Vitamin D Status and Bone Density in Recently Diagnosed Inflammatory Bowel Disease: The Manitoba IBD Cohort Study

William D. Leslie [1,2]
Norine Miller [1,2]
Linda Rogala [1,2]
Charles N. Bernstein [1,2]

1. Department of Medicine, University of Manitoba, Winnipeg, MB, Canada
2. University of Manitoba, Inflammatory Bowel Disease and Research Centre, University of Manitoba, Winnipeg, MB, Canada

Author for Correspondence and Reprints:

Dr. William D. Leslie, MD, MSc
Department of Medicine (C5121)
409 Tache Avenue
Winnipeg, Manitoba CANADA
R2H 2A6
Phone: 204-237-2311
Fax: 204-237-2007
Email: bleslie@sbgh.mb.ca

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ABSTRACT

Objectives. Bone mineral density (BMD) is usually normal at the time of inflammatory bowel disease (IBD) diagnosis. The purpose of this study was to evaluate the role of vitamin D metabolism in recently diagnosed IBD.

Methods. Adult subjects with recently diagnosed IBD (median 4 y) were recruited from the University of Manitoba IBD Research Registry into the Manitoba IBD Cohort Study. Baseline BMD and serum 25-hydroxy vitamin D (25OHD) were measured in a nested subgroup of 101 subjects of whom 94 had repeat BMD measurements 2.3 ± 0.3 y later.

Results. Only a minority (22 [21.8%]) of recently diagnosed IBD participants had optimal serum 25OHD levels (75 nmol/L or greater). Serum 25OHD was positively correlated with baseline BMD for the lumbar spine, total hip and total body (all $P<0.05$). MANOVA confirmed significant between-group differences in baseline T-scores when vitamin D status was categorized according to serum 25OHD quartile ($P<0.05$). Gain in total body BMD between the baseline and follow up DXA scans was positively correlated with 25OHD ($r=0.20$, $P<0.05$).

Conclusions. Poorer vitamin D status correlates with lower baseline BMD at all measurement sites and better vitamin D status is correlated with a gain in total body BMD. Early optimization of vitamin D may play an important role in preventing IBD-related bone disease.

Key Words: Bone mineral density; Crohn's disease; Dual energy x-ray absorptiometry; Inflammatory bowel disease; Osteoporosis; Ulcerative colitis; Vitamin D.

Study Highlights:

1) What is current knowledge

● Patients with IBD have an increased risk of osteoporosis and fractures.

● BMD is typically normal at the time of IBD diagnosis.

● Vitamin D deficiency/insufficiency is common in the general population and in chronic disease states, and is believed to contribute to the skeletal complications of IBD.

● Vitamin D is increasingly recognized as having important extra-skeletal actions including immune system regulation and tissue proliferation/differentiation.

2) What is new here

● Only a minority of recently diagnosed IBD participants had optimal vitamin D status.

● Serum 25OHD was positively correlated with baseline BMD.

● Each SD decrease in serum 25OHD was associated with a 2.0-3.4% reduction in baseline BMD.

● Gain in total body BMD was positively correlated with 25OHD.

INTRODUCTION

A higher incidence of osteoporosis and fractures is a recognized complication of inflammatory bowel disease (IBD). [1] Population-based studies confirm that IBD patients have a 21-40% increased risk of fractures compared with the general population. [2-4] Multiple factors have been implicated including nutritional deficiencies, systemic inflammation, malabsorption and corticosteroid use. [5]

The role of vitamin D insufficiency in IBD-related bone disease is uncertain. Vitamin D insufficiency has gained considerable attention in recent years following observations that vitamin D status is suboptimal in a large fraction of healthy and osteoporotic individuals worldwide. [6,7] Although the definition of optimal vitamin D status is still open to debate, many experts now define the beginning of the normal range as the serum 25-hydroxy vitamin D (25OHD) level associated with a serum PTH which is not further suppressed by increasing vitamin D intake. This translates to an optimal lower limit of 25OHD of 75 nmol/L. [8,9] This is also the approximate range where increases in intestinal calcium absorption and lower extremity muscle function appear to plateau. [10,11]

Several reports indicate that bone mineral density (BMD) is usually normal in individuals with newly-diagnosed IBD but may be followed by a period of accelerated bone loss. [12-14] Whether vitamin D insufficiency contributes to reduced BMD in early IBD had not been investigated. This has significant clinical implications since vitamin D supplementation is easy and inexpensive, with a high safety profile.

The Manitoba IBD Cohort Study is a research program with the aim of defining multiple health outcomes and their determinants in persons with IBD. Skeletal health in

IBD was one of the primary research themes and the current analysis was undertaken to determine the association between BMD and vitamin D status at baseline and follow-up in a population-based cohort of recently diagnosed IBD patients.

METHODS

Patient Population

The study design and patient population have been previously reported in detail[15]. Briefly, individuals within 7 years of IBD diagnosis were identified from the University of Manitoba IBD Research Registry, the largest population-based registry of IBD in North America. The University of Manitoba IBD Epidemiology Database and the University of Manitoba IBD Research Registry were created in 1995. [16] Patients eligible for inclusion were identified through the population-based administrative health registry of Manitoba Health (the single provincial health insurer that provides comprehensive coverage to all residents of Manitoba) using an administrative definition of IBD that was validated by random sample chart reviews. Questionnaires were mailed to patients identified in the Manitoba Health registry and those who agreed to be included in a research registry and to be contacted for future IBD related research studies were included in the University of Manitoba Inflammatory Bowel Disease Research Registry. This methodology was repeated in 2000 to enhance the numbers of patients in the Research Registry.

Individuals within seven years of IBD diagnosis and who were 18 years of age or older (N = 606) were identified from the Registry and were invited to participate in the study. A total of 418 (69.0%) individuals initially responded. Following this initial

contact, 19 individuals did not respond to the request to complete the baseline survey, another four individuals withdrew, and seven individuals were found to be ineligible, resulting in a final sample of 388 participants for the Manitoba IBD Cohort Study. From the initial study population, a subset was randomly selected for participation in more detailed investigations of bone health. All assessments were performed in non-winter months. The objective was to recruit 100 participants stratified by age (<50 years vs. >50 years) in equal proportion. Exclusion criteria for the bone substudy included current or recent pregnancy (within 6 months), current or recent breast feeding (within 6 months), other disorders known to affect bone metabolism independent of IBD and its treatment (rheumatoid arthritis or other inflammatory joint disease, thyroid disease, parathyroid disease, primary bone disease, severe neurologic disease impairing ambulation, non-cutaneous malignancies, liver disease, eating disorder, or renal dysfunction with serum creatinine > 0.150 mmol/L). Individuals that completed the baseline measurements were invited to return for follow-up BMD measurements 2.3 ± 0.3 years later. The study protocol was approved by the University of Manitoba Research Ethics Board and all participants provided signed informed consent.

Measurements

All subjects underwent measurement of lumbar spine, hip and total body BMD with dual-energy x-ray absorptiometry (DXA) (Hologic QDR-4500, Waltham, MA). Scans were acquired and analyzed using manufacturer specifications and White reference data to provide standard measures of areal BMD including T-scores and Z-scores. T-scores and Z-scores used sex-matched reference data (except for the total body for which male

reference data are not available). In accordance with BMD reporting recommendations from the International Society of Clinical Densitometry (ISCD), abnormal BMD was defined as T-score $\leq$ -2.5 in participants age 50 years or older, and by a Z-score $\leq$ -2.0 if age less than 50 years. [17,18] All results were reviewed by a single study investigator with extensive experience in clinical and research DXA (WDL). Daily quality control of the DXA device gave long-term precision error <0.5% and the in vivo coefficient of variation [CV] was 1.0-1.7% for the measurement sites.

Serum was collected for measurement of 25OHD, parathyroid hormone (PTH), creatinine, calcium, phosphate, albumin, total alkaline phosphatase (tALP), bone-specific alkaline phosphatase (BSAP), and N-telopeptide (NTX). Serum 25OHD was measured using a radioimmunoassay that detects both the D_2 and D_3 metabolites of vitamin D (Diasorin Inc., Stillwater, MN, USA) by a lab that participates in DEQAS Quality Assurance Program for vitamin D (inter-assay coefficient of variation [CV] 6 -13 %). Intact PTH was measured using an enzyme immunoassay (Diagnostics Products Corporation, LA, CA) run on an Immulite system (CV 9%).

Statistics

Univariate analyses were used to compare groups (Chi square test for categorical data and Student's T-test for continuous data). The association between continuous variables was assessed using simple and multiple linear regression. Analysis of covariance (ANCOVA) was used to test and exclude interactions with age and the underlying diagnosis. Serum 25OHD was categorized according to quartiles and also using prespecified cutoffs as deficient (< 25 nmol/L), insufficient (25-49 nmol/L), marginal

(50-74 nmol/L) and optimal (> 75 nmol/L). Multiple analysis of variance (MANOVA) was used to explore the relationship between 25OHD category and BMD measurements. Post hoc subgroup comparisons were performed using Tukey's test. Continuous data are expressed as mean ± SD unless otherwise stated. All statistical analyses were performed with Statistica version 7.1 (Statsoft, Inc., Tulsa, OK). A *P*-value of less than 0.05 was used to determine statistical significance.

RESULTS

Population

Baseline bone measurements were obtained in 101 subjects as described in Table 1. The median since IBD diagnosis was 4 years. There were slightly more women than men (59 [58%] vs 42 [42%]) which was comparable with the sex distribution in the overall study population. There was a similar number of individuals with Crohn's disease (56 [55%]) versus ulcerative colitis/proctitis (45 [45%]) in the bone substudy participants, and the proportion was similar to the overall study population. By design, one-half of the bone substudy participants were less than age 50 at the time of recruitment and the remainder were over age 50. The mean age (47 ± 16 years) was slightly older than in the overall study population, reflecting the age-stratified recruitment to the bone sub-study. Women were slightly younger than men (45 ± 14 years vs 50 ± 17 years, P=0.07) but the difference was not statistically significant. BMI was equal for men and women (28.7 ± 6.0 kg/m^2 vs 27.8 ± 6.8 kg/m^2, P=0.49). Most of the subjects (61 [60%]) reported a history of prior corticosteroid use. The median age of first corticosteroid exposure was 40 years (interquartile range 28-51) with median cumulative exposure 4 months

(interquartile range 2-9). Of the 101 participants, 17 (17%) admitted to current or past use of one or more bone-sparing medications (15 estrogen, 4 bisphosphonate, 4 raloxifene, 4 parenteral calcitonin). Self-reported disease activity based on the 6 months prior to the baseline visit was categorized as active in 24 (24%) of the participants and inactive in the remainder.

Baseline Measurements

Baseline skeletal measurements are summarized in Table 2. Mean Z-scores for the lumbar spine (-0.14 ± 1.25) and total hip (0.08 ± 0.99) indicate BMD close to the age-matched mean. Men had lower lumbar spine Z-scores then women ($P<0.01$) but total hip results were similar. Participants younger than age 50 years had lower Z-scores for all sites compared with those age 50 and older (all $P<0.01$), and this persisted after ANCOVA adjustment for weight and cumulative corticosteroid use (all $P<0.05$). Mean T-scores for the lumbar spine and total hip did not differ according to sex or age stratum. Total body T-scores and Z-scores were lower in women than men which may relate to the lack of male reference data. A diagnosis of Crohn's disease compared with ulcerative colitis/proctitis was associated with lower total hip and total body Z-scores, but lumbar spine Z-scores were similar. Nine (9%) of the subjects had at least one abnormal measurement (8 lumbar spine, 2 total hip and 1 total body). No significant difference in the distribution of abnormal bone density measurements was seen according to sex, age stratum or diagnosis.

The mean serum 25OHD was 58.6 ± 25.4 nmol/L, and was similar for sex and age stratum. Mean 25OHD for fall months (50.2 ± 19.3 nmol/L, N=37) were lower than

those from spring (64.1 ± 28.5 nmol/L, N=42) or summer months (62.3 ± 25.5 nmol/L, N=22; ANOVA *P*=0.038). Serum 25OHD did not differ between subjects with Crohn's disease (59.1 ± 28.1 nmol/L) and those with ulcerative colitis/proctitis (58.1 ± 21.8 nmol/L). Serum 25OHD did not differ between subjects with prior corticosteroid exposure (60.0 ± 26.6 nmol/L) versus those without corticosteroid exposure (59.0 ± 2.7 nmol/L). In subjects with prior corticosteroid exposure there was no significant correlation between serum 25OHD and age of first corticosteroid exposure (*P*=0.28) or cumulative exposure (*P*=0.14). Serum 25OHD was similar in the participants with active and inactive disease (58.9 ± 23.5 vs 57.9 ± 26.1 nmol/L, *P*=0.87).

Vitamin D status was categorized according to 25OHD as deficient (<25 nmol/L) in 6 (5.9%), insufficient (25-49 nmol/L) in 38 (37.6%), marginal (50-74 nmol/L) in 35 (34.7%), and optimal (>75 nmol/L) in 22 (21.8%). Mean values for the other biochemical parameters were within the laboratory reference ranges. Serum 25OHD was unrelated to age, sex, weight or BMI (all *P*>0.2). Serum creatinine was slightly higher in men than women, and in the older age stratum. Serum calcium was slightly lower in the younger age stratum while serum phosphate was slightly greater. As expected, there was an inverse correlation between serum 25OHD and PTH (r=-.42, *P*<0.001) (Table 3). In turn, serum PTH was positively correlated with all markers of bone turnover (tALP r=.28, *P*<0.01; BSAP r=.25, *P*<0.05; NTX r=.25, *P*<0.05). In addition, serum 25OHD showed a positive association with serum albumin (r=.30, *P*<0.01).

Correlates of BMD

Several of the biochemical parameters showed significant associations with baseline bone density (Table 4). Higher serum 25OHD was associated with greater bone density at all sites (lumbar spine r=.28, $P<0.01$; total hip r=.21, $P<0.05$; total body r=.21, $P<0.05$) and this was unchanged after adjustment for seasonal differences (lumbar spine r=.31, $P<0.01$; total hip r=.24, $P<0.05$; total body r=.23, $P<0.05$) and was unaffected by the underlying diagnosis ($P>0.1$ for all interaction terms). Each SD decrease in serum 25OHD was associated with a 3.4% reduction in spine BMD, a 2.9% reduction in total hip BMD and a 2.0% reduction in total body BMD. BMD showed a negative correlation with serum PTH and all markers of bone turnover. There was a positive correlation between bone density at the total hip and total body with and serum creatinine, and these were still statistically significant when adjusted for age and sex in multiple linear regression models (serum creatinine versus total hip BMD beta=0.34, $P<0.01$; serum creatinine versus total body BMD beta=0.27, $P<0.05$). Baseline BMD at all measurement sites was positively correlated with serum albumin.

Baseline bone density was studied in relation to vitamin D status. When analyzed according to serum 25OHD quartile, MANOVA confirmed significant differences in baseline bone density ($P<0.05$). There was a general trend of greater BMD for higher serum 25OHD subgroups (Figure 3), but in post hoc testing this was only statistically significant for quartile 2 versus quartile 4 ($P<0.05$). There was a non-significant trend ($P=0.08$) for differences in baseline bone density related to serum 25OHD categorized as deficient (< 25 nmol/L), insufficient (25-49 nmol/L), marginal (50-74 nmol/L) and optimal ($\geq$ 75 nmol/L). When the deficient (< 25 nmol/L) subgroup was compared with

higher levels of serum 25OHD (≥ 25 nmol/L), there was a significant difference in baseline BMD by MANOVA (P<0.05).

Change in BMD

Of the original 101 participants undergoing BMD measurements, 94 (53 women and 41 men) returned for repeat measurements 2.3 ± 0.3 years later. There was an increase in mean BMD at all measurement sites between the first and second scans: lumbar spine +0.017 ± 0.049 g/cm^2 (1.7%), total hip +0.003 ± 0.040 g/cm^2 (0.3%), and total body +0.003 ± 0.022 g/cm^2 (0.3%). The change was only statistically significant for the lumbar spine and may simply reflect age-related degenerative effects. No sex subgroup differences were seen in the change in bone density (data not shown). Subjects younger than age 50 had a larger increase in total body BMD than older subjects (+0.008 ± 0.021 g/cm^2 versus -0.002 ± 0.021 g/cm^2, P<0.05), but age subgroup was unrelated to change at the lumbar spine or total hip.

Gain in total body BMD between the baseline and follow up DXA scans was positively correlated with serum 25OHD (r=0.20, P<0.05) (Table 4). Serum 25OHD was unrelated to BMD change at the lumbar spine or total hip. No correlation was observed between serum PTH and change in BMD at any site. Higher levels of BSAP and NTX showed an association with increasing bone density (P<0.05 in 3 of 6 comparisons). Serum creatinine was correlated with a decrease in total hip BMD (r=-0.24, P<0.05) and tALP was correlated with a decrease in total body BMD (r=-0.24, P<0.05).

Parsimonious multiple linear regression models were constructed for predicting the change in BMD at each measurement site from age, osteoporosis medication use,

serum 25OHD, creatinine, and a single bone turnover marker, NTX (Table 5). Disease activity was not included in these models since it did not show a significant univariate relationship with baseline BMD, change in BMD, serum 25OHD or the other biochemical parameters (data not shown). For the lumbar spine and total hip, NTX ($P<0.05$) was a significant independent predictor of change in BMD with higher NTX associated with a larger increase. Higher serum creatinine was independently associated with a greater decrease in total hip BMD ($P<.01$). For the change in total body BMD, only serum 25OHD showed a significant effect ($P<0.05$) with higher values associated with a larger gain in BMD.

DISCUSSION

We have found that vitamin D status was associated with baseline BMD measurements in a population-based cohort with recently diagnosed IBD. There was a weaker association between baseline vitamin D status and change in total body BMD after mean 2.3 years of follow up. Markers of bone turnover were predictive of baseline BMD at all sites and with change at the lumbar spine and total hip, whereas serum creatinine appeared to have an effect limited to the total hip. It is unclear if this inconsistency between sites relates to true biologic differences or simply the relatively small size of the cohort. Age-adjusted Z-scores were lower in younger than in older participants, and this was unrelated to weight or corticosteroid exposure. The mechanism and clinical significance of this finding is unknown and warrants further study.

It is noteworthy that only a minority of the study participants (21.8%) had vitamin D status in the optimal range (75 nmol/L or greater). Our findings are not surprising

given the well established role of vitamin D on skeletal metabolism, although not all reports have found an association between vitamin D status and bone density. [19,20] One study failed to find an association between vitamin D intake and BMD in premenopausal women, but serological assessment provides a more complete picture since it also reflects skin synthesis. [20] Several studies have documented stability or an increase in BMD in IBD patients given calcium and vitamin D supplements. [21-24] These findings are consistent with observational studies [25-27] and randomized trials [28-31] conducted in the general population that have found better vitamin D status to be associated with higher BMD. It should be noted that the terms vitamin D "deficiency" and "insufficiency" have not been defined with universal agreement, and vitamin D is usually regarded as a continuum encompassing both terms. Deficiency of vitamin D has been used to describe advanced musculoskeletal effects of chronically low vitamin D: rickets in children and osteomalacia in adults. Vitamin D "insufficiency" is a milder form of deficiency, although the mild secondary hyperparathyroidism may cause increased bone turnover and bone loss.

Although this study has focused on the skeletal actions of vitamin D, this hormone also has important physiological actions outside of the musculoskeletal system.[32] Receptors for calcitriol and the enzyme involved in its synthesis (1-hydroxylase) are expressed by many tissues other than those concerned with calcium regulation, and in some tissues there appear to be important antiproliferative and prodifferentiating properties. [33] A recent systematic review yielded 63 observational studies of vitamin D status in relation to cancer risk. [34] The majority of these studies found a protective relationship between sufficient vitamin D status and lower risk of

cancer. Of relevance to IBD patients, a meta-analysis of colorectal cancer found that individuals with 25 mcg (1,000 IU) per day or more of oral vitamin D or serum 25OHD concentrations ≥82 nmol/l had 50% lower incidence of colorectal cancer compared to reference values. [35] Recently, a four-year RCT conducted in 1179 community-dwelling postmenopausal women showed that daily supplementation with calcium 1400–1500 mg and vitamin D_3 27.5 mcg (1100 IU) substantially reduced all-cancer risk. [36] Vitamin D is also involved in regulation of the immune system, and evidence is accumulating for its involvement in the pathogenesis of IBD. [37]

The advantage of our study was that it was conducted in a population-based sample of IBD patients who had relatively recent onset disease. Several limitations apply to our study. This was an observational study and therefore associations may not reflect causality. We did not include healthy controls and cannot say whether vitamin D status or BMD in our IBD patients differs from the general population. The Canadian population has a high prevalence of vitamin D insufficiency/deficiency. [38-40] In a population-based study of seasonal changes in 25OHD, 97% had 25OHD levels below 80 nmol/L in at least one of four measurements during the year. [38] Our results may have limited generalizability to geographic areas with different patterns of sun exposure. A global ecological study found that for each 10 degrees change in latitude from the equator, hip fracture probability increased by 0.6%. [6] Finally, biochemical analyses were based on a single measurement at baseline and may not reflect long term vitamin D status.

We have confirmed previous reports that abnormal BMD is uncommon within the first few years after IBD diagnosis. Our findings also suggest that vitamin D

supplementation is probably warranted for most IBD patients. In the absence of vitamin D supplementation, serum 25OHD should be measured. Markers of bone turnover may provide information on BMD that is independent of vitamin D status, but this requires further study. In summary, poorer vitamin D status correlates with lower baseline BMD and better vitamin D status is correlated with a gain in total body BMD. Early optimization of vitamin D may play an important role in preventing IBD-related bone disease.

Statement of Support, Contribution and Competing Interests

A. Who is the guarantor of the paper?

William D. Leslie

B. What is EACH AUTHOR'S contribution to the paper?

WDL: Concept, design, obtaining funding, data analysis, primary writing of the manuscript

NM: Concept, data collection, reviewing the manuscript

LR: Concept, data collection, reviewing the manuscript

CNB: Concept, design, obtaining funding, data analysis, reviewing the manuscript

All authors approved the final draft submitted.

C. What financial support was received?

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D. What may be the potential competing interests?

In the past 5 years WDL has received speaker fees, research honoraria, and unrestricted research grants from Merck Frosst Canada Ltd; research honoraria and unrestricted educational grants from Sanofi-Aventis and Proctor & Gamble Pharmaceuticals Canada, Inc.

Table 1. Population characteristics compared with all cohort subjects and all eligible registry subjects.

	Bone Substudy Subjects N=101	All Cohort Subjects N=384	Eligible From Registry N=606
Age (years)	46.9 ± 15.5	40.5 ± 14.7	43.7 ± 17.7
Men (%)	42 (42%)	156 (41%)	260 (43%)
Crohn's disease (%)	56 (55%)	188 (49%)	266 (44%)
Height (cm)	167.7 ± 9.8	N/A	N/A
Weight (kg)	79.0 ± 18.8	N/A	N/A
BMI (kg/m^2)	28.1 ± 6.5	N/A	N/A

Mean ± SD
BMI, body mass index; N/A, not available

Table 2. Baseline measurements stratified by sex, age and diagnosis subgroups.

Measurement	Combined N=101	Sex subgroups		Age subgroups		Diagnosis subgroups	
		Women N=59	Men N=42	Age < 50 y N=51	Age ≥ 50 y N=50	CD N=56	UC N=45
Lumbar spine (L1-4):							
BMD	0.987 ± 0.121	0.987 ± 0.1171	0.988 ± 0.1283	0.985 ± 0.096	0.990 ± 0.144	0.980 ± 0.124	0.996 ± 0.11
T-score	-0.71 ± 1.12	-0.55 ± 1.07	-0.93 ± 1.17	-0.69 ± 0.91	-0.73 ± 1.31	-0.76 ± 1.19	-0.65 ± 1.04
Z-score	-0.14 ± 1.25	0.12 ± 1.18**	-0.51 ± 1.26	-0.53 ± 0.94**	0.24 ± 1.40	-0.26 ± 1.32	-0.01 ± 1.15
Abnormal (%) [1]	8 (8%)	3 (5%)	4 (10%)	4 (8%)	4 (8%)	4 (7%)	4 (9%)
Total hip:							
BMD	0.937 ± 0.132	0.894 ±0.109***	0.997 ± 0.140	0.921 ± 0.106	0.953 ± 0.154	0.908 ± 0.127*	0.973 ± 0.13
T-score	-0.33 ± 0.90	-0.39 ± 0.89	-0.24 ± 0.93	-0.36 ± 0.79	0.29 ± 1.01	-0.51 ± 0.93*	-0.11 ± 0.83
Z-score	0.08 ± 0.99	0.05 ± 0.96	0.13 ± 1.04	-0.24 ± 0.84**	0.40 ± 1.04	-0.15 ± 0.97**	0.36 ± 0.95
Abnormal (%)	2 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)	2 (4%)	0 (0%)
Total body:							
BMD	1.132 ± 0.109	1.100 ± 0.090***	1.177 ± 0.114	1.128 ± 0.086	1.136 ± 0.13	1.118 ± 0.098	1.149 ± 0.12
T-score	0.34 ± 1.26	-0.03 ± 1.08***	0.86 ± 1.31	0.30 ± 0.98	0.39 ± 1.49	0.18 ± 1.13	0.55 ± 1.38
Z-score	1.09 ± 1.37	0.63 ± 1.10***	1.75 ± 1.46	0.61 ± 1.05***	1.57 ± 1.48	0.82 ± 1.17*	1.41 ± 1.52
Abnormal (%)	1 (1%)	1 (2%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	1 (2%)
25OHD	58.6 ± 25.4	59.8 ± 27.9	57 ± 21.5	60.8 ± 30.4	56.4 ± 19.1	59.1 ± 28.1	58.1 ± 21.8
PTH	33.5 ± 13.4	34.6 ± 14.0	31.8 ± 12.4	31.2 ± 10.2	35.8 ± 15.7	33.3 ± 12.3	33.7 ± 14.7
Creatinine	75.4 ± 20.4	65.9 ± 11.4***	88.7 ± 22.7	69.7 ± 14.1**	81.2 ± 24.0	71.9 ± 18.6	79.8 ± 21.8
Calcium	2.35 ± 0.10	2.35 ± 0.10	2.36 ± 0.10	2.32 ± 0.10**	2.38 ± 0.10	2.33 ± 0.10 *	2.37 ± 0.09
Phosphate	1.08 ± 0.19	1.11 ± 0.19	1.05 ± 0.18	1.12 ± 0.20*	1.04 ± 0.17	1.11 ± 0.19	1.05 ± 0.18
Albumin	38.6 ± 3.9	38.1 ± 3.3	39.4 ± 4.5	38.7 ± 5.0	38.4 ± 2.3	37.9 ± 4.6 *	39.4 ± 2.45
tALP	76.5 ± 20.7	78.5 ± 21.1	73.8 ± 20.0	74.5 ± 18.2	78.7 ± 22.9	78.7 ± 21.0	73.9 ± 20.2
BSAP	19.6 ± 8.2	20.9 ± 8.9	17.9 ± 6.8	20.5 ± 7.9	18.8 ± 8.5	19.8 ± 7.9	19.5 ± 8.67
NTX	12.3 ± 3.8	11.9 ± 3.8	12.9 ± 3.8	12.8 ± 3.8	11.8 ± 3.7	12.6 ± 3.8	11.9 ± 3.8

Mean ± SD
BMD, bone mineral density; CD, Crohn's disease; UC, ulcerative colitis/proctitis; 25OHD, 25-hydroxy vitamin D; PTH, parathyroid hormone; tALP, total alkaline phosphatase; BSAP, bone-specific alkaline phosphatase; NTX, N-telopeptide
[1] Abnormal defined as T-score $\leq$ -2.5 if age 50 years or older, Z-score $\leq$ -2.0 if age less than 50 years.
P-value * < 0.05, ** < 0.01, *** <0.001 between subgroups

Table 3. Pearson correlation coefficients between biochemical markers.

	25OHD	PTH	Creatinine	Calcium	Phosphate	Albumin	tALP	BSAP	NTX
25OHD	--	-0.42***	-0.02	0.19	-0.08	0.30**	-0.19	-0.20*	-0.18
PTH	-0.42***	--	0.00	-0.12	0.07	-0.15	0.28**	0.25*	0.25*
Creatinine	-0.02	0.00	--	0.24*	-0.12	0.19	-0.15	-0.30**	0.05
Calcium	0.19	-0.12	0.24*	--	-0.06	0.27**	-0.06	-0.02	0.02
Phosphate	-0.08	0.07	-0.12	-0.06	--	-0.17	0.00	-0.02	0.29**
Albumin	0.30**	-0.15	0.19	0.27**	-0.17	--	-0.36***	-0.03	-0.21*
tALP	-0.19	0.28**	-0.15	-0.06	0.00	-0.36***	--	0.61***	0.21*
BSAP	-0.20*	0.25*	-0.30**	-0.02	-0.02	-0.03	0.61***	--	0.26**
NTX	-0.18	0.25*	0.05	0.02	0.29**	-0.21*	0.21*	0.26**	--

25OHD, 25-hydroxy vitamin D; PTH, parathyroid hormone; tALP, total alkaline phosphatase; BSAP, bone-specific alkaline phosphatase; NTX, N-telopeptide
P-value * < 0.05, ** < 0.01, *** <0.001

Table 4. Pearson correlation coefficients between bone density and biochemical markers.

	Baseline BMD			Change in BMD		
	Lumbar spine	Total hip	Total body	Lumbar spine	Total hip	Total body
25OHD	.28**	.21*	.21*	-.02	.01	.20*
PTH	-.20*	-.28**	-.26*	-.04	.00	-.06
Creatinine	.14	.47***	.35***	.04	-.24*	.08
Calcium	.01	.06	-.06	-.04	-.03	.01
Phosphate	-.11	-.24*	-.25*	.06	.13	.05
Albumin	.28**	.27**	.30**	-.09	-.17	.10
tALP	-.25*	-.34**	-.27**	-.06	.04	-.24*
BSAP	-.24*	-.35***	-.32**	.12	.23*	.03
NTX	-.40***	-.36***	-.30*	.28**	.25*	.18

BMD, bone mineral density; 25OHD, 25-hydroxy vitamin D; PTH, parathyroid hormone; tALP, total alkaline phosphatase; BSAP, bone-specific alkaline phosphatase; NTX, N-telopeptide
P-value * < 0.05, ** < 0.01, *** <0.001

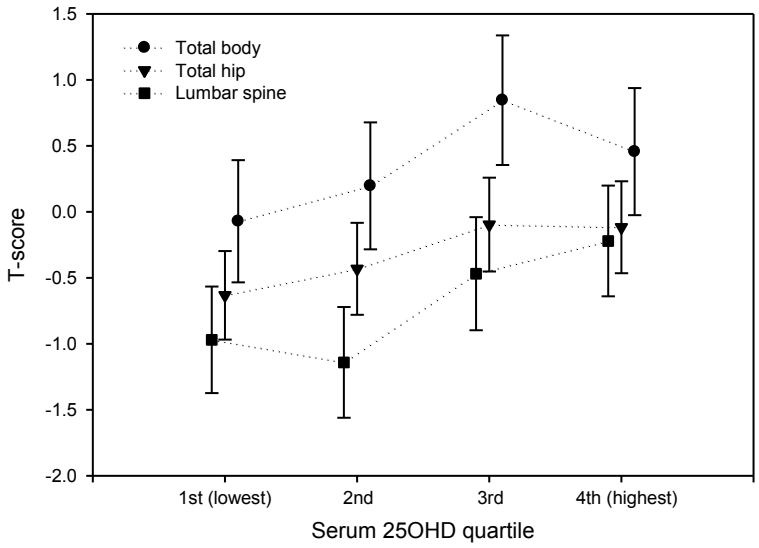
Table 5. Multiple regression analyses for change in bone density at the lumbar spine, total hip and total body.

	Change in lumbar spine BMD			Change in total hip BMD			Change in total body BMD		
	Beta	SE	*P*-value	Beta	SE	*P*-value	Beta	SE	*P*-value
Age	-0.037	0.119	NS	0.032	0.117	NS	-0.147	0.118	NS
Osteoporosis medication use	0.237	0.106	.023	0.215	0.104	.041	0.190	0.104	NS
25OHD	0.052	0.101	NS	0.077	0.099	NS	0.252	0.100	.014
Creatinine	0.004	0.112	NS	-0.304	0.110	.007	0.093	0.110	NS
NTX	0.252	0.109	.028	0.269	0.106	.013	0.157	0.107	NS

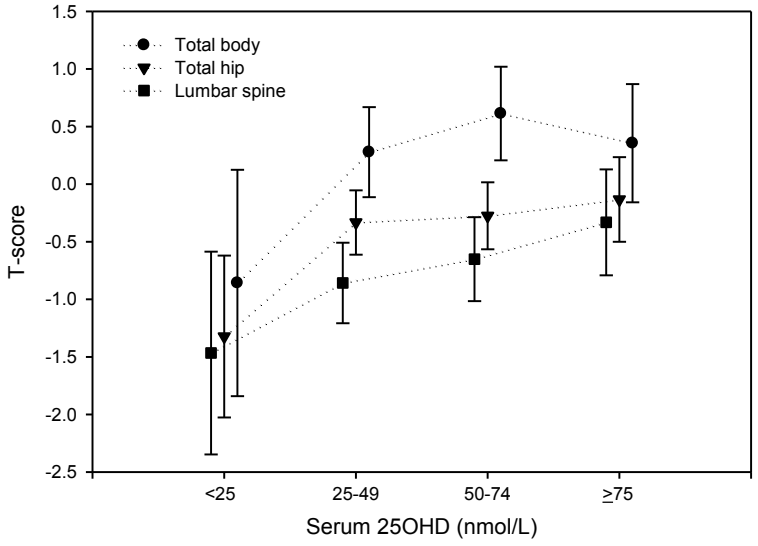
BMD, bone mineral density; 25OHD, 25-hydroxy vitamin D; NTX, N-telopeptide

Figure 1. Vitamin D status in relation to baseline bone density. (A) Quartiles of serum 25OHD. (B) Categorization of serum 25OHD as deficient (< 25 nmol/L), insufficient (25-49 nmol/L), marginal (50-74 nmol/L) and optimal (≥ 75 nmol/L). Error bars are 95 confidence intervals.

A)



B)



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