

The frequency of vitamin D deficiency in adults with Crohn's disease

Jesse S Siffledean BSc¹, Kerry Siminoski MD¹, Hillary Steinhart MD², Gordon Greenberg MD², Richard N Fedorak MD¹

JS Siffledean, K Siminoski, H Steinhart, G Greenberg, RN Fedorak. The frequency of vitamin D deficiency in adults with Crohn's disease. *Can J Gastroenterol* 2003;17(8): 473-478.

BACKGROUND: Vitamin D deficiency is a putative, pathogenic cofactor in the increase in osteopenia and osteoporosis seen in patients with Crohn's disease.

OBJECTIVE: To determine the frequency of low serum 25-hydroxyvitamin D₃ (25-OHD) levels and the associated alterations in bone mineral density in a cohort of adults with Crohn's disease.

METHODS: 25-OHD levels were determined in 242 consecutive patients with Crohn's disease seen in two tertiary inflammatory bowel disease referral centres. Bone mineral density was assessed by dual energy x-ray absorptiometry.

RESULTS: Nineteen (8%) patients exhibited vitamin D deficiency (25-OHD less than 25 nmol/L) and 52 (22%) patients exhibited vitamin D insufficiency (25-OHD less than 40 nmol/L). Mean T-scores at the lumbar spine, femoral neck, total hip and ultradistal radius in the group with low 25-OHD did not differ from those of the normal 25-OHD group. Serum alkaline phosphatase and parathyroid hormone levels were higher in the low 25-OHD group than in the normal group. Decreased red blood cell (RBC) folate predicted low 25-OHD in male patients, while smoking, RBC folate and serum iron predicted low 25-OHD in female patients. The rate of low 25-OHD deficiency in the winter was significantly higher than that in the summer (11.9% versus 2.8%, respectively).

CONCLUSION: Vitamin D-deficient Crohn's disease patients exhibit biochemical evidence of metabolic bone disease, without detectable differences in bone mineral density. Sunlight exposure, nutrition and smoking status were predictors of vitamin D deficiency in this patient cohort.

Key Words: Bone mineral density; Crohn's disease; Inflammatory bowel disease; Osteopenia; Osteoporosis; Vitamin D

Bone mineral density (BMD) is reduced in patients with Crohn's disease (1-7). The mechanism underlying the lower bone mineral density is not clearly understood; however, multiple etiologies are likely involved. These include disease activity, corticosteroid therapy, calcium and vitamin D deficiency, acute inflammatory cytokine action on osteoclast and osteoblast activity, sex hormone deficiency, smoking and overall poor nutrition (5-14).

Previous studies have demonstrated that between 17% and 68% of Crohn's disease patients exhibit frank serum 25-hydroxyvitamin D₃ (25-OHD) deficiency, defined as a val-

Fréquence du déficit en vitamine D chez les adultes atteints de la maladie de Crohn

CONTEXTE : La carence en vitamine D est un cofacteur pathogène et généralement admis de la fréquence accrue de l'ostéopénie et de l'ostéoporose observées chez les patients atteints de la maladie de Crohn.

OBJECTIF : Déterminer la fréquence de faibles taux sanguins de 25-hydroxyvitamine D₃ (25-OHD) et les modifications de la densité minérale osseuse qui y sont associées dans une cohorte d'adultes atteints de la maladie de Crohn.

MÉTHODE : Les taux de 25-OHD ont été mesurés chez 242 patients consécutifs, atteints de la maladie de Crohn, dans deux centres de soins tertiaires, spécialisés dans les maladies inflammatoires de l'intestin. La densité minérale de l'os a été évaluée au moyen de l'absorptiométrie doublé énergie à rayons X.

RÉSULTATS : Une carence en vitamine D (25-OHD < 25 nmol/l) a été décelée chez 19 patients (8 %) et une insuffisance en vitamine D (25-OHD < 40 nmol/l), chez 52 patients (22 %). Les scores T moyens mesurés au niveau de la colonne lombaire, au col du fémur, à la hanche et au radius ultradistal chez les patients présentant un faible taux de vitamine D ne différaient pas de ceux enregistrés chez les patients présentant un taux normal de vitamine D. Les taux sériques de phosphatase alcaline et de parathormone étaient plus élevés dans le groupe affichant de faibles valeurs de 25-OHD que dans le groupe normal. Une diminution de la concentration de folates dans les globules rouges (GRs) s'est avérée un prédicteur de faible taux de 25-OHD chez les hommes, tandis que, chez les femmes, ce sont le tabagisme, la concentration de folates dans les GR et le fer sérique. Le taux de déficit en vitamine D s'est montré significativement plus élevé en hiver qu'en été (11,9 % contre 2,8 % respectivement).

CONCLUSION : Les patients atteints de la maladie de Crohn qui présentent un déficit en vitamine D montrent des signes biochimiques d'ostéopathie métabolique mais pas de différences décelables de la densité minérale osseuse. L'exposition au soleil, l'alimentation et le tabagisme se sont révélés des prédicteurs de déficit en vitamine D dans la cohorte à l'étude.

ue less than 25 nmol/L (10 ng/mL) (3,8,12,15-20). Several studies have identified an association between bone mineral density and 25-OHD status (8,12,20), and reported that vitamin D supplementation improved bone mineral density in Crohn's disease patients (21). In contrast, other studies have failed to show an association between 25-OHD deficiency and reduced bone mineral density (1,3-7,15,19).

25-OHD serves as the major precursor and most objective measure of the active hormone 1,25-dihydroxyvitamin D₃ (1,25-OHD) undergoing 1-alpha hydroxylation in the kidney. 1,25-OHD increases calcium and phosphate absorption and

¹Division of Gastroenterology and Division of Endocrinology, Department of Medicine, University of Alberta, Edmonton, Alberta;

²Division of Gastroenterology, University of Toronto, Toronto, Ontario

Correspondence: Dr RN Fedorak, Division of Gastroenterology, University of Alberta, 519 Robert Newton Research Building, Edmonton, Alberta

T6G 2C1. Telephone 780-492-6941, fax 780-492-8121, e-mail richard.fedorak@ualberta.ca

Received for publication October 31, 2002. Accepted April 28, 2003

reabsorption in the gastrointestinal tract and kidneys, respectively, thus maintaining normal mineralization of newly formed bone (18,22). A low serum concentration of 1,25-OHD delays osteoid deposition by osteoblasts, increases parathyroid hormone (PTH) release and activity and results in increased bone resorption. Prolonged vitamin D deficiency results in osteomalacia in adults (22).

The main source of vitamin D in healthy individuals is cutaneous ultraviolet irradiation of 7-dehydrocholesterol, with a minor contribution from the intake of foods containing, or fortified with, vitamin D (22,23). Serum vitamin D deficiency is rarely, if ever, reported in healthy North American adults (24,25). Serum 25-OHD levels vary seasonally, reaching the highest levels in the summer months, and are lowest in the late winter months (12,22-26). The absence of vitamin D deficiency in North American adults in the winter is most likely due to the ingestion of foods fortified with vitamin D (22,25). When ingested, vitamin D is absorbed mainly at the jejunum of the small intestine (27). Furthermore, enterohepatic recirculation at the ileum normally prevents excessive excretion of vitamin D (12,23). Any, or all, of these sources of vitamin D may be affected in Crohn's disease.

The aim of the present study is to determine, in a Canadian cohort of adult patients with Crohn's disease, the frequency of low serum 25-OHD levels, the associated risk of low bone mineral density and to identify the causes of lowered vitamin D in these patients.

PATIENTS AND METHODS

Patients

Between 1998 and 2000, 224 patients with Crohn's disease who had attended the Inflammatory Bowel Disease Research Centre and Clinic, at the University of Alberta Hospital, Edmonton, Alberta, were consecutively enrolled in a prospective longitudinal investigation to assess the clinical efficacy of bisphosphonate therapy on bone mineral density in Crohn's disease patients. An additional 18 patients from Mount Sinai Hospital, Toronto, Ontario, were also enrolled, giving a total of 242 patients. Data relevant to vitamin D were gathered at baseline assessment. Informed consent from each patient was received in writing before being enrolled. Crohn's disease and the site of the disease was diagnosed on the basis of endoscopic, radiological and histological examination. Those patients with a serum 25-OHD concentration below 25 nmol/L were classified as being vitamin D deficient and were separately analyzed for the purposes of this study.

The following exclusion criteria were applied at baseline: age less than 18 years; patients with known bone disorders other than osteoporosis (such as hyperparathyroidism, Paget's disease, renal osteodystrophy and documented osteomalacia); patients with abnormal thyroid function; patients with significant renal impairment (serum creatinine twice the normal level); patients with clinical short bowel syndrome; patients on total parenteral or enteral nutrition; and patients with spinal anatomy that did not allow adequate assessment with dual energy x-ray absorptiometry (DEXA, Hologic 4500, Hologic Inc, USA). In addition, patients who had received bisphosphonate or fluoride supplement in the 24 months before the data collection or pharmacological doses of calcium (greater than 1.0 g/day) or vitamin D (greater than 800 IU/day) in the six months before entry were excluded from the study.

Patient demographics and disease information, such as age at baseline, diagnosis date, gender, smoking status, Crohn's disease

activity in the last year and corticosteroid use in the last year, were obtained through a questionnaire conducted at baseline. Other baseline information was gathered by reviewing patients' records, and by blood, urine and DEXA analysis. Weight and height were obtained to determine body mass index (BMI) (in kg/m²), and disease site was recorded as colonic, ileal, ileocolonic or jejunal/duodenal.

BMD measurements

BMD (g/cm²) of the lumbar spine (L1-L4), the femoral neck, total hip and ultradistal radius was measured at baseline by DEXA using standard protocols. Vertebral BMD values were normalized using the Hologic reference database; hip values were normalized using the National Health And Nutrition Examination Study III database (28). Osteopenia (T-values greater than -1.0 but less than or equal to -2.5) and osteoporosis (T-values less than -2.5) were diagnosed using the lowest value of the lumbar vertebrae (L1-L4 inclusive, unless there was a specific reason to exclude a given vertebra), total hip density or femoral neck density (29).

Biochemical measurements

Blood samples were drawn at baseline to determine the following serum values: serum alkaline phosphatase (U/L), phosphorous (mmol/L), calcium (mmol/L), PTH (pmol/L), 25-OHD (nmol/L), albumin (g/L), sodium (mmol/L), potassium (mmol/L), chloride (mmol/L), creatinine (μmol/L), random glucose (mmol/L), platelet count ($\times 10^9$ /L), white blood cell (WBC) count ($\times 10^9$ /L), hemoglobin (g/L), total protein (g/L), magnesium (mmol/L), C-reactive protein (CRP) (mg/L), iron (μmol/L), total iron binding capacity (μmol/L), ferritin (μg/L), carotene (μmol/L), vitamin B₁₂ (pmol/L), red blood cell folate (nmol/L), testosterone (nmol/L), follicle stimulating hormone (U/L), luteinizing hormone (U/L), estradiol (pmol/L), and thyroid stimulating hormone (U/L). Urine samples were also collected for a 24 h period at baseline to measure urine N-telopeptide (nmol/mmol urine creatinine) and urine creatinine (mmol/24 h). CRP, WBC, platelet count and serum ferritin served as markers of inflammation and Crohn's disease activity (30,31). Urinary N-telopeptide was measured as a marker of bone resorption (32).

25-OHD determination

Serum levels of 25-OHD were determined by prior extraction with acetonitrile. Quantification of 25-OHD was performed by competitive radioimmunoassay (DIASORIN 25-OHD Radioimmunoassay kit, DIASORIN Corporation, USA). The intra- and interassay coefficients of variance were less than 12% each, respectively.

Statistical analysis

Descriptive statistics were reported as a mean ± SD for continuous variables that were normally distributed, unless otherwise stated. Results for the group of Crohn's disease patients with serum 25-OHD deficiency were compared with those of the patient group with normal levels of 25-OHD. For comparison of means of continuous variables between two groups, an unpaired *t*-test was performed. If the data was not normally distributed the Mann-Whitney rank test was employed. Comparison of discontinuous variables was performed using a χ^2 test.

To determine if any continuous variables could predict vitamin D status in the Crohn's disease patients, a univariate analysis of the data from the 242 patients was first performed using Pearson's correlation test. All significantly associated variables were then

TABLE 1
Demographic characteristics of 19 patients with Crohn's Disease and serum 25-hydroxyvitamin D₃ (25-OHD) deficiency, compared with 219 Crohn's disease patients with normal serum 25-OHD

Demographic characteristics	Normal 25-OHD	Deficient 25-OHD
Age (years) Mean ± SD	38.5±12.4	40.4±10.0
Sex (M/F)	97/122	09/10
Age at diagnosis (years) Mean ± SD	27.2±12.0	30.3±9.9
Years since Crohn's disease diagnosis Mean ± SD	11.3±8.7	10.1±8.9
Crohn's disease location: (% of patients)		
Colon	21.7	11.8
Ileum	37.7	41.2
Colon and ileum	40.1	41.2
Jejunum/duodenum	0.5	5.8
% smokers (at any time)	35.6	62.5
BMI (kg/m ²) (Mean ± SD)	24.9±4.6	27.1±4.6
Number of flares requiring a physician's visit in last year (% of patients)		
≤ 2	78.8	80.4
3-6	20.3	18.8
7-10	0.9	0.8
Patients who used steroids in last year (%)	51.6	47.4
Patients with normal bone density (%)	37.2	36.8
Patients with osteopenia (%)	48.6	57.9
Patients with osteoporosis (%)	14.2	5.3

25-OHD deficiency is defined as a serum 25-OHD concentration below 25 nmol/L. BMI Body mass index; F Female; M Male

subjected to forward stepwise regression analysis to develop a multivariate model.

For all tests, significance was defined as P<0.05 (95% CI), with normality and variance defined at P<0.01. The statistical program Sigma Stat (V.2.03, SPSS Inc, USA 1998) was used for all statistical procedures.

RESULTS

Serum 25-OHD deficiency

Demographic characteristics of the patients with normal serum 25-OHD and those with serum 25-OHD deficiency are given in Table 1. Of the 242 consecutive patients assessed at baseline, 19 (8%) were found to have a serum 25-OHD of less than 25 nmol/L. Of those patients with 25-OHD deficiency, 11 (57.9%) were osteopenic, one (5.3%) was osteoporotic and seven (36.8%) had normal BMD. These BMD results did not differ significantly from the prevalence of low BMD in the non-25-OHD deficient Crohn's disease cohort (48.6% osteopenia and 14.2% osteoporosis, P>0.05). In the 25-OHD deficient patients, decreased bone mineral density was most pronounced at the femoral neck and lumbar spine (47.4% and 42.1%, respectively). This distribution of decreased bone mineral density did not differ from that of patients with normal levels of 25-OHD (51.9% femoral neck and 46.1% lumbar spine, P>0.05). Age at baseline, disease duration, BMI, corticosteroid use, and disease location did not differ between the deficient and normal 25-OHD group. There were a greater percentage of smokers in the 25-OHD deficient group than in the normal group (62.5% and 35.6%, respectively); however, this difference did not show statistical significance ($\chi^2=5.929$, P=0.115).

TABLE 2
Biochemical and bone mineral density characteristics in Crohn's disease patients with normal and deficient serum levels of 25-hydroxyvitamin D₃ (25-OHD)

Biochemical characteristics	Normal 25-OHD (n=219)	Deficient 25-OHD (n=19)
Alkaline phosphatase (U/L)	83.8±31.9	108.9±52.8*
Phosphate (mmol/L)	1.1±0.2	1.1±0.2
Calcium (mmol/L)	2.3±0.1	2.2±0.1
Parathyroid hormone (pmol/L)	3.5±1.8	5.0±3.1*
Albumin (g/L)	40.1±4.0	37.4±4.9*
Carotene (μmol/L)	1.9±0.9	1.2±0.7*
RBC Folate (nmol/L)	970.7±361.3	916.8±381.8
N-telopeptide (nmol/mmol creatinine)	46.3±40.0	53.6±29.8
T Score		
L1-L4 T-score	-0.84±1.16	-0.67±1.09
Femoral neck T-score	-0.90±1.10	-0.73±1.14
Total hip T-score	-0.42±1.07	-0.49±1.08
UD radius T-score	-1.08±1.04	-1.28±0.94

Values are reported as mean ± SD. 25-OHD deficiency is defined as a serum 25-OHD concentration below 25 nmol/L. *Denotes statistically significant difference relative to normal 25-OHD group (P<0.05, CI=95%); L1-L4 Lumbar vertebra 1 to 4; RBC Red blood cell; UD Ultradistal.

Serum 25-OHD insufficiency

Vitamin D insufficiency has been defined conservatively as a serum 25-OHD level below 40 nmol/L. Long term exposure to 25-OHD at this level may also be of clinical importance, although the effect on BMD has not been determined. Fifty-two patients (22%) demonstrated serum 25-OHD insufficiency (levels below 40 nmol/L).

Biochemical parameters

The biochemical characteristics of the 25-OHD deficient and normal 25-OHD groups are compared in Table 2. Serum alkaline phosphatase levels were higher in the 25-OHD deficient group than in the normal 25-OHD group (108.9±52.8 U/L versus 83.8±31.9 U/L, respectively, P=0.015), as was serum PTH (5.0±3.1 pmol/L versus 3.5±1.8 pmol/L, respectively, P=0.012). In contrast to this evidence of metabolic bone disease, no significant differences in urine N-telopeptide levels, a measure of bone resorption, were demonstrable between the two groups (Table 2). Serum albumin was lower in the 25-OHD-deficient group compared with the normal 25-OHD group (37.4±4.9 g/L versus 40.1±4.0 g/L, respectively, P=0.006), as was serum carotene (1.2±0.7 μmol/L versus 1.9±0.9 μmol/L, respectively, P<0.001). No other biochemical parameter differed significantly between the two groups.

Biochemical characteristics for the 25-OHD-insufficient group were similar to those in the 25-OHD-deficient group (Table 3).

BMD

The mean T-scores at the lumbar spine, femoral neck, total hip and ultradistal radius for the normal 25-OHD and 25-OHD-deficient groups is shown in Table 2. No significant differences in the T-scores at any of these skeletal sites were found. In the 25-OHD-deficient group, age at diagnosis was the only variable to correlate, negatively, with low T-scores, and only at the lumbar spine and femoral neck (-0.587 and -0.625, respectively, P<0.05) (Figure 1). Furthermore, when

TABLE 3
Biochemical and bone mineral density characteristics in Crohn's disease patients with normal and insufficient serum levels of 25-hydroxyvitamin D₃ (25-OHD) (25-OHD levels of less than 40 nmol/L)

Characteristics	Normal 25-OHD (n=190)	Deficient 25-OHD (n=25)
Alkaline phosphatase (U/L)	82.9±31.0	94.1±41.0*
Phosphate (mmol/L)	1.1±0.2	1.1±0.2
Calcium (mmol/L)	2.3±0.1	2.3±0.1
Parathyroid hormone (pmol/L)	3.3±1.7	4.6±2.4*
N-telopeptides (nmol/mmol creatinine)	43.8±28.4	48.4±26.2

Values are reported as mean ± SD. 25-OHD insufficiency is defined as a serum 25-OHD concentration below 40 nmol/L. *Denotes statistically significant difference relative to normal 25-OHD group ($P<0.05$, CI=95%).

regression analysis was performed, only age at diagnosis was predictive of low T-scores at these two sites ($r=0.605$ and $r=0.666$, respectively, $P<0.05$). Similar results were seen in the 25-OHD-insufficient group (data not shown).

Determinants of 25-OHD deficiency

Pearson correlation was carried out between serum 25-OHD and clinical characteristics in the Crohn's disease population. Carotene and RBC folate both positively correlated with serum 25-OHD ($r=0.250$ and $r=0.349$, respectively, $P<0.05$) in the male population. In addition to the positive correlation with carotene and RBC folate (0.292, and 0.184, respectively, $P<0.05$) in the female Crohn's disease population, smoking negatively correlated with serum 25-OHD ($r=-0.258$, $P<0.05$), and serum creatinine and iron positively correlated with serum 25-OHD ($r=0.186$, 0.226, respectively, $P<0.05$).

When forward regression analysis was performed, only RBC folate predicted serum 25-OHD ($r=0.325$, $P<0.05$) in the male Crohn's disease population. In the female Crohn's disease population, smoking, RBC folate and serum iron predicted serum 25-OHD ($r=0.418$, $P<0.05$). PTH was excluded from this analysis because of its known relationship to vitamin D.

When serum levels of 25-OHD were compared in smokers and nonsmokers with Crohn's disease, 25-OHD was significantly lower in smokers (49.4±21.6 versus 58.3±23.3, respectively, $P<0.005$).

Finally, only two of the 19 patients with low serum 25-OHD had elevated serum PTH levels above the upper limit of normal (greater than 6.8 pmol/L) at baseline. Both patients reported being smokers and one of these patients had a BMI of 17.6 kg/m². In both cases, serum alkaline phosphatase levels were within normal range (30 to 130 IU/L).

Seasonal variation of 25-OHD levels

Table 4 shows the number of patients with deficient and normal 25-OHD levels, relative to the seasonal time of measurement (ie, winter, October to March, and summer, April to September). Sixteen (84.2%) of the 19 patients with deficient 25-OHD levels were identified during the winter. When the frequency of 25-OHD deficiency in patients during summer and winter periods was compared, patients in the winter period exhibited a significantly higher frequency of vitamin D deficiency than did patients in the summer period (11.9% versus 2.8%, respectively, $P=0.02$).

DISCUSSION

This study, comprising 242 patients, demonstrates that 8% of adult patients with Crohn's disease exhibit deficient serum levels of 25-OHD. This finding is highly significant, considering that deficient levels of 25-OHD are almost never reported in the healthy North American population (24,25). Nevertheless, the frequency of 25-OHD deficiency in our cohort is lower than the previously reported range of 17% to 68% (3,8,12,15-20). This difference may be explained by the fact that some of the previous studies used heterogeneous inflammatory bowel disease populations, others focused on small subsets of Crohn's patients with small bowel involvement, and most were characterized by small study populations. Furthermore, global variation in exposure to sunlight and dietary intake and sources of vitamin D could account for the difference in the reported incidence of serum 25-OHD deficiency between this current study and others that have been reported from Norway (19), Finland (15), Austria (12,17), Denmark (20), Netherlands (3), Germany (8), Wales (18) and Chicago, USA (16). Edmonton, the site of the present research, receives approximately 728 h of sunlight between October and March, and approximately 1577 h of sunlight between April and September, a variation sufficient to alter serum 25-OHD levels between seasons (33).

Many experts in the area of vitamin D research also examine the issue of 25-OHD insufficiency, defined conservatively as a serum 25-OHD below 40 nmol/L. In our study, 52 patients (22%) with Crohn's disease demonstrated serum 25-OHD insufficiency (levels below 40 nmol/L) at the time of analysis. Similar to the 25-OHD-deficient group, the 25-OHD-insufficient group demonstrated no significant differences in T-scores at the lumbar spine, femoral neck, total hip or ultradistal radius, and continued to demonstrate elevated levels of alkaline phosphatase and PTH, but not N-telopeptide (Table 3). Nevertheless, patients with 25-OHD below 25 nmol/L demonstrated even higher levels of PTH and alkaline phosphatase.

It is conceivable that our results represent an under-reporting of the incidence of 25-OHD-deficiency. Our study observed that the majority of patients exhibiting deficient serum levels had their 25-OHD levels assessed during the winter months (84.1%), yet the total distribution of measurements was equal between the winter and summer months. We would have expected a higher incidence of serum 25-OHD deficiency if all measurements were carried out within a specified time period during the winter months. In fact, when only those patients who had been assessed for vitamin D status during the winter months were considered, almost 12% demonstrated 25-OHD deficiency. Our results further demonstrated that there was a four-fold higher probability of 25-OHD deficiency if patients were assessed in the winter months, rather than in the summer. This would concur with the results of Vogelsang et al (12), who observed that 25-OHD levels in Crohn's disease patients were higher in the summer than in the winter and that of the patients with low 25-OHD levels in the winter, less than half continued to exhibit low levels in the summer.

Our study confirms previous reports that serum levels of 25-OHD do not correlate with, or predict, bone mineral status in patients with Crohn's disease (1,3-7,15,19). Furthermore, while 63% of patients with deficient serum 25-OHD exhibited low BMD, this frequency did not differ from the study population with normal levels of 25-OHD (Table 2). This has led to the conclusion that factors other than 25-OHD insufficiency

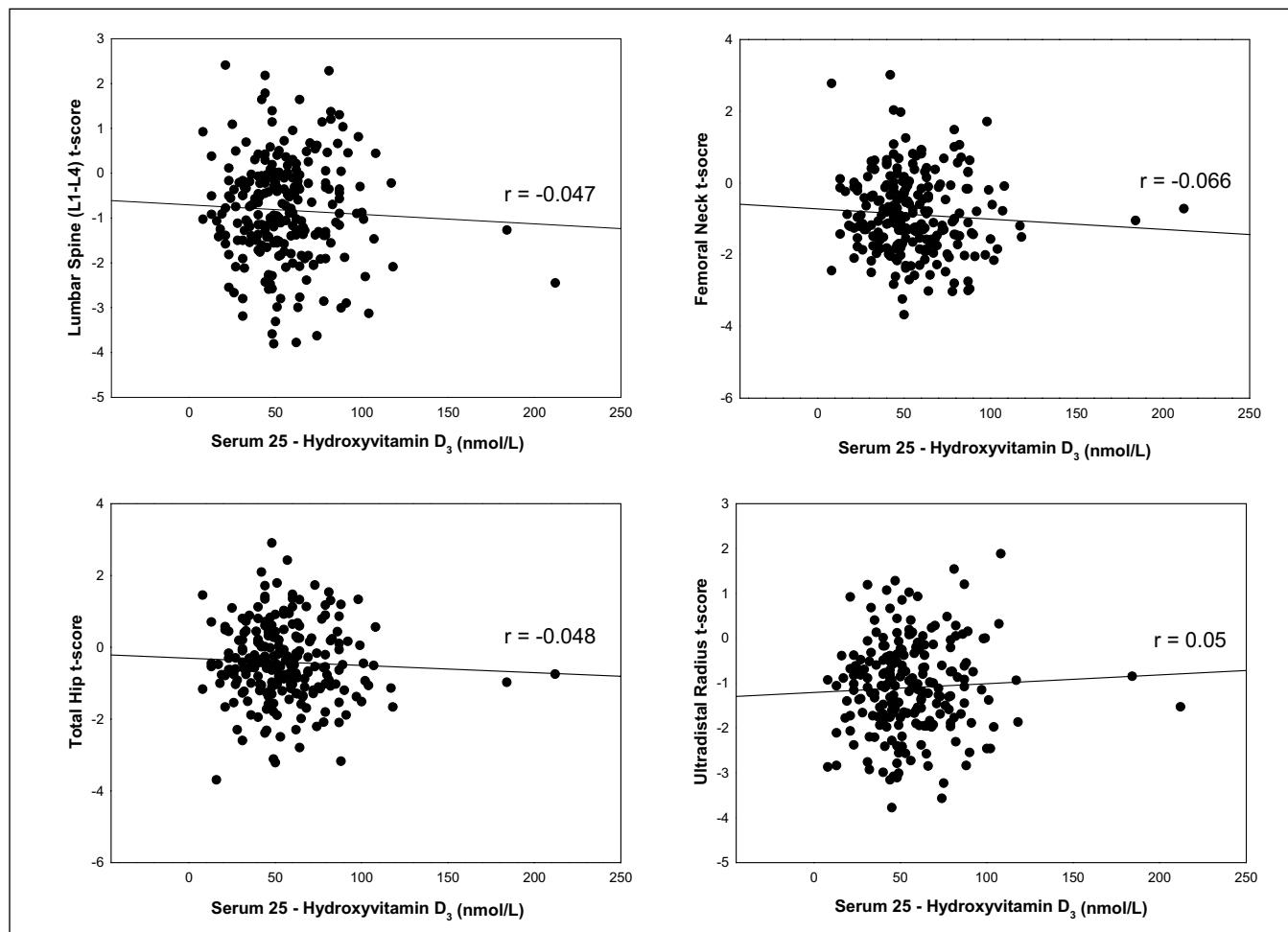


Figure 1) Relationship of serum 25-hydroxyvitamin D₃ (25-OHD) to T-scores at the lumbar spine, femoral neck, total hip and ultradistal radius in 242 patients with Crohn's disease. Bone mineral density was assessed by dual energy x-ray absorptiometry. *r-values represent Pearson correlation coefficients between 25-OHD and the different skeletal sites assessed. No statistically significant relationship was demonstrated between 25-OHD and T-scores at any skeletal site assessed ($P>0.05$)

may be primarily responsible for the increased incidence of osteopenia and osteoporosis in patients with Crohn's disease. Nevertheless, it does appear that a deficiency in 25-OHD did have adverse effects on bone metabolism. Patients with low serum 25-OHD experienced a higher mean alkaline phosphatase and serum PTH, when compared to the patients with normal 25-OHD levels. Similar findings have been previously reported in other studies (8,19). It has been recognized that secondary hyperparathyroidism, as a consequence of 25-OHD deficiency, along with an increase in bone formation rate mark the beginnings of 25-OHD deficiency-induced metabolic bone disease, which is often asymptomatic and subclinical (16,34-36). Furthermore, a long term borderline 25-OHD serum level will contribute to a nutritional deficiency disorder resulting in osteoporosis (35,36).

25-OHD deficiency may occur in patients with Crohn's disease for a number of reasons, including low sunlight exposure, malabsorption and reduced vitamin D intake. In this study, very few patients demonstrated low 25-OHD in the summer months. Indeed, only three of the 108 patients who were assessed between April and September exhibited low serum 25-OHD, two of them being assessed in the first week of April. This observation is in agreement with the accepted notion that

skin generation of 25-OHD through sunlight exposure is likely a significant source of 25-OHD in patients with Crohn's disease (12,16,18,23). Low serum RBC folate levels was predictive of 25-OHD deficiency in both males and females. Furthermore, patients with low serum 25-OHD exhibited lower albumin and carotene levels than those with normal levels of circulating 25-OHD. These findings imply that nutritional status, possibly related to the dietary intake of foods fortified with 25-OHD, plays an important role in determining 25-OHD deficiency in our patients with Crohn's disease.

Intestinal vitamin D malabsorption did not appear to be a major factor in determining 25-OHD status in our patients, as disease location did not influence serum 25-OHD levels. This is likely explained by the fact that the site of vitamin D absorption, the proximal jejunum, is not frequently involved in Crohn's disease (27). Only two out of 242 patients in our study reported jejunal disease involvement. In addition, enterohepatic interruption, an important aspect of vitamin D absorption, remained intact since vitamin B₁₂, a measure of ileal dysfunction, did not appear to be related to, or influence, serum levels of 25-OHD.

Interestingly, our data observed that smoking in females predicted deficient serum levels of 25-OHD. Whether smok-

TABLE 4
The number of patients with deficient and normal 25-hydroxyvitamin D₃ (25-OHD) levels, relative to the seasonal time of measurement (ie, winter, October to March and summer, April to September)

Patient group	Summer n (%)	Winter n (%)
Normal serum 25-OHD levels (n=218)	105 (47.1)	118 (52.9)
Deficient serum 25-OHD levels (n=19)	3 (15.8)	16 (84.2)
Percentage 25-OHD deficiency	(2.8)	(11.9)*

25-OHD deficiency is defined as a serum 25-OHD concentration below 25 nmol/L. *P=0.02 relative to summer months

ing has a 25-OHD-lowering effect in females has never been previously reported in patients with Crohn's disease, but a few mechanisms are proposed. One study has suggested women who smoke are thinner than their nonsmoking counterparts (37), yet we did not find any relationship between BMI and 25-OHD in the present study. Smoking has also

REFERENCES

- Habtezion A, Silverberg MS, Parkes R, Mikolainis S, Steinhart AH. Risk factors for low bone density in Crohn's disease. *Inflamm Bowel Dis* 2002;8:87-92.
- Dinca M, Fries W, Luisetto G, et al. Evolution of osteopenia in inflammatory bowel disease. *Am J Gastroenterol* 1999;94:1292-7.
- Schoon EJ, Geerling BJ, Van Dooren IMA, et al. Abnormal bone turnover in long-standing Crohn's disease in remission. *Aliment Pharmacol Ther* 2001;15:783-92.
- Ghosh S, Cowen S, Hannan J, Ferguson A. Low bone mineral density in Crohn's disease, but not in Ulcerative Colitis, at diagnosis. *Gastroenterology* 1994;107:1031-9.
- Compston JE, Judd D, Crawley EO, et al. Osteoporosis in patients with inflammatory bowel disease. *Gut* 1987;28:410-5.
- Dressner-Pollak R, Karmeli F, Eliakim R, et al. Femoral neck osteopenia in patients with inflammatory bowel disease. *Am J Gastroenterol* 1998;93:1483-90.
- Martin JP, Tonge KA, Bhonsle U, Jacyna MR, Levi J. Bone mineral density in patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 1999;11:537-41.
- Bischoff S, Herrmann A, Goke M, et al. Altered bone metabolism in inflammatory bowel disease. *Am J Gastroenterol* 1997;92:1157-63.
- Silvennoinen J, Lehtola J, Niemela S. Smoking is a risk factor for osteoporosis in women with inflammatory bowel disease. *Scand J Gastroenterol* 1996;31:367-71.
- Sylvester FA, Wyzga N, Hyams JS, Gronowicz GA. Effect of Crohn's disease on bone metabolism in vitro: A role for Interleukin 6. *J Bone Miner Res* 2002;17:695-702.
- Robinson RJ, Iqbal SJ, Al-Azzawi F, Abrams K, Mayberry JF. Sex hormone status and bone metabolism in men with Crohn's disease. *Aliment Pharmacol Ther* 1998;12:21-5.
- Vogelsang H, Ferenci P, Woloszczuk W, et al. Bone disease in vitamin D deficient patients with Crohn's disease. *Dig Dis Sci* 1989;34:1094-9.
- Ardizzone S, Bollani S, Bettica P, et al. Altered bone metabolism in inflammatory bowel disease: There is a difference between Crohn's disease and ulcerative colitis. *J Intern Med* 2000;247:63-70.
- Siffledeen J, Fedorak RN, Siminoski K, et al. Bones and Crohn's: Risk factors associated with low bone mineral density in patients with Crohn's disease. *Inflammatory Bowel Disease*, 2002. (In press).
- Silvennoinen J. Relationships between vitamin D, parathyroid hormone, and bone mineral density in inflammatory bowel disease. *J Intern Med* 1996;239:131-7.
- Driscoll RH, Meredith SC, Sitrin M, Rosenberg IH. Vitamin D deficiency and bone disease in patients with Crohn's disease. *Gastroenterology* 1982;83:1252-8.
- Vogelsang H, Schofl R, Tillinger W, Ferenci P, Gangl A. 25-Hydroxyvitamin D absorption in patients with Crohn's disease and with pancreatic insufficiency. *Wien Klin Wochenschr* 1997;109:678-82.
- Harries AD, Brown R, Heatley RV, Williams LA, Woodhead S. Vitamin D status in Crohn's disease: Association with nutrition and disease activity. *Gut* 1985;26:1197-203.
- Jahnsen J, Falch JA, Mowinckel P, Aadland E. Vitamin D status, parathyroid hormone, and bone mineral density in patients with inflammatory bowel disease. *Scand J Gastroenterol* 2002;37:192-9.
- Andreassen H, Rix M, Brot C, Eskildsen P. Regulators of calcium homeostasis and bone mineral density in patients with Crohn's disease. *Scand J Gastroenterol* 1998;33:1087-93.
- Vogelsang H, Ferenci P, Resch H, Kiss A, Gangl A. Prevention of bone mineral loss in patients with Crohn's disease by long-term oral vitamin D supplementation. *Eur J Gastroenterol Hepatol* 1995;7:609-14.
- McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. *Am J Med* 1992;93:69-77.
- Buchman A. Bones and Crohn's: Problems and solutions. *Inflamm Bowel Dis* 1999;5:212-27.
- Styrd RP, Gilbertson TJ, Brunden MN. A seasonal variation study of 25-hydroxyvitamin D₃ serum levels in normal humans. *J Clin Endocrinol Metab* 1979;48:771-5.
- Chesney RW, Rosen JF, Hamstra AJ, et al. Absence of seasonal variation in serum concentrations of 1,25-dihydroxyvitamin D despite a rise in dihydroxyvitamin D in the summer. *J Clin Endocrinol Metab* 1981;95:139-42.
- Veith R, Cole DE, Hawker GA, Trang HM, Rubin LA. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. *Eur J Clin Nutr* 2001;55:1091-7.
- Hollander MD. Mechanism and site of small intestinal uptake of vitamin D in pharmacological concentrations. *Am J Clin Nutr* 1976;29:970-5.
- Looker AC, Orwoll ES, Johnston CC, et al. Prevalence of low femoral bone density in older US adults from NHANES III. *J Bone Miner Res* 1997;12:1761-8.
- WHO Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organization Technical Report Series* 1994;843:1-129.
- Rosalki SB. C-reactive protein. *Int J Clin Pract* 2001;55:269-70.
- Kapsoritakis A, Koukourakis MI, Sfiridakis A, et al. Mean platelet volume: A useful marker of inflammatory bowel disease activity. *Am J Gastroenterol* 2001;96:776-81.
- Dressner-Pollak R, Karmeli F, Eliakim R, Ackerman Z, Rachmilewitz D. Increased urinary N-telopeptide cross-linked type 1 collagen predicts bone loss in patients with inflammatory bowel disease. *Am J Gastroenterol* 2000;95:699-704.
- Environment Canada, Government of Canada. Climate Station Information. <www.msc-smc.ec.gc.ca/climate/station_catalogue/index_e.cfm>. (Version current at June 24, 2003)
- Parfitt AM, Rao DS, Stanciu J, et al. Irreversible bone loss in osteomalacia. *J Clin Invest* 1985;76:2403-12.
- Peacock M. Effects of calcium and vitamin D insufficiency on the skeleton. *Osteoporos Int* 1998;8(Suppl 2):S45-51.
- Heaney RP. Vitamin D: How much do we need, and how much is too much? *Osteoporos Int* 2000;11:553-5.
- Jensen GF. Osteoporosis of the slender smoker revisited by epidemiologic approach. *Eur J Clin Invest* 1986;16:239-42.
- MacMahon B, Trichopoulos D, Cole P, Brown J. Cigarette smoking and urinary estrogens. *N Engl J Med* 1982;307:1062-5.
- Heikkinen A, Parviaainen MT, Tuppurainen MT, et al. Effects of postmenopausal hormone replacement therapy with and without vitamin D₃ on circulating levels of 25-hydroxyvitamin D. *Calcif Tissue Int* 1998;62:26-30.

been suggested to decrease estrogen production in females (38), conceivably leading to decreased intestinal uptake of vitamin D, decreased renal production of 1,25-OHD, and reduced levels of 25-OHD (39). Further studies are required to elucidate the precise mechanism behind the 25-OHD-reducing effect of cigarette smoke in Crohn's disease patients, and to determine what implications this has for their BMD. This study did not examine the effects of small intestinal surgery, fat malabsorption or dietary intake on 25-OHD levels.

In summary, 25-OHD-deficient Crohn's disease patients exhibit biochemical evidence of metabolic bone disease, although no detectable difference in BMD was observed. Sunlight exposure, nutrition and smoking status were predictors of 25-OHD deficiency in this patient cohort.

ACKNOWLEDGEMENTS: The present study was supported by the Crohn's and Colitis Foundation of Canada, and by Proctor and Gamble Pharmaceuticals, Canada.



The Hindawi logo consists of two interlocking circles, one blue and one green, forming a stylized infinity or double helix symbol.

Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

