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Adalimumab Therapy Has a Beneficial Effect on Bone Metabolism in Patients with Crohn's Disease.

Sundaram G. Veerappan  
*Royal College of Surgeons in Ireland*

Martin Healy  
*St. James's Hospital, Dublin*

Bernard J. Walsh  
*St. James's Hospital, Dublin*

Colm A. O'Morain  
*Adelaide & Meath Hospital, Dublin*

Jacqueline Daly  
*Royal College of Surgeons in Ireland, jdaly@rcsi.ie*

*See next page for additional authors*

Citation


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TITLE PAGE

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Authors: Sundaram G. Veerappan, Martin Healy, Bernard J. Walsh, Colm A. O Morain, Jacqueline S. Daly, Barbara M. Ryan.

E-mail and Postal address of authors:

Sundaram G. Veerappan; Division of Biology, Department of Anatomy, Royal College of Surgeons in Ireland, Dublin 2, Republic of Ireland & Department of Gastroenterology, Adelaide & Meath Hospital, Tallaght, Dublin 24, Republic of Ireland;

sveerappan78@gmail.com

Martin Healy; Department of Biochemistry, St. James’s Hospital, Dublin 8, Republic of Ireland;

mhealy@stjames.ie

Bernard J. Walsh; Department of Gerontology, St. James’s Hospital, Dublin 8, Republic of Ireland;

jbwalsh@tcd.ie

Colm A. O'Morain; Department of Gastroenterology, Adelaide & Meath Hospital, Tallaght, Dublin 24, Republic of Ireland;

gastroenterology@amnch.ie
Jacqueline S. Daly; Division of Biology, Department of Anatomy, Royal College of Surgeons in Ireland, Dublin 2, Republic of Ireland Republic of Ireland; jdaly@rcsi.ie

Barbara M. Ryan; Department of Gastroenterology, Adelaide & Meath Hospital, Tallaght, Dublin 24, Republic of Ireland; barbara.ryan2@amnch.ie

Corresponding author: Dr. Sundaram G. Veerappan

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ABSTRACT

Background:
Infliximab have been shown to have beneficial effects on bone metabolism in patients with Crohn’s disease (CD) although as yet the exact mechanisms have not been fully elucidated.

Aim:
To evaluate the impact of Adalimumab therapy on bone metabolism using a combined in vivo and in vitro model.

Methods:
Parathyroid hormone, Vitamin D, bone formation markers, bone resorption marker, pro-inflammatory cytokines, anti-inflammatory cytokines, osteoprotegerin and sRANKL were measured in control patients, and pre and post treatment with Adalimumab in CD patients. The effect of control patients’ and pre and post treatment CD patients’ sera on human osteoblasts (hFOB 1.19) in vitro cell viability and differentiation were also analysed.

Results:
There was a significant increase in bone formation markers osteocalcin ($P < 0.05$) and pro-collagen type 1 N propeptide ($P < 0.01$) at 1 and 3 months post treatment. Moreover
there was a sustained but not significant fall in serum CTx, a bone resorption marker. No significant change was seen over time with other parameters measured. Serum from pre-Adalimumab treatment Crohn’s patients increased osteoblast viability compared to post treatment sera at 6 months ($P = 0.002$) and controls. However post-Adalimumab treatment sera at 6 months appeared to increase osteoblast differentiation ($P = 0.001$), which is likely to be important in new bone formation.

**Conclusions:**

This first study evaluating the role of Adalimumab as a possible bone protector in Crohn’s disease patients has shown that similar to infliximab, Adalimumab has complex and potentially beneficial effects on bone metabolism.

**KEYWORDS**

Adalimumab; Crohn’s disease; Osteoporosis; bone metabolism
INTRODUCTION

Osteoporosis in patients with inflammatory bowel disease (IBD) is common and was first reported approximately 30 years ago [1]. As many as 75% and 40% of IBD patients are osteopenic (T-score < -1) [2], and osteoporotic (T-score < -2.5) [3] at the spine and at the femoral neck respectively. In a recent population based cohort study, the relative risk of hip fracture was 1.41 (0.94-2.11) for ulcerative colitis and 1.68 (1.01-2.78) for CD patients [4].

The pathogenesis of reduced bone mineral density (BMD) in IBD is multi-factorial. Factors such as age, gender, oestrogen deficiency, alterations in calcium homeostasis, nutritional and dietary factors, smoking, alcohol and immobility are all likely to play a role, as in the general population. However, current evidence suggests that these effects and associations are relatively weak and are overshadowed by the effect of the IBD itself [5]. Findings that newly diagnosed patients with untreated CD have reduced BMD [6], suggests that demineralization in patients with IBD may also occur as a direct consequence of intestinal inflammation. Elevated pro-inflammatory cytokines have been implicated in the pathogenesis of bone resorption in rheumatoid arthritis and postmenopausal osteoporosis [7,8].

Circulating pro-inflammatory cytokine levels have of course also been shown to be elevated in IBD patients with active inflammation [9-11] suggesting that disease activity and
High cytokine levels could also play a role in IBD-related bone mineral density loss. A rat model of colitis was associated with a dramatic 33% loss in trabecular bone and an even greater suppression in bone formation rate [12]. Healing of colitis was associated with an increased bone formation rate and a return of bone measurements to normal levels. Serum from children with CD was shown to affect bone mineralization in an organ culture model without altering bone resorption [13]. These observations suggest that mediators produced during intestinal inflammation may alter osteoblast function and bone formation.

It has been observed that osteoporotic patients with IBD have higher serum interleukin (IL)-6 levels than non-osteoporotic patients [10]. Tumour necrosis factor (TNF)-α, IL-1β and IL-6, amongst others, are potent activators of bone resorption at low concentrations in vitro [14,15]. These interactions suggest that TNF blockade may have beneficial effects on bone generally.

To date, data describing the effects of in vivo TNF-α inhibition on general bone metabolism in patients with IBD are still limited and restricted to infliximab treatment [16-20]. These studies have shown that infliximab appears to have a beneficial effect on bone metabolism in CD patients. The beneficial effects appear to be primarily due to an increase in bone formation, while reduction in bone resorption seems to play a lesser role [17,18]. These effects seem to be independent of whether the patients are classified as responders or non-responders to infliximab treatment based on clinical scores, and of whether the patients are on steroid therapy [16-18]. Patients who received infliximab have also been shown to have increased BMD based on DXA findings [21-23]. It's likely that several different mechanisms play a role in this positive effect of infliximab on bone metabolism.
Adalimumab is a human IgG1 monoclonal antibody specific for human TNF and is used for the treatment of active CD. To date, there are no published data investigating the effect of Adalimumab on bone metabolism in IBD patients. However, a recently published study in rheumatoid arthritis patients showed an improvement in BMD in Adalimumab-treated patients over a 4-year period [24] indicating that Adalimumab may have similar effects to infliximab on bone metabolism. The aim of our study was to explore the effects of Adalimumab treatment on bone metabolism in active CD patients by measuring the effect of induction/maintenance Adalimumab therapy on biochemical markers of bone turnover, bone nutrients, pro-inflammatory cytokines, anti-inflammatory cytokines and osteoclastogenesis markers. We also examined in an in vitro model the effect of sera of Adalimumab-treated patients on human osteoblast cell cultures and compared to the effect of healthy control sera on the same cell cultures.

MATERIALS AND METHODS

Study design and protocol

This study was a prospective, single centre open label study involving moderate-to-severe CD patients who failed to respond to conventional medical therapy (5-aminosalicylates, Mercaptopurine or azathioprine) or who had lost response or were non-responders to infliximab and who were started on Adalimumab therapy. This study was reviewed and approved by our institution’s ethics committee and included a healthy control population matched for age and sex with our IBD patients.

Patients
Twenty patients with CD who received Adalimumab were studied. Inclusion criteria included: a confirmed diagnosis of CD using conventional methods, Adalimumab naïve, at least 12 weeks since last dose of infliximab before commencing Adalimumab (if receiving Adalimumab due to loss of response or intolerance to infliximab), normal serum calcium and phosphate levels, stable drug regime for the previous 2 months and active disease. Active disease was defined as one of the following: a single or multiple perianal or enterocutaneous draining fistula(e) as a complication of CD, resistant to conventional treatment for at least 3 months; moderate-to-severely active CD based on Crohn's disease activity index (CDAI) and confirmed by radiography or endoscopy; disease refractory or dependent on oral (> 7.5mg/day prednisolone or 3mg budesonide) and/or non-responding to immunosuppressive agents (azathioprine, Mercaptopurine or methotrexate). Patients with confounding factors such as concomitant bone diseases (including Paget, osteomalacia, hyperparathyroidism and other endocrinopathies with bone alterations), and chronic alcoholism were excluded from this study.

All patients had active disease despite standard therapy. Adalimumab was given subcutaneously using an induction regime of 160mg at week 0, 80mg at week 2, and 40mg every other week thereafter. Patients receiving Adalimumab were followed up for 6 months post commencement of treatment and sera were obtained prior to each injection at baseline, 1 month, 3 month, and 6 month and for analysis. Disease activity was assessed using the CDAI.

**Control patients**
Twenty healthy normal controls matched for age and gender with our CD patients were also studied. People with confounding factors as described previously were excluded from acting as controls in this study. Control patients’ sera were obtained on a once-off occasion for analysis. Patient and control characteristics are shown in Table 1.

**Laboratory analyses**

All blood samples obtained from the subjects in this study were early morning fasting blood samples. Total procollagen type 1 N-terminal propeptide (P1NP) and N-MID Osteocalcin (OC) were chosen as markers of bone formation and C-telopeptide of type-1 collagen (CTx) serum concentration as a marker of bone resorption. The pro-inflammatory cytokines IL-1β, IL-6 and TNF-α were measured along with the anti-inflammatory cytokines; IL-10 and IL-13. OPG and sRANKL were measured as markers for osteoclastogenesis. All samples and standards were assayed using the appropriate Enzyme-linked immunosorbent assay techniques in duplicates and average reading was obtained for analysis. Parathyroid hormone (PTH) levels were measured using the Elecsys intact PTH assay and Vitamin D was measured using the DiaSorin 25-hydroxycalciferol assay. C-reactive protein (CRP) was measured using standard methods.

**Dual Energy X-ray absorptiometry (DXA)**

All BMD measurements were obtained by DXA scan at our institution’s diagnostic imaging department. All BMD readings were carried out by a single radiologist to reduce variability from the use of different densitometers and inter-observer variability. Meas-
urement of BMD were assessed at the posterior-anterior spine (region L1-L4) and left femur (region neck) using the local reference population provided by the manufacturer of the DXA machine. Osteopenia and osteoporosis were defined according to World Health Organization recommendations.

**In-vitro studies (cell source)**

The osteoblast cell line used in this study was the hFOB 1.19, Homo sapiens (Human) fetal osteoblast 1.19. This conditionally immortalized cell line was established with the temperature sensitive expression vector pUCSVtsA58 and the neomycin resistance expression vector pSV2-neo. This cell line has also been shown to be capable of osteoblastic differentiation, and to demonstrate osteoblast physiology and cytokine effects on osteoblasts [25].

**In-vitro studies (cell viability)**

Cell viability was measured using the alamarBlue assay. The alamarBlue assay incorporates a fluorometric/colorimetric growth indicator based on detection of metabolic activity. Specifically, the system incorporates an oxidation-reduction (REDOX) indicator that both fluoresces and changes colour in response to chemical reduction of growth medium resulting from cell growth [26]. Briefly, a 100μl of 0.5 x 10^6 cells/ml concentration was pipetted in triplicate onto a 96 well plate. 3 x 96 well plates were prepared for reading the viability at Day 2, Day 7 and Day 14 incubations. The 96 well plates were incubated at 34°C for 24 hours. After 24 hours, the waste media from each of the wells were removed. 100μl of serum free media (SFM) was then added in the wells that contained
cells and left incubated for further 24 hours. Following 24 hours of incubation, SFM was removed from each of the wells and 10% serum from study subjects was added into the wells in triplicates. The day of serum exposure on the hFOB 1.19 cells were taken as day 0 of incubation. After 2 days of incubation, i.e. on Day 2, 10μl of alamarBlue was added into in 96 well plates labeled Day 2. The plate was then incubated for 4 hours at 34°C and the absorbance was read on a spectrophotometer at 540nm and 620nm. The viability of each of the wells was calculated as described by the manufactures. The media was changed every 3 days for this entire experiment. On Day 7 and Day 14 of incubation respectively, the appropriate plate’s absorbance was read as previously.

**In-vitro studies (cell functionality)**

A marker of early-stage differentiation by the human osteoblast cell is the expression of the alkaline phosphatase (ALP) which aids bone formation. The p-Nitrophenyl Phosphate (pNPP) solution based assay was used in this study to measure ALP production. This assay is based on the principle of ALP hydrolysis of p-Nitrophenyl Phosphate to p-Nitrophenyl. pNPP is a chromogenic substrate for alkaline and other phosphatases. The reaction yields p-nitrophenol, which becomes an intense yellow soluble product under alkaline conditions and can be measured at 405 nm on a spectrophotometer.

As previously described in cell viability experiments, similarly 3 x 96 plates were prepared with cells. After 2 days of incubation, i.e. on Day 2, the media was removed from the wells that contains cells in Day 2 plate and rinsed twice with 100μl of phosphate buffer solution. Following this 100μl of pNPP assay was added into each well and the
plate was incubated for 30 minutes in a 34°C incubator and the absorbance was read at 405nm. The media was changed every 3 days for this entire experiment. On Day 7 and Day 14 of incubation respectively, the appropriate plate’s absorbance was read as described above.

**Statistical analysis**

Calculations were made with SPSS version 17.0 software. All data were initially analysed to see if they were parametric or non-parametric in distribution. Paired Student’s t-test was used in parametric distribution cohorts and Wilcoxon Signed Ranks test was used in non-parametric distribution cohorts to compare values at baseline and after 1 month, 3 months and 6 months of CD treated Adalimumab patients. Independent Student’s t-test was used in parametric distribution cohorts and Mann- Whitney test was used in non-parametric distribution cohorts to compare values between the control population and baseline in CD treated Adalimumab patients. A P value of < 0.05 was considered significant. The results are presented as mean +/- SD.

**RESULTS**

**Patient’s characteristics**

*Duration of disease*

The mean CD duration (+/- SD) was 6.45 +/- 6.77 years (range 0.1-27 years).

*Doses of antecedent treatment:*
The majority of the CD patients were on 5-ASA maintenance therapy (70%) and nearly half were on immunomodulators (9/20; 7 of these were azathioprine and the remaining 2 were on Mercaptopurine). The 5-ASA was commenced in all patients at the time of their respective initial diagnosis and the immunomodulators were introduced in the 9 patients at a mean (+/- SD) of 4.1 +/- 6.47 years (range 0-17 years) from the time of their initial diagnosis. Of the 9 patients who were on immunomodulators; 6 had ileo-colonic disease, 2 had fistulizing disease and 1 had colonic disease.

*Concurrent diseases:*

Of the 20 CD patients, 3 patients had other concurrent diagnosis (1 had known diagnosis of ankylosing spondylitis made 23 years prior to the CD diagnosis; 1 had a diagnosis of lactose intolerance and 1 patients had a diagnosis of non-specific connective tissue disease).

*Nutritional status:*

The mean body mass index provided in our Table 1 indicates that our CD patients had mean BMI within the normal range. 18/20 (90%) patients had a normal albumin level prior to commencement of Adalimumab. Of the remaining 2 patients in whom the albumin levels were low, one patient was noted to have severe nutritional deficiency as documented by albumin level of 17 pre-treatment and the other patient had moderate deficiency with an albumin level of 28 pre-treatment. Other causes of hypoalbuminaemia were excluded.

*Individual actual treatment for CD or other disorders:*
All the medications each of the CD patients were taking during the study is provided in Table 2 (Sub-analysis of patient’s characteristics).

**Control patient’s characteristics**

Two of the control patients had a known diagnosis of hypertension which was well controlled on single antihypertensive agents at the time of inclusion in the study (both of them had a normal DXA). The remaining 18 control subjects had no documented or other known diseases at the time of their enrolment in this study and were not on any regular medications. Of the 20 control patients, two patients had a distant history of a short one week course of corticosteroid use for chest infections. All patients had a normal clinical examination and normal blood parameters prior to inclusion. 7 of the control patients were current smokers with the mean +/- SD years of smoking being 16 +/- 7.2 years (range 10-30 years) and further 4 control patients were previous smokers with the mean +/- SD stopping smoking being 5.65 +/- 9.57 years (range 0.6 - 20 years).

**Disease activity**

The mean disease activity measured by CDAI score appreciably decreased from 304.65 at baseline to 113.7 after 1 month, 61.65 after 3 months and 27.7 after 6 months ($P < 0.001$ at all time points compared with baseline) (Figure 1A). To investigate any relationship between disease activity and change in bone metabolism markers, patients were stratified into those with a fall in CDAI of 70 -100 points (n=5), 100-150 points (n=4) and those with 150 points or greater (n=11) at 1 month after treatment with Adalimumab. Mean CRP (mg/L) at baseline was 15.92 and fell significantly following Adalimumab therapy at all time points measured ($P < 0.05$) (Figure 1B). At baseline, 16
patients were not on steroids and 4 patients were on steroids with a mean dosage of 4.2. At 6 months, only 1 patient remained on steroids with a mean dosage of 0.125. Although a trend was observed, the mean dosage of steroid that patients were on at 6 months did not fall significantly ($P = 0.098$) following treatment. (Figures not shown).

**Bone mineral density in active CD patients compare to control**

Four of 20 CD patients had osteoporosis (T score ≤ -2.5) and further 10 patients had osteopenia (T score of -1.0 to -2.5) prior to Adalimumab treatment. In control patients two of 20 patients had osteoporosis (T score ≤ -2.5) and further 11 patients had osteopenia (T score of -1.0 to -2.5) (Figure 2). The two control patients who had osteoporosis were both males and were ex-smokers of 20pack and 6 year respectively. The mean +/- SD of the DXA in the control group is -1.3 +/- -0.9 (range from -0.3 to -3.8) and in the CD group is -1.5 +/- -1.1 (range from -0.1 to -3.8).

**The effect of Adalimumab on bone markers**

Bone formation markers, OC and P1NP (ng/ml) were significantly increased at 1 month ($P = 0.015$ and $P = 0.008$ respectively) and 3 months ($P = 0.038$ and $P = 0.006$ respectively) compared to baseline, but not at 6 months (Figure 3A and 3B). The bone resorption marker, CTx serum (ng/ml), decreased from baseline (0.312) to 1 month (0.3051) and remained decreased for the remaining 6 months (0.2734), but this decrease was not statistically significant (Figure 3C). Serum concentration of OC was lower in CD before Adalimumab treatment than in controls while they returned to normal levels after
Adalimumab treatment (Figure 3A). Surprisingly, P1NP levels were lower and CTx serum levels were higher in controls compared to CD patients at baseline, although these differences were not statistically significant. (Figure 3 B-C).

The effect of Adalimumab on bone nutrients

At baseline CD patients had lower Vitamin D levels and higher PTH levels compared to controls (48.02 nmol/L compared to 53.45 nmol/L and 35.08 pg/ml compared to 24.85 pg/ml respectively). However this was not statistically significant. With Adalimumab treatment, both PTH and Vitamin D levels showed a non significant trend towards increase at 6 months (figures not shown).

The effect of Adalimumab on serum cytokines

Overall, changes in serum pro-inflammatory (IL-1β, IL-6 and TNF-α) and anti-inflammatory (IL-13 and IL-10) cytokines from baseline to 6 months were not statistically significant. Control patients had a lower serum levels of all the cytokines measured compared to CD patients at baseline (figures not shown).

The effect of Adalimumab on osteoclastogenesis markers

Serum levels of RANKL and OPG decreased after Adalimumab therapy. At baseline, both OPG and RANKL levels were higher in CD patients compared to control, and with therapy the levels decreased towards that of control. None of these measured changes reached statistical significance however (figures not shown).
The effect of Adalimumab on human osteoblast cell viability

Pre-treatment CD sera were associated with increased viable osteoblast cell number compared to controls, and Adalimumab treatment resulted in a reduction in viability back towards control levels (Figure 4A). Exposure of hFOB cells to CD sera pre and post Adalimumab treatment showed that compared to pretreatment baseline sera, post treatment sera reduced in vitro hFOB viability at all time points measured. However the effect was significant only for 6 months post commencement of treatment at day 14 of incubation.

The effect of Adalimumab on human osteoblast cell functionality

Osteoblasts exposed to control sera secreted significantly higher levels of ALP than those exposed to pretreatment, baseline CD sera ($P < 0.001$ at Day 2, $P < 0.001$ at Day 7 and $P = 0.001$ at Day 14). Post Adalimumab treatment sera resulted in higher levels of ALP secretion from osteoblasts than pretreatment baseline sera and this reached significance at 6 months of Day 14 exposure ($P = 0.001$). Moreover, ALP secretion following exposure to post treatment CD sera, no longer differed from the levels secreted following exposure to control sera (Figure 4B).

DISCUSSION

In this study, we show that Adalimumab treatment had a favorable effect on bone metabolism in patients with active CD. There was a significant and rapid increase in OC and P1NP, markers of bone formation, and a decrease, albeit non-significant, in sCTx, a marker of bone resorption. Our results are comparable to those found in studies looking
at the effect of infliximab on bone metabolism in CD patients [17,18]. These favourable effects were not associated with baseline CDAI’s, DEXA findings, disease location, concomitant medical therapy, previous TNF-α exposure, current steroid therapy or indeed on biological response to Adalimumab. Although the control group had a lower PINP and a higher CTx than CD patients pretreatment this was not statistically significant. However the other bone formation marker measured osteocalcin was noted to be lower in CD patients before treatment compared to control. Most importantly, a rise was noted in the formation markers, and a decrease was noted in the resorption marker with Adalimumab therapy which is the main study outcome measured. The number of patients and controls in this study was relatively small, and it was an unanticipated that so many controls would prove to have low BMD. It would certainly be interesting to study these markers in a larger group of age- and sex -matched controls who have documented normal BMD to see if in that instance higher baseline PINP and lower CTx would be found in controls compared to patients. This will be studied in future, larger studies.

Although one might have expected improvement in bone nutrients with Adalimumab therapy secondary to increase in absorption of the critical nutrients required for bone modelling and increased dietary intake with overall clinical improvement, our study failed to show any significant effect. Interestingly, even our control patients had below normal Vitamin D levels (defined as ≥ 80nmol/l); again highlighting that Vitamin D insufficiency and deficiency is a pandemic problem.
We found a non-significant trend towards higher levels of OPG and sRANKL in CD patients at baseline, compared to controls and those levels of OPG and sRANKL decreased after Adalimumab therapy. Although these results did not reach significance, they are consistent with findings of two previous studies which reported firstly higher concentrations of OPG in active CD patients compared to healthy controls [27] and secondly, decrease OPG levels following infliximab therapy [28]. The fact that similar, albeit at first glance somewhat counterintuitive changes have been reported after treatment with two different anti TNF-α treatments, suggest that the observed changes may be a group effect of TNF-α blockade. Therefore given the fact that healthy subjects have both lower OPG and sRANKL compared to active CD at baseline, and that Adalimumab therapy leads to a decrease in both parameters to approximate levels of control subjects, it would appear that the inflammatory response leads to counter-regulatory changes in bone homeostasis controls and that dampening down the inflammatory response with biologic therapy leads to a re-setting of the controls towards normal. However, given the lack of statistical significance, these results cannot be over interpreted.

It has been suggested that bone loss in IBD patients is a result of cytokines (IL-1, IL-6 and TNF-α) released from inflamed intestine directly influencing osteoblast and osteoclast function [12,28]. In this study there was no significant association found between the serum cytokine levels and bone turnover markers or Adalimumab therapy. Our patient numbers were small, and larger studies might well find such an association. However, a previous study in patients treated with infliximab found that mucosal but not system-
ic measurements of pro-inflammatory cytokines mirrored response to therapy [29]. We did not measure mucosal cytokine levels in this study.

The results of our in vitro studies are particularly interesting. Exposure of osteoblasts to sera from active CD-Adalimumab treated patients showed consistently higher levels of viable cells compared to control subjects at every time point measured. Therapy led to a decrease in viability levels towards those of controls. This suggests that Adalimumab treatment does in fact have an effect of osteoblast activity. Higher osteoblast cell viability observed pretreatment is most likely an inflammatory driven response. More important than osteoblast cell viability alone however, is osteoblast differentiation and functionality. Although higher osteoblast viability was found pretreatment with Adalimumab, the amount of ALP secreted was significantly lower in the pretreatment group CD group compared to control patients. ALP is an early differentiation marker of the osteoblasts and higher levels found post Adalimumab treatment indicate that the osteoblasts which were present, had better functionality. This strongly suggests that Adalimumab treatment has a direct effect on the human osteoblasts in vitro and that the increase in bone formation markers identified in vivo is reflection of this beneficial effect.

We did not look at osteoclast function in vitro as to date there is no good human in vitro osteoclast model.

Two different TNF alpha inhibitors (Infliximab and Adalimumab) have now been shown to improve bone metabolism in IBD and also Rheumatoid arthritis patients [24], another inflammatory condition. A potential question arises as to whether anti TNF agents might also be advantageous in non - inflammatory, age related osteoporosis / osteopaenia? Given current established therapies for osteoporosis such as bisphosponate therapy or 6
monthly sub cut Denosumab injection, both of which are widely available and much cheaper than the anti-TNF agents, this question is not likely to be addressed for the time being.

Our study has a number of limitations. Firstly, this was not a randomized study and the number of patients included was small, therefore meaningful analyses of various subgroups were restricted. Secondly, this was a heterogeneous group of medically refractory CD patients receiving different parallel medical therapies that may have affected the overall effect of Adalimumab therapy on the studied parameters. Thirdly, markers of bone turnover, bone nutrients, osteoclastogenesis, and cytokines varied from patient to patient in this study, suggesting that the aetiology of bone loss in CD may be heterogeneous in nature. However, to counter for this, each patient acted as his/her own control. Clinical remission rates were high in this study, and this is likely to be playing a significant role in the observed changes. Interestingly, our study did not show significant changes in the chosen measured cytokines over the duration of the study, which raises the possibility that other pro-inflammatory cytokines may be playing an important role, or indeed that this study was too small to detect such changes. Fourthly, BMD was measured at baseline only, as the study was 6 months in duration and it would be unlikely that DXA scans would show any meaningful changes over this time period. Previous studies looking at the effects of bisphosphonates therapy on BMD in CD patients only showed appreciable change in BMD on DXA after 1 year of therapy [30]. Repeat DXA scan after more prolonged therapy would be fruitful in future studies of longer duration to see whether the beneficial effects on bone formation markers and osteoblast function translate into meaningful improvements in DXA scan, and by extrapolation into reduction in
fracture risk. Finally, it is debatable as to whether all our control patients can be considered as ‘real’ controls given that 2 of them had osteoporosis and 11 of them had osteopenia based on DXA scan measurement. The fact that more than half of our control patients were either active smokers or previous heavy smokers is more than likely a major contributor to the findings of the very similar DXA results (reduced BMD) amongst patients and controls. This was an unanticipated limitation of our study, as although the controls appeared physically and biochemically healthy, they may not in fact have acted as ‘real’ controls in this particular study outcome. This highlights the fact that even among presumed healthy subjects in the general population many people have undetected lower BMD and vitamin D levels. However, this sub group of control patients did not exhibit significant differences in any of the parameters measured in this study as compared to remaining control patients with normal BMD.

Notwithstanding these limitations, this is the first study to have examined the effect of Adalimumab on bone metabolism in CD patients. As a result, this is the first study to demonstrate that Adalimumab treatment has a beneficial effect on bone metabolism in CD patients both \textit{in vivo} and \textit{in vitro} and that Adalimumab therapy has a direct effect on osteoblasts. The observed effects are similar to some previously reported effects of infliximab therapy and hence are likely to represent a class effect. While this may have seemed intuitive, it is important to have demonstrated this.

A larger study is needed to confirm these results and to investigate the long-term effect of Adalimumab therapy on bone metabolism and bone mineral density. It will also be inter-
esting to assess the effect of cessation of therapy on these parameters and to determine whether the effects are transient or enduring. This will be the goal of future studies.

REFERENCES


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<td>Table 1: Patients characteristics (N/A=not applicable)</td>
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<td>1 (5)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Previous infliximab exposure, n (%)</strong></td>
<td>13 (65)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Other concomitant medications, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium/Vitamin D</td>
<td>6 (30)</td>
<td>N/A</td>
</tr>
<tr>
<td>Bisphosphonates</td>
<td>0 (0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Patient</td>
<td>Duration of disease prior to commencement of Adalimumab (years)</td>
<td>List of medications</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Patient 1</td>
<td>2</td>
<td>5-ASA; AZA; Cal/Vit D; CS</td>
</tr>
<tr>
<td>Patient 2</td>
<td>5</td>
<td>AZA</td>
</tr>
<tr>
<td>Patient 3</td>
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<td>5-ASA; CS; Cal/Vit D</td>
</tr>
<tr>
<td>Patient 4</td>
<td>11</td>
<td>5-ASA; Multivitamins; Lomotil (antidiarrhoeal)</td>
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<td>Patient 5</td>
<td>7</td>
<td>5-ASA; Cal/Vit D; Tramadol (analgesia)</td>
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<tr>
<td>Patient 6</td>
<td>5</td>
<td>AZA; PPI</td>
</tr>
<tr>
<td>Patient 7</td>
<td>0.1</td>
<td>AZA; CS</td>
</tr>
<tr>
<td>Patient 8</td>
<td>2</td>
<td>AZA; PPI</td>
</tr>
<tr>
<td>Patient 9</td>
<td>9</td>
<td>5-ASA; antihypertensive; B12 injections</td>
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<tr>
<td>Patient 2</td>
<td>2</td>
<td>5-ASA</td>
</tr>
<tr>
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<td>10</td>
<td>5-ASA</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>5-ASA; 6-MP; PPI; SSRI; Antiemetics</td>
</tr>
<tr>
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<td>8</td>
<td>5-ASA; 6MP; PPI; Cal/Vit D</td>
</tr>
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<td>13</td>
<td>0.8</td>
<td>5-ASA</td>
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<td>2</td>
<td>None</td>
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<tr>
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</tr>
<tr>
<td>17</td>
<td>2</td>
<td>CS</td>
</tr>
<tr>
<td>18</td>
<td>5-ASA; Cal/Vit D</td>
<td>IFX (ineffective)</td>
</tr>
<tr>
<td>19</td>
<td>27</td>
<td>5-ASA; AZA; Cal/Vit D</td>
</tr>
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**Table 2: Sub-analysis of CD patient characteristics**

*Abbreviations: 5-ASA: 5 Aminosalicylic acid; AZA: Azathioprine; 6MP: 6-Mercaptopurine; CS: Corticosteroids; Cal/Vit D: Calcium and vitamin D supplements; PPI: Proton pump inhibitors; IFX: Infliximab; SSRI: Selective serotonin receptor inhibitors; S/E: side effects*
FIGURES

**Figure 1A**: CDAI scores in control and CD patients

**Figure 1B**: CRP levels in control and CD patients
Figure 2: Distribution of BMD based on DXA score in control and Crohn’s patients
Figure 3 A

Osteocalcin (OC)

Figure 3 B

P1NP

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Control  Adalimumab treated

Months

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p=0.038  p=0.015

p=0.006  p=0.008

---

Figure 3 A

Figure 3 B
Figure 3 (A-C) Changes in median Osteocalcin (OC), Pro-Collagen type 1 N propeptide (P1NP) and Carboxyterminal N-telopeptide (CTx) serum levels during treatment with Adalimumab.
Figure 4A: Number of viable hFOB cells on exposure to serum from Adalimumab treated CD and control over 14 days.
Figure 4B: Amount of ALP secreted on exposure of serum from control and CD Adalimumab treated patients over 14 days.