J Gastroenterol (2010) 45:399–405
DOI 10.1007/s00535-009-0165-0

ORIGINAL ARTICLE—ALIMENTARY TRACT

Effect of exclusive enteral nutrition on bone turnover in children with Crohn's disease

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Received: 5 August 2009 / Accepted: 8 November 2009 / Published online: 3 December 2009

Abstract
Background Poor bone acquisition and increased fracture risk are significant complications associated with Crohn's disease (CD). The aim of this study was to determine the effects of 8 weeks of exclusive enteral nutrition (EEN) therapy upon markers of bone turnover in children with newly diagnosed CD.
Methods Twenty-three children with newly diagnosed CD and 20 controls (without CD) were enrolled. Children with CD were treated with 8 weeks of EEN. Inflammatory markers [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, platelets], nutritional markers (height, weight), and bone markers [C-terminal telopeptides of Type-1 collagen (CTX) and bone specific alkaline phosphatase (BAP)] were measured prior to and following therapy.
Results At diagnosis, children with CD had elevated serum CTX (2.967 ± 0.881 ng/ml) compared to controls (2.059 ± 0.568 ng/ml; $P = 0.0003$). Following the period of EEN, CTX levels fell significantly (2.260 ± 0.547 ng/ml; $P = 0.002$), while serum BAP levels (51.24 ± 31.31 μg/L at diagnosis; control serum BAP = 66.80 ± 23.23 μg/L; $P = 0.07$) increased significantly (64.82 ± 30.51 μg/L; $P = 0.02$), with both normalizing to control levels.
Conclusions As well as reducing inflammation, decreasing disease activity, and improving nutrition in children with newly diagnosed CD, EEN therapy also normalized serum markers of bone turnover, suggesting an improvement in bone health. Further investigations of short- and long-term effects of EEN on bone density and overall bone health are now required.

Keywords Crohn's disease · Children · Bone turnover · Exclusive enteral nutrition

This work was presented in part at the ESPGHAN Annual Meeting, Dresden, Germany (June 2006) and at Australian Gastroenterology Week, Perth, Australia (October 2006).

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Introduction

Crohn's disease (CD), a chronic inflammatory disorder of the gastrointestinal tract, may also impact upon a number of extraintestinal sites, including bone. Reduced bone mineral density (BMD) has been reported in more than 40% of adult patients with inflammatory bowel disease (IBD) in cross-sectional studies [1–3] and is more prevalent in CD than in ulcerative colitis (UC) [1, 4–8]. These findings also extend to children with IBD, with several studies demonstrating reduced bone mass in comparison to healthy children [4, 7, 9–12].

Various factors may contribute to bone loss in CD. These include malnutrition, malabsorption, vitamin D and K deficiency, corticosteroid therapy, and the inflammatory process itself. For instance, interleukin (IL)-6, a proinflammatory cytokine, is released by inflammatory cells, stimulates osteoclast activity, [13] and is hypothesized to be a mediator of bone loss [14]. Although attention has focused upon the effects of corticosteroid therapy on BMD, most studies report only a weak relationship [1, 4, 8, 15], while others fail to observe a relationship [14, 16, 17]. A recent study found 56% of steroid-naïve children newly diagnosed with CD had BMD *Z* scores less than −1 SD at the lumbar spine [18]. These findings suggest that inflammatory mediators released from inflamed bowel may contribute directly to low bone mass in children with CD.

As no known cure for CD exists, the goal of treatment is to achieve and maintain remission. Standard therapies to decrease inflammation include corticosteroids, antibiotics, and more recently, enteral nutrition. Exclusive enteral nutrition (EEN) is the therapy of choice at Sydney Children's Hospital to induce remission, with 80% of newly diagnosed patients entering clinical remission after 8 weeks of therapy [19]. Although only a small number of trials have been conducted in children, there is consensus that enteral feeds and steroids have similar efficacy [20].

Various beneficial effects ascribed to EEN include reduced disease activity scores, histological healing, and downregulation of mucosal proinflammatory cytokines [21]. Nutritional therapy has also been shown to cause less reduction in BMD as compared to patients treated with corticosteroids [22]. Use of EEN also leads to improved nutritional status [23]. Bannerjee et al. [24] showed that improvements in growth-related proteins [such as insulin-like growth factor (IGF)-1] and reduction in IL-6 preceded changes in nutritional parameters (such as weight gain) after just 1 week of EEN, suggesting that the anti-inflammatory effects of EEN lead onto nutritional restitution, rather than the reverse.

Evaluating the effect of a chronic inflammatory disease or a given treatment, such as corticosteroids or EEN, on bone metabolism can involve the assessment of several aspects including bone turnover, BMD, and fracture incidence. Measuring biochemical markers of bone turnover serves as a relatively simple and noninvasive means of assessing bone metabolism. Markers of bone turnover can rapidly reflect the effects of therapy, and further, are independent predictors of the risk of osteoporotic fractures in adults [25].

The aim of this study was to investigate the effects of EEN upon markers of bone turnover in children with active CD and to further advance our understanding of the complex interactions between gut inflammation and bone metabolism.

Methods

Patients

Children were enrolled prospectively at the time of diagnosis with CD at Sydney Children's Hospital, Randwick. The included children were aged between 12 months and 18 years and were diagnosed with CD according to standard radiological, histological, and endoscopic criteria. Exclusion criteria were previous diagnosis of CD or UC, severe colitis requiring intensive medical or surgical management, and use of antibiotics or anti-inflammatory agents in the previous 4 weeks. A non-IBD control group was also enrolled, consisting of children presenting with gastrointestinal complaints, but without organic intestinal disease based on standard radiological, histological, and endoscopic findings.

Patients were weighed wearing light clothing on the same calibrated digital scales to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using the same fixed, calibrated stadiometer. Age and sex-specific SD scores (*Z* scores) for weight and height were calculated using the National Center for Health Statistics 2000 growth data in Epi Info (Centers for Disease Control and Prevention, Atlanta GA, USA). Body mass index (BMI) [26] and BMI *Z* scores were calculated using available weight and height measurements for 22 patients aged 2–18 years.

Basic demographic data, details of presenting features, and results of standard markers of inflammation [C-reactive protein (CRP), albumin, erythrocyte sedimentation rate (ESR), and platelets] were recorded and stored in a dedicated database. Disease location was defined according to the Montreal classification [27] and symptom duration prior to diagnosis was recorded for each patient with CD.

Management of CD and sample collection

All the children diagnosed with CD were managed with 8 weeks of EEN as sole therapy to induce remission. EEN was provided as a 1 kcal per mL polymeric formula (Osmolite; Abbott Australasia, Sydney, NSW, Australia). In addition to Osmolite, patients were able to drink water and chew sugarless gum only, as per standard protocol [19]. Formula volume was prescribed according to estimated energy requirements, calculated using the Schofield equation [28] and ideal body weight for height as per standard protocol [19]. Formula volumes were modified throughout the 8-week period based on adequacy of weight gains. Regular outpatient clinical review was undertaken, along with close liaison by phone throughout the period of EEN.

Serum separated from peripheral blood samples was collected from each subject prior to EEN (baseline) and at

completion of EEN immediately prior to recommencement of normal diet (post EEN) and then stored at −80°C until assayed. A single serum sample was collected from all control subjects.

Informed consent was obtained from each patient or their caregiver(s). The South Eastern Sydney and Illawarra Area Health Service Research Ethics Committee approved the study.

Monitoring of disease activity and measurement of serum bone markers

The Pediatric Crohn's Disease Activity Index (PCDAI) [29] was calculated using clinical and laboratory markers (ESR, albumin, and hematocrit) to assess disease activity in each patient at diagnosis and at the completion of EEN. Remission was defined as PCDAI less than 15.

The following proteins were measured in stored serum by solid phase immunoassays, using the protocol supplied by the manufacturers: degradation products of C-terminal telopeptides of Type-1 collagen (CTX; Nordic Biosciences Diagnostics, Herlev, Denmark) and bone specific alkaline phosphatase (BAP; Immunodiagnostics Systems, Tyne & Wear, UK). The CTX and BAP assays had detection limits of 0.020 ng/ml and 0.7 μg/L, respectively. In addition to defining the levels of these proteins at the two time points for each patient, the change in these markers was also defined (Delta or Δ).

Statistics

All graphs and statistics were generated using Prism 4.0 for Windows (GraphPad Software, San Diego, CA, USA). The χ^2 test was utilized to compare gender between the two groups. All other comparisons between the CD and control groups were by *t* tests, and comparison between baseline and post EEN in the CD group was by paired *t* tests. Spearman's correlation was used for all correlations. Results are presented as mean (SD). Significance was accepted at $P < 0.05$.

Results

Patient demographics and inflammatory markers

Twenty-three children with CD and 20 controls were enrolled in the study. There were no differences between the CD and control groups in terms of age and gender (Table 1). At diagnosis of CD, 13 children had mild disease (all with PCDAI < 29), while ten children had moderate to severe disease (PCDAI ≥ 30). The mean PCDAI at diagnosis was 28.1 (±13.2; Table 2). The mean length of symptoms prior to diagnosis of CD was 47 weeks (SD 43 weeks). All children with CD had upper gastrointestinal involvement (L4), one child had L4 disease only, one child had terminal ileal disease only (L1), seven children had colonic disease (L2), and fourteen children had ileocolonic disease (L3). Further, perianal disease was present at diagnosis in nine (39%) of the 23 children.

Table 1 Demographics of patients with Crohn's disease and non-IBD controls

	CD ($n = 23$)	Non-IBD controls ($n = 20$)
Age, years, mean (SD)	10.72 (3.77)	8.64 (3.23)
Gender (% male)	17 male (74%)	13 males (65%)

The disease group and controls did not differ in terms of age and gender ($P > 0.05$ for both)

IBD inflammatory bowel disease

Table 2 Growth and inflammatory marker data from children with Crohn's disease at diagnosis and following 8 weeks of exclusive enteral nutrition (EEN)

	N	Pre EEN treatment	Post EEN treatment	*P*
Weight	23	35.04 (13.92)	37.42 (14.04)	<0.0001
Weight *Z* score	23	−0.672 (1.275)	−0.311 (1.107)	<0.0001
Height	23	1.395 (0.240)	1.404 (0.234)	0.005
Height *Z* score	23	−0.485 (1.054)	−0.448 (1.027)	0.34
PCDAI	23	28.1 (13.2)	10.0 (6.1)	<0.0001
BMI *Z* score	22	−0.687 (1.334)	−0.110 (1.073)	<0.0001
CRP	23	25.9 (55.8)	5.8 (7.0)	0.08
ESR	23	28.5 (20.7)	19.8 (13.4)	0.032
Albumin	23	31.0 (7.3)	35.8 (3.4)	0.002
Platelets	22	443 (116)	356 (95)	<0.0001

Results are presented as mean (SD). Analysis by paired *t* test

PCDAI Pediatric Crohn's Disease Activity Index, *BMI* body mass index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate

Following 8 weeks of EEN therapy, disease activity scores were decreased, with mean PCDAI scores falling to 10 (±6.1); $P < 0.0001$ (Table 2). Sixteen (70%) of the 23 patients entered remission (PCDAI < 15) and the remaining seven patients had PCDAI scores between 15 and 20 at the completion of EEN. Mean weight, albumin, and height, as well as weight *Z* scores and BMI *Z* scores increased significantly over the 8-week period of EEN (Table 2). ESR and platelet levels fell significantly, while CRP levels fell, but this change did not reach significance (Table 2).

Serum markers of bone metabolism

CTX was elevated in children with CD at diagnosis (2.967 ± 0.881 ng/ml) compared to controls (2.059 ± 0.568 ng/ml;

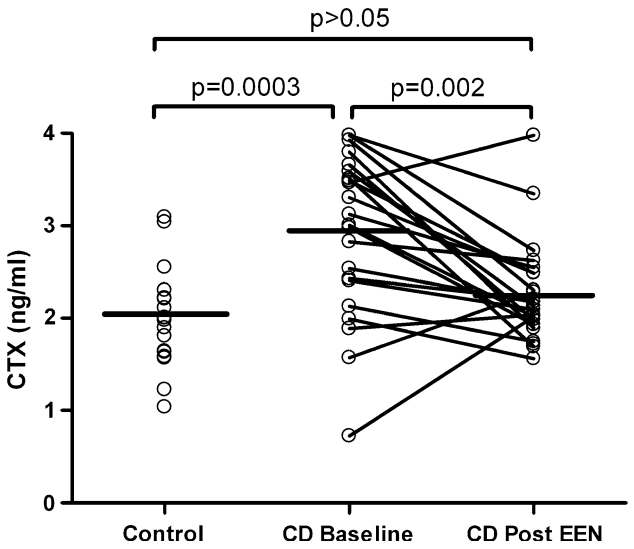

Fig. 1 Serum C-terminal telopeptides of Type-1 collagen (CTX) levels in children with Crohn's disease (CD) and non-inflammatory bowel disease (IBD) controls. CTX was elevated in children with CD at diagnosis (2.967 ± 0.881 ng/ml; $P = 0.0003$) compared to controls (2.059 ± 0.568 ng/ml) and fell to be equivalent to controls following 8 weeks of exclusive enteral nutrition (EEN) treatment (2.260 ± 0.547 ng/ml; $P = 0.002$). *Horizontal line* indicates the mean

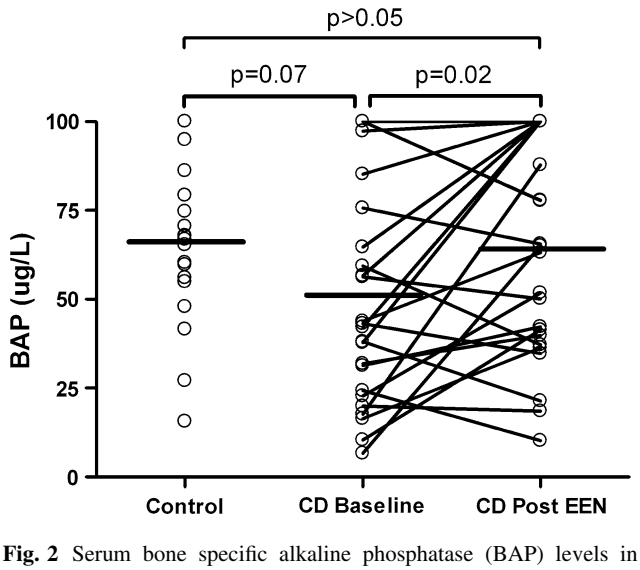

Fig. 2 Serum bone specific alkaline phosphatase (BAP) levels in children with CD and non-IBD controls. BAP was low but not significantly different in children with CD at diagnosis (51.24 ± 31.31 μg/L; $P = 0.07$) compared to controls (66.80 ± 23.23 μg/L), but increased significantly with 8 weeks of EEN treatment (64.82 ± 30.51 μg/L; $P = 0.02$). *Horizontal line* indicates the mean

$P = 0.0003$; Fig. 1). CTX levels fell significantly in children with CD after 8 weeks of EEN (2.260 ± 0.547 ng/ml; $P = 0.002$; Fig. 1), to reach levels similar to those in control patients.

Serum BAP levels were low at diagnosis of CD (51.24 ± 31.31 μg/L) and approached a significant difference from control levels (66.80 ± 23.23 μg/L; $P = 0.07$; Fig. 2). BAP levels increased significantly (64.82 ± 30.51 μg/L; $P = 0.02$) with EEN, to be equivalent to control levels post treatment (Fig. 2).

Correlation of serum bone markers with disease factors

Serum BAP correlated with a number of inflammatory markers. There were significant correlations between BAP and albumin ($R = 0.418$; $P = 0.006$), CRP ($R = -0.55$; $P = 0.0001$), platelets ($R = -0.533$; $P = 0.0005$), and PCDAI ($R = -0.459$; $P = 0.002$), but not with ESR or any growth parameter. CTX did not correlate with any serum inflammatory marker or growth parameter (data not shown).

The change in weight *Z* scores (Δweight *Z*), change in BMI *Z* scores (ΔBMI *Z*), and change in PCDAI (ΔPCDAI) over the period of EEN was compared to the change in CTX (ΔCTX) and BAP (ΔBAP) over the same period. There was a close correlation between Δweight *Z* and ΔBAP ($R = 0.7645$; $P < 0.0001$; Fig. 3), but not with ΔCTX. Not unexpectedly, ΔBMI *Z* also correlated with

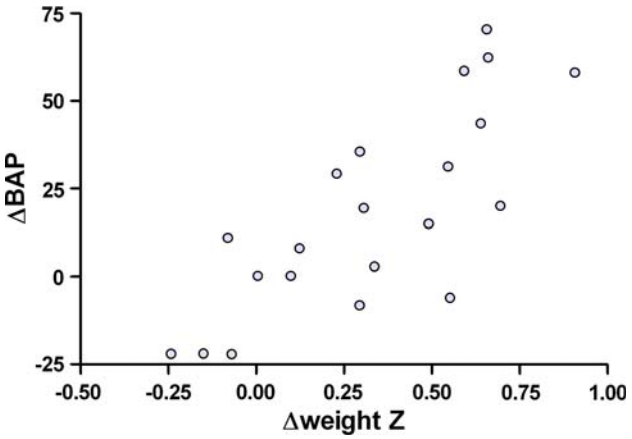

Fig. 3 Correlation between change in weight *Z* score and change in serum BAP with EEN treatment. The change in weight *Z* score in children with CD significantly correlated ($R = 0.7645$; $P < 0.0001$) with the change in serum BAP levels over the period of EEN treatment

ΔBAP ($R = 0.6388$; $P = 0.0024$) and not with ΔCTX. ΔPCDAI did not correlate with ΔCTX or ΔBAP (data not shown). However, a significant correlation with a negative slope was observed between ΔBMI *Z* and ΔPCDAI ($R = -0.4980$; $P = 0.0183$; Fig. 4), indicating that an increase in the BMI *Z* score is associated with a reduction in the PCDAI.

We also investigated whether symptom duration, entering remission at week 8, presence of perianal disease, or disease location influenced the change in serum bone markers (ΔCTX and ΔBAP) over the course of EEN. There

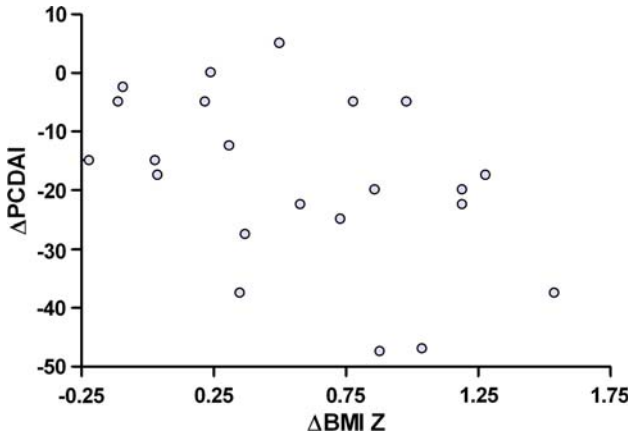

Fig. 4 Correlation between change in body mass index (BMI) *Z* score and change in Pediatric Crohn's Disease Activity Index (PCDAI) with EEN treatment. The change in BMI *Z* score in children with CD significantly correlated ($R = -0.4980$; $P = 0.0183$) with the change in PCDAI over the period of EEN treatment

was no correlation between symptom duration and the growth parameters or ΔCTX; however, the correlation between symptom duration and ΔBAP approached significance ($R = -0.457$; $P = 0.065$). Neither ΔCTX nor ΔBAP were affected by remission, presence of perianal disease, or disease location (data not shown).

Discussion

The present study has shown that an 8-week course of EEN as the sole treatment for children with active CD resulted in a significant and rapid normalization in markers of bone turnover. At diagnosis, children with CD had increased bone resorption and lower bone formation markers compared with controls. After 8 weeks of therapy these changes in bone turnover were reversed to control levels.

Adult studies have also shown increased bone resorption in IBD [30–32]. However, in contrast to our findings, two pediatric studies found that children with newly diagnosed CD [33] and children with IBD [34] had significantly lower markers of bone resorption relative to healthy controls. One explanation for these contrasting findings may be the age of the populations. In the previous studies of children with CD the children had a mean age at diagnosis of 13 years, whereas the children in the present study had a mean age at diagnosis of 10.7 years. Further, Sylvester et al. [33] reported a large variation in bone turnover markers in their cohort due to pubertal status (defined as Tanner stage). In contrast, variation in pubertal status for most of the children in the present study was likely not relevant, given their younger, prepubertal age. Further, changes in puberty were unlikely to impact upon the results of the present study, due to its short duration of 8 weeks. In our study, although mean height (at −0.485 *Z* score) was significantly decreased at baseline, neither bone formation nor resorption markers correlated with growth.

Recently, a further study evaluated serum and urine markers of bone in 110 children with CD at two time points, 6 months apart [35]. Similar to our findings in children at diagnosis, CD was associated with lower markers of bone formation (BAP) and higher markers of bone resorption [urine deoxypyridinoline/creatinine (DPD)] than seen in 220 control subjects. These authors noted the confounding effects of growth and pubertal status upon interpretation of the biomarkers; however, the study time points were much greater than in the present study, as was the age of the subjects. Once adjusted for confounding variables, however, CD was still associated with lower BAP and greater DPD. This study and previous pediatric studies have not examined the impact of specific therapies upon the markers of bone turnover.

In the present study, markers of both bone resorption and bone formation normalized following treatment. These markers returned to control levels over the 8-week period of EEN, without other specific intervention. This improvement in bone turnover after 8 weeks of EEN therapy is comparable to the findings of Franchimont et al. [36], who used biochemical markers of bone turnover to evaluate the effect of infliximab therapy on bone loss in adult CD. Similar to the present study, the baseline serum concentration of CTX, a marker of bone resorption, was significantly elevated in these adult patients with CD. An increase in bone formation or a decrease in bone resorption was then observed in 59% of patients following infliximab treatment. As infliximab treatment targets inflammation alone, together these findings suggest that the normalization in bone turnover may be specifically due to amelioration of inflammatory processes. Cytokines produced in, and released from, the inflamed mucosa in active CD are also known to enhance bone resorption and subsequent bone loss [37]. Therefore, as EEN has been shown to reduce inflammation, [21, 38, 39] this nutritional therapy may normalize bone turnover through a reduction in inflammation. Certainly, the concurrent fall in inflammatory scores with EEN therapy in the present study supports this supposition, as does the correlation of BAP (but not CTX) with inflammatory markers.

In addition to improvements in bone turnover and reduced inflammation in children with CD, the present study clearly showed improvements in nutritional status with EEN therapy. The nutritional markers of albumin, mean weight, mean height, weight *Z* score, and BMI *Z* score all improved after 8 weeks of therapy. Further, improved nutrition, as measured by BMI *Z* scores, was associated with a greater reduction in disease activity, as

measured by PCDAI. The effect of nutritional, as distinct from anti-inflammatory, therapy on bone turnover has previously been investigated in 19 female adolescent inpatients with anorexia nervosa [40]. These girls had reduced markers of bone formation and elevated markers of bone resorption. After 15 weeks of a supervised eating plan designed to meet caloric requirements, body weight, BMI, lean body mass, and serum BAP significantly increased and serum CTX concentrations significantly decreased. A further pediatric IBD study conducted over a 2-year period also observed clinical improvement and improved nutrition coinciding with improved bone formation markers in children with IBD [34]. These children, however, were treated with a variety of medical therapies and few received EEN. Interestingly, markers of bone resorption remained stable over the 2 years, as did BMD *Z* scores. The study authors commented that the delay in bone mineralization catch up may have been consequent to ongoing mild inflammation [34]. Nevertheless, the results of these studies suggest that improved nutrition, as seen with EEN therapy in the present study, may be a further mechanism of normalization of bone markers.

Bone mineral density was not investigated in the present study. Consequently, we are unable to determine whether the children in the present study had normal or reduced bone mass, or to define the impact of improved markers of bone turnover upon BMD. Previous reports have established reduced BMD in children at diagnosis of CD [34] and during their illness [12, 30]. Normalization of bone turnover may lead to improved BMD over time, but likely only if ongoing inflammation is also controlled. Building upon the data provided in this present study, future work could compare changes in markers of bone turnover in groups treated with EEN and other therapies (such as corticosteroids).

In summary, this study has shown that children at diagnosis of CD have elevated serum markers of bone resorption and a trend to lower markers of bone formation, suggesting an imbalance favoring decreased bone acquisition, or perhaps even bone loss. Further, following 8 weeks of EEN therapy, serum bone markers normalized, suggesting normalization in bone turnover. In addition, EEN therapy was shown to both reduce inflammation and improve nutrition. The combination of these two mechanisms may have resulted in the normalization of serum bone markers. Further studies are now required to establish the effects of EEN therapy on these and other parameters of bone health in CD, over longer periods of time.

Acknowledgments The study was funded in part by the Sydney Children's Hospital Foundation. Osmolite was supplied by Abbott Australasia. Laboratory research was undertaken in the Westfield Research Laboratories.

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