or use of glucocorticoids.

Keywords

Calcium; Crohn's disease; Malabsorption; TNF- α ; Vitamin D

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1 Introduction

Vitamin D is important for optimal intestinal absorption of calcium for proper mineralization of the skeleton and to maintain normal calcium homeostasis for other cellular processes [1]. The risk of osteoporosis and osteoporotic fractures is increased in conditions associated with impaired intestinal calcium absorption [2]. These conditions are also often associated with increased inflammation [3,4]. Crohn's disease (CD), rheumatoid arthritis (RA) and menopause are examples of conditions associated with increased fracture risk [5,6], impaired calcium absorption [7–9] and an increased inflammatory state [10].

Vitamin D regulates intestinal calcium absorption by several proteins expressed in the small intestine. These proteins include calbindin 9K, TRPV6, PMCA1 and NCX1 [11–13]. Vitamin D, obtained from the diet (as ergocalciferol (vitamin D₂) or cholecalciferol (vitamin D₃)) or made in the skin (vitamin D₃), is converted to the active hormonal form 1,25-dihydroxyvitamin D (1,25(OH)₂D) following two sequential hydroxylations by the liver and kidney. The 1,25(OH)₂D hormone binds to the vitamin D nuclear receptor in the enterocytes to increase the expression of the calcium transporter proteins in the intestine, and thus facilitate gut calcium absorption [13,14].

Calcium absorption in CD may be impaired due to vitamin D deficiency [5], magnesium deficiency [15,16], glucocorticoid use [17] and/or intestinal resection [18]. Another potential mechanism for decreased calcium absorption in inflammatory diseases, such as CD, is inflammation-induced resistance to vitamin D action at the level of the enterocytes [8]. Inflammatory bowel diseases such as CD are characterized by increased systemic and epithelial TNF- α and IL-1 concentrations [19,20]. Of interest, treatment with 1,25(OH)₂D₃ (calcitriol) has been shown to reduce systemic TNF- α levels and markers of bone turnover in patients with CD [21,22]. These data suggest that vitamin D nutriture may improve calcium absorption, in part, by increasing gut calcium transporter proteins, decreasing mucosal inflammation and/or overcoming intestinal resistance to vitamin D. However, calcium transport has not been tested in clinical studies of CD patients given vitamin D.

The objective of our study was to determine the efficacy of calcium absorption in subjects with CD and matched healthy controls in response to increasing doses of calcitriol (1,25(OH)₂D₃). We assessed fractional calcium absorption (FCA) using the dual calcium isotope method in both CD and healthy control subjects in response to increasing doses of calcitriol.

2 Materials and methods

The study was approved by the Emory Institutional Review board (IRB) and registered at clinicaltrials.gov under study # NCT00427804. The calcitriol capsules were purchased from Teva Pharmaceuticals USA (North Wales, PA, USA). Calcium isotopes were purchased from Trace Sciences International (Wilmington, DE, USA). Oral suspensions of ⁴⁴Ca of 3 mg/mL and intravenous suspensions of ⁴²Ca of 0.4 mg/mL were prepared and sterilized and vacuum packaged in 5mL vials by AnazaoHealth (Tampa, FL, USA). Samples of each batch underwent pyrogenicity testing by AnazaoHealth. The concentrations of the calcium isotope solutions were confirmed independently at Emory University Hospital's chemistry laboratory.

2.1 Subjects

Two groups of subjects were recruited into the study, those with biopsy proven, stable Crohn's disease (cases) and those without a history of inflammatory disorders of the GI tract or RA (controls). Subjects with CD were considered clinically stable if they were in remission [23] or had mild-to-moderate disease [23] on mesalamine, sulfasalazine or azathioprine therapy.

CD subjects on therapy with corticosteroids or anti-TNF agents (Infliximab, Adalimumab or Certolizumab) were excluded from the study to eliminate the ameliorating effect of these agents on TNF- α levels [24–26]. All patients were recruited from the Atlanta Veterans Administration Medical Center (VAMC) and the Emory Clinic. Only male patients were recruited who were between the ages of 18 and 50. The study was limited to younger male subjects to remove the effect of sex steroid hormone insufficiency on calcium absorption [27]. Subjects were excluded for the following: if they were taking activated vitamin D medications such as calcitriol, paricalcitol, doxercalciferol, had a history of nephrolithiasis, hypercalcemia or hypercalciuria, short bowel disease due to intestinal resection, use of osteoporosis medications (bisphosphonate, calcitonin or teriparatide), chronic kidney disease (calculated GFR < 60 mL/min/1.73m²), history of hyperparathyroidism (parathyroid hormone greater than upper limit of normal) or hypoparathyroidism (parathyroid hormone below lower limit of normal). A Harvey–Bradshaw score was calculated to indicate disease severity in the CD patients [28].

2.2 Study design

This was a prospective case-control study. All subjects were recruited from July of 2007 to August of 2008. Pre-screening of electronic medical records and informational fliers were used to identify subjects. Subjects were then approached and informed about the study on their routine follow-up visits to the Emory Clinic or Atlanta VAMC Gastroenterology or other clinics. All subjects who agreed to participate gave written informed consent.

The design of the study is detailed in Fig. 1. Screening serum and 24 h urine calcium/creatinine values were collected for all patients. A focused food frequency questionnaire was used to determine mean daily calcium and vitamin D consumption [29]. All subjects who met inclusion criteria were asked to return to the clinical studies center for baseline calculation of the FCA using the dual calcium isotope method [30]. Dual isotope FCA testing was performed for each subject at baseline, the morning after completion of a 7day course of calcitriol 0.25 mcg BID, and the morning after completion of a 7-day course of calcitriol 0.5 mcg BID (Fig. 1). A minimum 7-day washout period was required between the two calcitriol treatment courses. Serum was collected on the morning of and prior to each FCA test for determination of calcium and 25(OH)D, and creatinine levels.

2.3 FCA measured by stable dual isotopes

Fifteen milligrams of the ⁴⁴Ca suspension (total dose 45 mg) was equilibrated overnight with 120 mL of Lactaid fat free milk to reach a concentration of 0.125 mg/mL of ⁴⁴Ca. The hospital pharmacy dispensed the 5 mL of the ⁴⁴Ca suspension (total dose 2 mg) to the study coordinator the morning of each FCA test. Subjects were allowed to maintain their regular diet, but to take no more than 600 mg of supplemental calcium and no more than 400 IU of supplemental vitamin D throughout the study.

All subjects reported to the study center at 8 a.m. after an overnight fast. They were asked to void upon arrival. Subjects were then given an infusion of suspension of the ⁴²Ca (2 mg total) over 3–5 min through an intravenous catheter following the baseline blood draw. Following the labeled calcium infusion, a standardized breakfast of orange juice (noncalcium fortified), cinnamon raisin bread, a fruit cup and the ⁴⁴Ca milk suspension was served. Subjects were asked to rinse their milk cups with orange juice to ensure consumption of the entire ⁴⁴Ca milk suspension. All subjects were then given a urine jug and instructed to collect their urine over the next 24 h. Subjects returned their 24 h urine collection to the Atlanta VAMC the following morning.

2.4 Calcium absorption analyses

Calcium absorption analyses were performed at Baylor College of Medicine Houston, Texas, USA, following previously published methods [31]. Ammonium oxalate was used to precipitate Ca isotopes in the urine samples [32]. The amount of extracted calcium was then used for calcium isotope ratio measurements. This amount was determined using magnetic sector thermal ionization spectrometry (MAT 261; Finnigan, Bremen, Germany). FCA was calculated using previously published formulas [31].

2.5 Biochemical analyses

Venous blood was collected at baseline and prior to each FCA test. The blood was centrifuged, aliquoted and frozen at -80°C until batch analysis for 25(OH)D and TNF- α concentrations. Serum concentrations of 25(OH)D and TNF- α were assessed using ELISA (IDS Fountain Hills, AZ, USA and R&D Systems, Minneapolis, MN, USA, respectively). A comprehensive blood metabolic profile, including tests of electrolytes, and renal and hepatic function were performed at Atlanta VAMC main hospital lab using standard hospital methods.

Each of the 24 h urine collections were first assessed for total volume. Approximately 50 mL of each collection was then aliquoted and frozen at -80°C for later determination of the FCA. Twenty-four hour urine calcium and creatinine concentrations were measured by the Atlanta VAMC main hospital laboratory using standard methods.

2.6 Baseline calcium and vitamin D intake assessment

Mean daily intake of vitamin D and calcium by subjects was estimated using a modified food frequency questionnaire adapted from a previously published version [29]. A serving size for each food item was described to the subjects. Subjects were then asked to recall the number of serving sizes they consumed for each food item within a given day, week or month. For each food item, the amount of vitamin D contained in each serving size was multiplied by the frequency of intake for each subject. Total vitamin D intake was then calculated, and averaged to represent mean intake *per day*.

2.7 Statistical analysis

Microsoft Excel 2003 (Microsoft, Seattle, WA, USA) and Graphpad Prism version 5.02 (San Diego, CA, USA) was used to conduct the statistical analysis. Descriptive statistics were used for demographic and laboratory data. We reported SEM except where noted. Two-way ANOVA was used to evaluate the effect of intervention, group and their interaction on FCA, serum calcium, 24 h urine calcium. A *p* value <0.05 was considered to be statistically significant. Baseline characteristics, follow-up measures and clinical outcomes were compared on an intention-to-treat basis.

3 Results

3.1 Subject demographics

Baseline characteristics of study subjects are given in Table 1. The majority of subjects in both groups were African American (4/4 in the CD group, 4/5 in the control group). Subjects in the CD group were clinically stable with a median Harvey–Bradshaw score of 4 (range 1–7). There were no statistically significant differences in baseline blood concentrations of calcium, albumin or 25(OH)D, 24 h urine calcium excretion, creatinine clearance or body mass index (BMI). Baseline serum TNF- α was not detectable in either group. Intake of calcium and vitamin D by food frequency questionnaire was also similar in both groups. Of note, all subjects in the CD and control groups were vitamin D insufficient, defined as blood 25(OH)D levels $<30\text{ ng/mL}$. However, as noted above, there was no significant difference in mean 25(OH)D levels

between groups. Of the four CD subjects, two were on medical therapy for their CD. One patient was on mesalamine and azathioprine and the other on sulfasalazine alone.

3.2 Serum and urine calcium in response to calcitriol

Calcium levels were similar at baseline between the two groups. Serum calcium levels did not change significantly in response to low- or high-dose calcitriol therapy in the control group (9.62 ± 0.08 , 9.67 ± 0.2 and 9.62 ± 0.08 mg/dL at baseline, low- and high-dose calcitriol, respectively). Similarly, there was no significant change in serum calcium levels in the CD group (9.58 ± 0.08 , 9.85 ± 0.06 and 9.53 ± 0.7 mg/dL at baseline, low- and high-dose calcitriol, respectively).

Levels of 24 h urine calcium were similar between groups at baseline. In the control group, 24 h urine calcium levels incrementally increased after low- and high-dose calcitriol, respectively, with an increase from 100 mg/day at baseline to 165 mg/day with high-dose calcitriol; however, this did not reach statistical significance ($p = 0.134$). The CD group did not experience any significant changes in 24 h urine calcium in response to low- and high-dose calcitriol (Fig. 2).

3.3 FCA in response to calcitriol

In the CD group, FCA significantly increased with low-dose calcitriol as compared with baseline. This group was able to further increase their FCA in response to high-dose calcitriol (Fig. 3). There was no significant difference in the FCA in response to low- and high-dose calcitriol comparing the CD to the control group ($p = 0.264$). The FCA in both groups reached a plateau of around 60% after high-dose calcitriol. The increase in FCA from baseline did not differ between the CD and control group with low- and high-dose calcitriol ($p = 0.6$ and $p = 0.8$, respectively). The CD subjects had a mean individual change in FCA in response to low- and high-dose calcitriol of $83.4\% \pm 40\%$ and $114.8\% \pm 70\%$ from baseline and the control subjects had a $99.0\% \pm 50\%$ and $71.8\% \pm 50\%$ change in FCA from baseline, respectively.

3.4 Adverse events

Mild hypercalcemia (Ca 11.2 mg/dL) was noted in only one subject with CD after high-dose calcitriol therapy. There were otherwise no adverse events such as nephrolithiasis or hypercalciuria noted in response to intravenous calcium isotope infusions and oral calcitriol in either the CD or control subjects.

4 Discussion

In this prospective case-control pilot study, we have demonstrated that young male subjects with stable CD do not have impaired intestinal calcium absorption at baseline when compared with controls. Further, vitamin D therapy similarly increased calcium absorption in CD and controls. Our findings from this pilot study suggest that patients with clinically stable CD have maintained intestinal calcium absorption at baseline and in response to calcitriol therapy compared with subjects without CD.

All subjects in both groups were vitamin D insufficient at baseline and both groups had similar mean 25(OH)D concentrations. These findings suggest that the intrinsic CD inflammatory state does not impair calcium absorption independently of vitamin D status. In agreement with our study, Kravitt *et al.* found that only four out of 31 subjects with CD have a negative calcium balance as evaluated by metabolic and calcium isotope studies [9].

Calcium absorption is impaired in other diseases associated with increased inflammation. Sambrook *et al.* [7] demonstrated that calcium absorption measured by dual isotope method was impaired in post-menopausal women with RA compared with healthy controls. Similarly,

Abrams *et al.* [33] found that calcium absorption was impaired in children with juvenile RA compared with healthy controls. A recently developed mouse model of CD characterized by elevated TNF- α concentrations demonstrated decreased intestinal expression of key proteins involved in calcium transport including TRPV6, calbindin-D_{9k} and PMCA1b [34]. New therapies to lower TNF levels have been developed for the treatment of CD and RA [35,36]. Although their effects on calcium absorption have not been evaluated, there appears to be a favorable effect of anti-TNF therapies on bone mineral density [36,37].

Aging, a condition associated with increased inflammation, has been associated with decreased absorption of calcium. Pattanaungkul *et al.* [8] examined younger and older women and found a positive relationship between serum 1,25-dihydroxyvitamin D₃ levels only in young but not old women, suggesting that aging resulted in vitamin D resistance in intestinal absorption of calcium. Walters *et al.* found decreased intestinal expression with age of the vitamin D-dependant calcium transport protein TRPV6 and vitamin D receptor with aging in women, confirming the increased intestinal vitamin D resistance with age [38].

Another aspect of our study was to examine the role of low- and high-dose calcitriol on calcium absorption in a model of inflammation such as CD. We sought to assess whether the intrinsic inflammatory state of CD would prevent a rise in calcium absorption in response to vitamin D when compared with controls. Furthermore, we sought to assess whether higher doses of calcitriol could overcome this so-called vitamin D resistance. Treatment with 1,25(OH)₂D (calcitriol) has been shown to reduce TNF- α levels and markers of bone turnover in patients with CD [21,22]. We did not find impaired calcium absorption in response to vitamin D in our subjects with mild CD.

There are limitations to this study. Our sample size was small. We only examined CD patients in remission or with mild disease, half of whom were on 5-ASA agents or azathioprine. We calculate that with this number of subjects enrolled, we had 80% power to exclude a difference of 35% in FCA between the two groups. This information is clinically useful since this approximates the expected rise in FCA in healthy controls comparing baseline to calcitriol-stimulated FCA. We can reject our initial hypothesis that CD patients have no increase in FCA in response to calcitriol. We lacked sufficient power to detect more subtle differences in FCA between these two groups. In order to detect smaller differences, we would require 40 or 150 subjects *per* group to detect a difference of 10 or 5%, respectively. Furthermore, our CD group may therefore not have had significant local inflammation of the intestine. None of our CD subjects had detectable systemic TNF- α levels. It could thus be argued that the level of inflammation in our group of CD subjects may not have been enough to interfere with vitamin D-mediated intestinal calcium absorption, and that greater degrees of inflammation may still play a role in impairing vitamin D-mediated calcium absorption. However, studies conducted on CD subjects with similar disease activity have elevated local TNF- α on jejunal biopsy samples [20]. Our findings are also limited to men since we did not enroll women. There may be differences in response to 1,25(OH)₂D with aging. Walters *et al.* demonstrated that men had increases in TRPV6 with serum 1,25(OH)₂D levels in contrast to women where there was no relationship [38]. Finally, our subjects were primarily African-American and therefore our results may not be generalized to other ethnicities. This study measured the absorption of calcium but did not assess the loss of calcium *via* intestinal secretions. It is possible that these are increased in CD, but unlikely that this would be affected by vitamin D therapy.

In conclusion, our study demonstrates that subjects with CD do not have impaired calcium absorption in response to the hormonal form of vitamin D, 1,25(OH)₂D (calcitriol), compared with matched controls. The implications of this finding are that stable CD patients may not require increased amounts of calcium and vitamin D supplementation since neither the response to vitamin D nor the intestinal absorption of calcium appears to be impaired. Future studies

should evaluate whether impaired calcium absorption occurs in active CD and whether these individuals are resistant to calcium absorption in response to vitamin D.

Abbreviations

1,25(OH) ₂ D	1,25-dihydroxyvitamin D
CD	Crohn's disease
FCA	fractional calcium absorption
RA	rheumatoid arthritis

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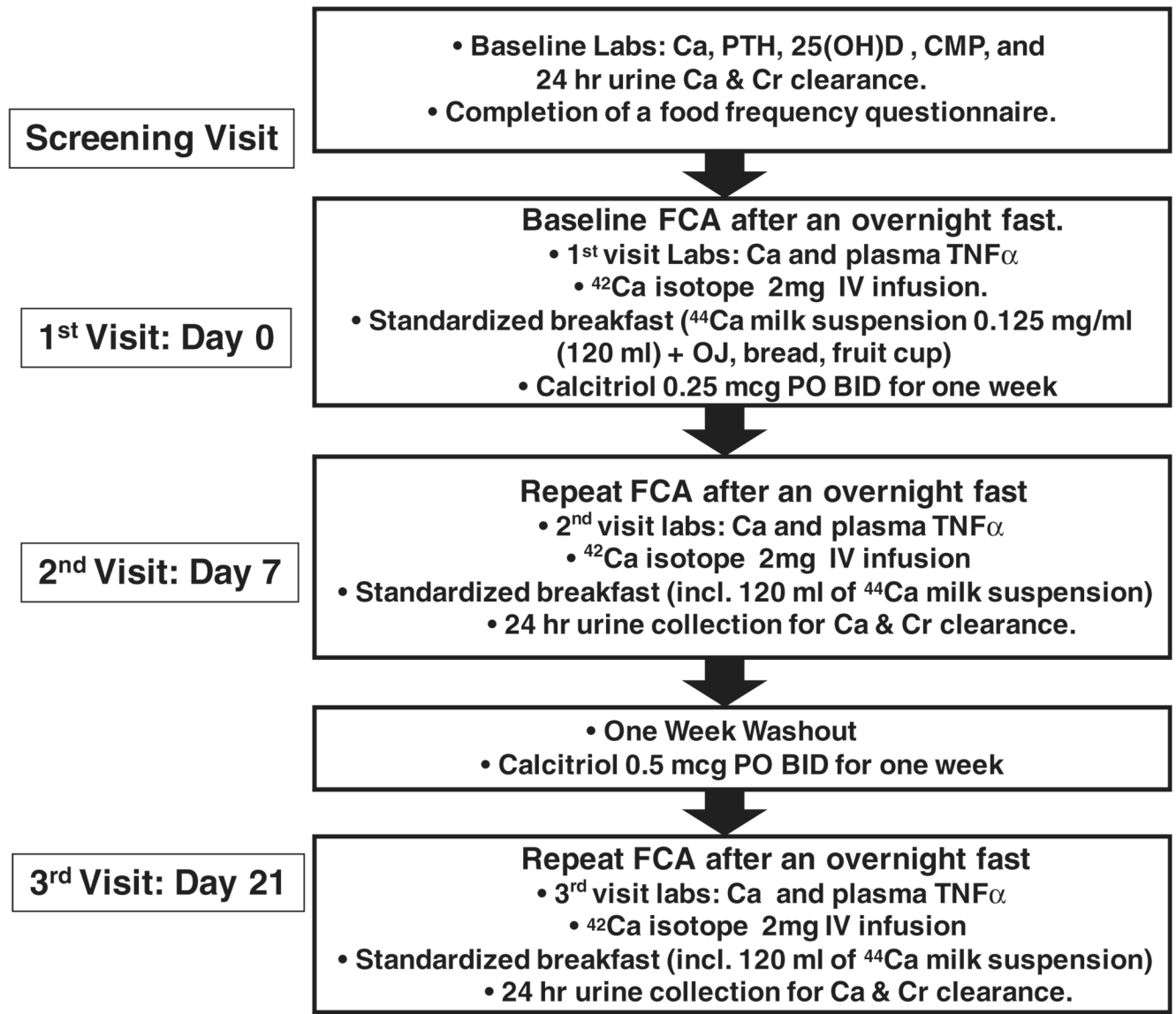


Figure 1.
Experimental methodology.

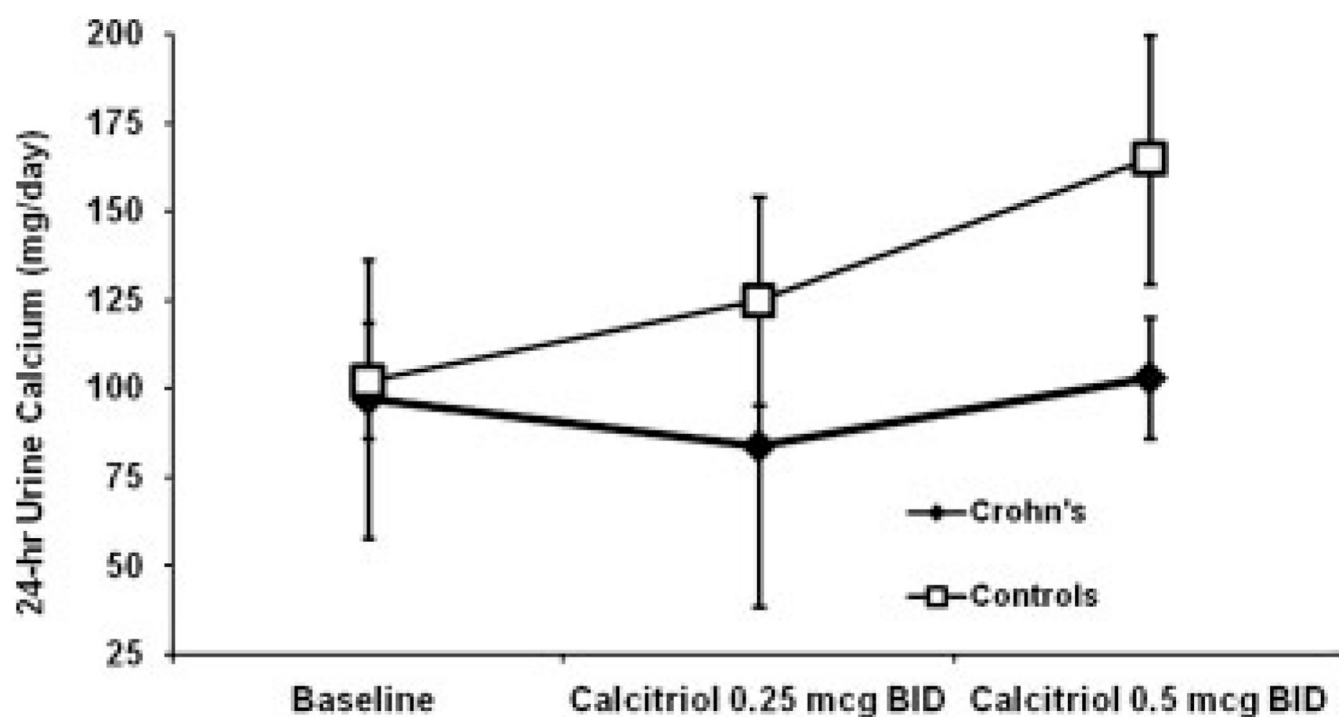


Figure 2.

Twenty-four-hour urine calcium levels in CD subjects and controls. Male subjects with CD (black diamonds) and healthy matched controls (white boxes) underwent determination for 24 h urine calcium excretion at baseline and in response to calcitriol at two doses (0.25 mcg twice daily and 0.50 mcg twice daily). There were no significant differences in the 24 h excretion of calcium comparing the CD and control groups.

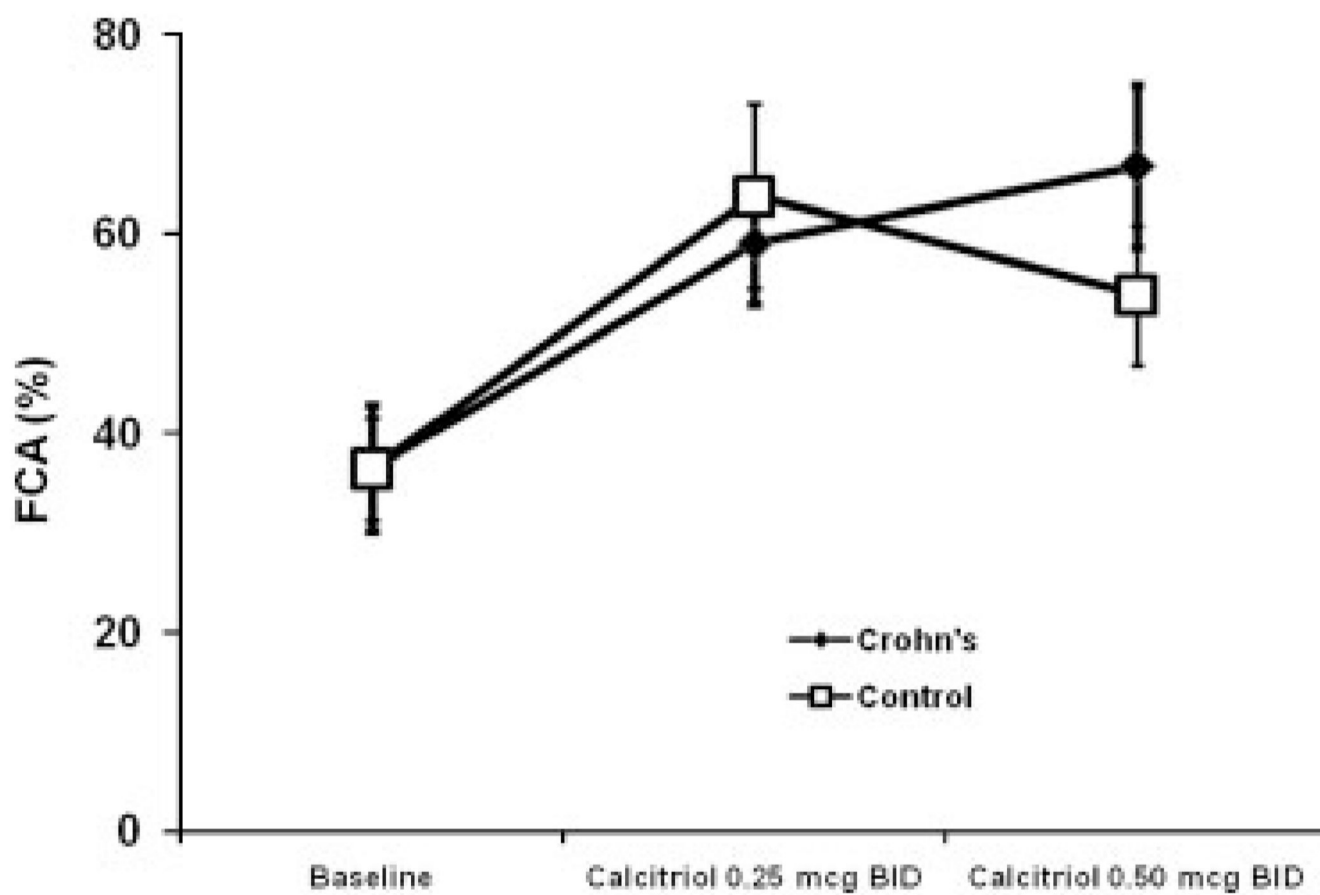


Figure 3. FCA in CD subjects and controls. Male subjects with CD (black diamonds) and healthy matched controls (white boxes) underwent determination of FCA using dual stable calcium isotopes at baseline and in response to calcitriol at two doses (0.25 mcg twice daily and 0.50 mcg twice daily). Both groups had similar FCA at baseline. There were no significant differences in FCA in response to both doses of calcitriol comparing the CD and control groups.

Table 1

Baseline characteristics of study population

	CD (n = 4)	Control (n = 5)	p-Value
Age (years)	35.5 ± 9.75	42.40 ± 5.13	0.105
Sex			
Male	4	5	–
Female	0	0	–
Race			
Black	4	4	–
White	0	1	–
BMI	26.3 ± 6.40	28.14 ± 5.09	0.336
Years of diagnosis	12.75 ± 8.54	N/A	–
Creatinine clearance (mL/min)	90.2 ± 5.8	96.9 ± 3.3	0.178
Serum			
Serum calcium (mg/dl)	9.58 ± 0.17	9.62 ± 0.18	0.03
Albumin (gm/dL)	4.30 ± 0.14	4.48 ± 0.24	0.114
25(OH)D (ng/mL)	16.26 ± 5.10	17.97 ± 3.84	0.295
TNF-α (pg/mL)	N/D	N/D	–
Vitamin D intake (IU)	240.50 ± 119.92	211.60 ± 132.11	0.372
Calcium intake (mg)	458.75 ± 135.36	357.09 ± 116.10	0.132

Mean ± SD.