Background: Vitamin D deficiency is common among patients with Crohn’s disease (CD) and has been proposed as a risk factor for the development of the inflammatory process or a cause. Furthermore, most studies have relied on radioimmunoassays to measure 25-hydroxyvitamin D (25(OH)D), which may be less accurate than the accepted gold standard of liquid chromatography tandem mass spectrometry (LC/MS/MS). Circulating 25(OH)D is metabolised to the metabolically active 1,25(OH)2D. The alternative pathway involves the production of the inactive 24,25(OH)2D via 24-hydroxylase prior to elimination. The ratio of 25(OH)D:24,25(OH)D may be a more accurate measure of vitamin D status than 25(OH)D alone. We aimed to characterise vitamin D metabolism in patients with active and inactive CD using LC/MS/MS.

Methods: We report the baseline cross-sectional results of a prospective cohort study. Patients were included if they had active CD defined as ulceration at endoscopy; or a Crohn’s disease Activity Index (CDAI) >220 and a CRP >10 mg/l or faecal calprotectin >250 mg/kg. Remission was defined as a CDAI <150 and normal inflammatory biomarkers. Patients were excluded if they received corticosteroids or vitamin D supplementation in the preceding 4 weeks. Serum was tested for 25(OH)D, 1,25(OH)2D and 24,25(OH)2D using an LC/MS/MS assay. Validated questionnaires were used to estimate vitamin D exposure from diet and sunlight. Spearman’s correlation coefficient was used to test correlations and unpaired t-tests to test differences between active and remission CD groups.

Results: Forty-seven consecutive patients with CD (20 active and 27 remission) were recruited; 55% were male. Median age was 37 years (range 23 to 76 yr). Fewer patients in the active group were on immunomodulators (30% vs. 61% p = 0.03) or TNF inhibitors (25% vs. 89% p < 0.001). There was no difference in serum 25(OH)D, 1,25(OH)2D or 24,25(OH)2D between the groups. Serum 24,25(OH)2D levels were significantly lower in the active group (mean 1.3 vs. 2.5 ng/ml p < 0.001) and thus the 25(OH)D:24,25(OH)2D ratio was higher (49.4 vs. 26.1 p < 0.001). There was an inverse correlation between CDAI and 24,25(OH)2D levels (r2 = 0.33; p = 0.01). Dietary vitamin D intake and sunlight exposure were not different between the groups.

Conclusions: In the setting of active inflammation, levels of 24,25(OH)2D are maintained by shifting the metabolism of 25(OH)D to 1,25(OH)2D rather than 24,25(OH)2D, suggesting a reduction in 24-hydroxylase activity to maintain the active metabolite. The ratio of 25(OH)D:24,25(OH)2D is increased in active disease and may be a more sensitive marker of vitamin D status in patients with CD.

Vitamin D activates human intestinal fibroblasts


1University of Valencia, Pharmacology, Valencia, Spain, 2Fisabio Hospital Dr Peset, Valencia, Spain, 3University of Valencia, Medicine, Valencia, Spain, 4CIBEReh, Valencia, Spain

Background: Vitamin D signals through the vitamin D receptor (VDR) which is a member of the nuclear receptor family of transcription factors and regulates expression of several genes involved in disease development. It is known that vitamin D has an inhibitory effect on human intestinal fibroblastic cells. We studied the effect of vitamin D on human intestinal fibroblastic cells in an in vitro model with the aim of determining whether vitamin D reduces the proliferation of these cells and its effect on the expression of some markers of intestinal fibrosis.

Methods: Human intestinal fibroblastic cells (HIFC) were obtained from healthy human volunteers and cultured in 2D and 3D culture models. The effect of vitamin D on cell proliferation was evaluated using the cell proliferation kit (CCK-8) and the expression of fibrotic markers was evaluated using immunocytochemistry and Western Blot.

Results: Treatment with 1,25(OH)2D decreased cell proliferation by 25% (p < 0.05). The expression of fibrotic markers, such as α-SMA and collagen type I, was also decreased by 50% (p < 0.05) in vitamin D treated HIFC.

Conclusions: Vitamin D decreases the proliferation of human intestinal fibroblastic cells and reduces the expression of fibrotic markers, suggesting a potential anti-fibrotic effect of vitamin D. Further studies are needed to confirm these findings and to evaluate the potential use of vitamin D as a therapy for intestinal fibrosis.
factors that play an immunoregulatory role in the gut. Defective signalling due to vitamin D deficiency or decreased mucosal VDR levels has been related to Crohn’s disease (CD). We aim to analyse the acute effects of Vitamin D in the activation of human intestinal fibroblasts.

**Methods:** Fibroblasts were isolated from non-damaged and damaged intestinal resection of CD patients and control patients (non-damaged intestine from colon cancer). Fibroblasts were treated with 1,25 Vitamin D$_3$ (10 nM and 100 nM) for 24 h. Gene expression of pro-inflammatory cytokines and COL1A1 was quantified by qPCR and protein levels were determined by western blot. Statistical significance was measured by ANOVA.

**Results:** Vitamin D increased the mRNA expression of VDR in fibroblasts obtained from the inflamed and non-inflamed mucosa of CD patients (Figure 1A) and it increased the mRNA of CYP24A1, a VDR target (Figure 1B). Treatment with vitamin D rised in a dose-dependent manner COL1A1 mRNA expression in fibroblasts from CD patients (Figure 1C) and in parallel it induced the expression of pro-inflammatory cytokines (IL1β, IL6) (Figure 1D, 1E). Protein levels of phospho-NFκB and phospho-STAT3 were also higher in fibroblasts treated with Vitamin D from CD patients.

**Conclusions:** Our study indicates that an acute treatment of Vitamin D activates an inflammatory pathway and a collagen I expression in human intestinal fibroblasts which may be involved in the initial response in the wound healing.

**P123**

The effect of necrosis inhibition on acute DSS colitis model of inflammatory bowel disease

D. Lee$^{1,2}$, J.S. Koo$^2$, C.H. Kim$^1$, S.H. Hwang$^1$, J.W. Choe$^2$, S.Y. Kim$^2$, S.W. Jung$^2$, H.J. Yim$^2$, Y.T. Jeen$^2$, S.W. Lee$^2$

$^1$Korea University Ansan Hospital, Internal Medicine, Seoul, South Korea, $^2$Korea University College of Medicine, Seoul, South Korea

**Background:** Inflammatory bowel diseases (IBD) were characterised by uncontrolled chronic inflammation, which lead to cell death and organ damage. In contrast to apoptotic cell death, necrosis is characterised by destruction of cell membrane, which released substances from the cells causing the inflammatory reaction and a cascade of vicious inflammatory cycle resulting in increased necrosis. Although necrosis is thought be a main cell death mechanism of IBD, few attempts have been made to reduce necrosis in IBD. A novel necrosis inhibitor (NI, NecroX-7) is recently developed, which blocks the opening of mitochondrial permeability transition pore and inhibits necrosis effectively. The aim of this study investigated the effect of necrosis inhibition in acute murine colitis model and in-vitro study.

**Methods:** Cleaved PARP-1 fragment band was analysed using western blot assay in intestinal epithelial cell line (IEC-18, rat) in order to confirm the necrosis inhibition effect of NI. And acute dextran-sodium sulfate (DSS) induced colitis was generated in C57BL/6 mice. NI (30 mg/kg) was administered once a day via oral gavage for 8 days from the day before DSS administration. The severity of colitis was assessed by weight, colon length and histologic score. And HMGB1 immunochemistry was performed on harvested intestine for evaluating necrotic cell death qualitatively. The inflammatory cytokines mRNA expressions were measured by quantitative RT-PCR.

**Results:** In the necrosis inhibition group, the expression of cleaved PARP-1 (55kDa, necrosis marker) was reduced compared with the control group, whereas the cleaved PARP-1 fragment (89 kDa, apoptosis marker) was not different between groups. In vivo study, NI treatment significantly reduced colitis represented by colon length and histologic score.