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The effect of vitamin D on intestinal inflammation and faecal microbiota in patients with ulcerative colitis

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- Dr Philip Hendy: acquisition of data, critical appraisal of manuscript for important intellectual content, final approval of submitted version;
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ABSTRACT

Background and Aims

There is evidence vitamin D may be immunomodulatory and alter faecal microbiota, but results from clinical studies in humans to date have been inconclusive. This study aimed to assess the effect of vitamin D replacement in vitamin D deficient patients with and without ulcerative colitis (UC) on inflammation and faecal microbiota.

Methods

Vitamin D was replaced over 8 weeks in patients with active UC (defined by faecal calprotectin ≥100 µg/g), inactive UC (faecal calprotectin <100 µg/g), and non-IBD controls with baseline 25(OH) vitamin D <50 nmol/L, and markers of inflammation and faecal microbiota analysed.

Results

Eight patients with active UC, 9 with inactive UC and 8 non-IBD controls received 40,000 units cholecalciferol weekly for 8 weeks. Mean baseline 25(OH) vitamin D increased from 34 (range 12-49) nmol/L to 111 (71-158) nmol/L ($p < 0.001$), with no difference across the groups ($p = 0.32$). In patients with active UC, faecal calprotectin levels reduced from median 275 to 111µg/g ($p = 0.02$), platelet count reduced (mean 375 to 313×10^9/L, $p = 0.03$), and albumin increased (mean 43 to 45g/L, $p = 0.04$). These parameters did not change in patients with inactive UC or non-IBD controls. No changes in overall faecal bacterial diversity were noted although a significant increase in *Enterobacteriaceae* abundance was observed in patients with UC ($p = 0.03$).

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Conclusions

Vitamin D supplementation was associated with reduced intestinal inflammation in patients with active UC, with a concomitant increase in *Enterobacteriaceae* but no change in overall faecal microbial diversity.

Key words: Basic science, experimental models and pathophysiology; clinical trials

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INTRODUCTION

With expanding therapeutic options for inflammatory bowel diseases (IBD), costs associated with medical therapies have risen disproportionately to those associated with disease complications [1]. Numerous epidemiological and laboratory-based immunological studies support the role of vitamin D as a potential inexpensive immunomodulator, and serum 25(OH) vitamin D (25(OH)D) status has been shown to be inversely proportional to intestinal inflammation in patients with IBD [2-8]. However, there remains a paucity of interventional data supporting it as a treatment for patients with IBD.

Dysbiosis, or dysregulation of the gut microbiota, is a recognised feature of IBD, and is thought to play a role in the pathogenesis and perpetuation of inflammation [9]. Patients with UC have reduced bacterial species richness, as well as temporal instability of the microbiota profile in clinical remission and in active disease, compared with healthy controls [10-12]. Members of the Firmicutes and Bacteroidetes phyla have been demonstrated to be reduced [10]. Increases in pathobiont bacterial species including *Fusobacterium nucleatum* and *Escherichia coli* have been shown in the mucosa and faeces of patients with UC [13, 14], whilst the immunoregulatory species *Faecalibacterium prausnitzii*, has been shown to be under-represented [15]. Mucolytic bacterial species including *Ruminococcus gnavus* and *Ruminococcus torques* are also disproportionately increased in abundance in patients with IBD with the suggestion being that increased numbers contributes towards the gut environment changes seen as the disease progresses [16]. Therapeutically targeting the microbiota using the broad approach of faecal microbiota transfusion has been shown to improve outcomes in patients with UC [17-19].

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There is evidence to suggest that vitamin D may modify the gut microbiota. Specifically, vitamin D supplementation has been shown to suppress intra-macrophage *Escherichia coli* survival in *in vitro* studies [20]. Vitamin D has also been shown to regulate anti-microbial peptide production [20-22]. Vitamin D deficient and vitamin D receptor (VDR) knockout mice have reduced ileal Paneth cell alpha defensin secretion, increased abundance of *Helicobacter hepaticus*, and reduced abundance of *Akkermansia muciniphila*, compared with control or wild-type mice [23]. Studies have also shown that VDR negatively regulates bacterial-induced intestinal epithelial NFκB activation and response to infection [24]. Conversely, a cross-sectional study of 150 young healthy adults found an inverse correlation between 25(OH)D status and faecal abundance of the butyrate-producing bacterium *Coprococcus* and *Bifidobacterium*, both of which may theoretically mediate an anti-inflammatory effect [25]. It is currently unknown whether vitamin D supplementation in patients with UC affects pro-inflammatory or anti-inflammatory gut microbiota as part of a strategy to influence disease activity.

This prospective pilot study aimed to evaluate change in subjective and objective markers of intestinal inflammation, and within the faecal microbiota, following vitamin D replacement in patients with active and inactive UC, and non-IBD controls.

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MATERIALS AND METHODS

Subjects and study protocol

Consecutive patients with vitamin D deficiency (defined by 25(OH)D < 50 nmol/L) attending outpatient clinics at St Mark's Hospital were invited to participate. Three groups were studied: (1) those without IBD or other known gastrointestinal malabsorptive condition, (2) those with inactive UC (defined as faecal calprotectin < 100 µg/g), and (3) those with active UC (faecal calprotectin ≥ 100 µg/g) [26, 27]. Inclusion criteria for patients with UC comprised partial Mayo score of ≤ 4, and stable therapy including mesalazine (≥2 months) and immunomodulatory or anti-tumour necrosis factor therapy (≥3 months) with no change in therapy planned for at least 12 weeks as per the patient's treating clinician. Exclusion criteria included other significant gastrointestinal disease, pregnancy (current or planned within 6 months), hypercalcaemia or evidence of primary or tertiary hyperparathyroidism, chronic kidney or severe cardiovascular disease, antibiotics within the previous 2 months or bowel preparation within the previous 4 weeks.

Demographic and disease characteristics and activity as assessed by Simple Clinical Colitis Activity Index (SCCAI)[28, 29] and Partial Mayo Index[30] were recorded, patients were asked to complete a food diary, and blood tests collected for markers of inflammation. Serum 25(OH) levels were quantified using liquid chromatography tandem mass spectrometry. Patients were asked to provide two faecal specimens with the assistance of Stoolcatcher™ (TagHemi, Zeijen, The Netherlands) as per manufacturer's instructions, and supplied with an ice pack for transport to the hospital within 2 hours. One container was analysed for calprotectin (by enzyme linked immunosorbent assay, ELISA, Schottdorf Laboratories, Germany), and the second stored at -80 degrees Celsius for microbiota analysis.

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Patients were prescribed vitamin D replacement according to the London North West Healthcare NHS Trust guidelines, at a dose of 40,000 IU once weekly for 8 weeks using 2 capsules of 20,000 IU vitamin D3 (Plenachol, Encap, West Lothian, UK). Following replacement, patients were re-assessed symptomatically and by objective markers of inflammation, with repeat faecal microbiota analysis. Adherence was checked by direct patient questioning of number of capsules remaining.

Faecal microbiota analysis

DNA Extraction

All samples were extracted within one month of collection using the Stratech PSP Spin Stool DNA kit following the manufacturer's instructions.

PCR amplification and sequencing. The V3-4 region of the 16S rRNA gene was amplified using Bakt_341F and Bakt_805R primers, as described previously [31], then pooled and purified using AMPure XP (Beckman Coulter, Brea, California, USA). The samples were then indexed using the Nextera XT Index Kit V2 (Illumina, San Diego, California, USA) and KAPA HiFi Hotstart ReadyMix (Kapa Biosystems, Cape Town, South Africa) with libraries were quantified using Quant-iT™ dsDNA Assay Kit HS (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed using an Illumina MiSeq sequencer (Illumina, San Diego, California, USA) using Illumina V3 chemistry and paired-end 2×300 base pair reads. Further details regarding PCR amplification are presented in Supplementary Material 1.

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Bioinformatic Analysis

Quality of the sequences was assessed using FastQC (version 0.11.3) [32]. The V3-V4 primer sequences at the 5' end of reads were hard trimmed using TrimGalore! (version 0.4.0) [33]. Sequences were analysed using DADA2 (version 1.3.1) to produce sequence variants. Taxonomy was assigned against the GreenGenes 13.8 database [34]. The outcome sequence variant table was converted to biom format using biomformat (version 2.1.3) [35]. Further details regarding bioinformatic analysis are presented in Supplementary Material 1.

Diversity analyses including Simpson Index for alpha diversity and Bray-Curtis for beta diversity were performed using the core_diversity_analyses.py script from QIIME (version 1.9.0) with a subsampling level of 19505 to ensure that all samples were included [36]. Taxa numbers at each taxonomic level were also produced. LEfSe analysis was carried out using the Huttenhower Galaxy Server (http://huttenhower.sph.harvard.edu/galaxy/) to identify any potential biomarkers associated with sample types [37]. DESeq2 analysis [38], Wilcoxon Rank sum tests and Kruskall-Wallis tests for significant changes in abundance in relation to sample type were carried out in R. A p-value of ≤ 0.05 was considered statistically significant, with the exception of DESeq2 analysis where an adjusted p value of ≤ 0.05 was used. Figures were made using ggplot2 in RStudio. PCoA plots were visualised using Emperor [39].

Statistical considerations

Statistical analyses for non-microbiota results were performed using SPSS v23 (IBM Corporation, Armonk, New York, 2015) and GraphPad Prism v5.04 (Graphpad software, La Jolla, California, 2010). Dependent and independent samples t-tests, Mann Whitney U test, analysis of variance (ANOVA) and Kruskall-Wallis tests were used where appropriate.

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Associations with increases in 25(OH)D were examined by bivariate correlations. A p-value of ≤ 0.05 was considered statistically significant.

Ethical statement

The protocol for this study was approved by the Office of Research and Ethics at London Northwest Healthcare NHS Trust, and was performed in accordance with United Kingdom regulations and the principles of the Declaration of Helsinki 1954 and its later amendments. Informed consent was obtained from all individual participants included in this study.

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RESULTS

Twenty-five patients participated in this study from August to September 2015 (late Summer to Spring at 52^0 North), with baseline characteristics outlined in Table 1. No significant demographic differences were noted across the three groups. A trend to higher BMI and waist circumference in non-IBD controls was seen. Most patients with UC had left-sided or extensive colitis of variable duration.

Routine laboratory indices and circulating components of the vitamin D axis amongst participants at baseline are outlined in Table 2. As expected, faecal calprotectin was significantly higher amongst patients with active disease, and platelet counts higher, with a trend towards higher C-reactive protein. No significant differences across the groups in serum 25(OH) D, calcium, phosphate or parathyroid hormone were noted. Baseline dietary patterns of the participants are outlined in Supplementary Table 1. Seven of the 25 participants had a vegetarian diet, with self-reported vegetable intake reported as >35 standard serves per week by the majority (14 of 25) participants.

The follow-up visit took place following 8 weeks of vitamin D replacement (mean duration 58 days). Vitamin D replacement resulted in an increase in serum 25(OH)D across all participants from a mean of 34 (range 12-49) nmol/L to 111 (71-183) nmol/L ($p < 0.001$, paired t-test) (Figure 1). There was no significant difference in the increase in 25 (OH) vitamin D between the three groups ($p = 0.316$). All patients reported completion of the full course of supplementation (320,000 IU vitamin D) except two non-IBD controls (240,000 IU each), one patient each with inactive and active UC (280,000 IU each). One patient with active UC ceased mesalazine tablets (taken at 3.2g daily) during the course of therapy in the context of symptomatic improvement. All other patients continued usual therapy. No

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significant change in dietary patterns over the study period were observed across most subjects.

Symptomatic disease activity indices significantly declined amongst patients with inactive and active colitis following vitamin D supplementation, reaching significance for SCCAI (p = 0.04 and p = 0.01, respectively) but not for the Partial Mayo Score (p = 0.10 and p = 0.09, respectively). In patients with active UC, objective markers of disease activity significantly improved following vitamin D supplementation: faecal calprotectin (median 257 [range 110->2000] to median 111 [5-2000] µg/g, p = 0.02); platelet count (mean 375 [255-509] to mean 313 [243-461] x 10^9/L, p = 0.03); and albumin (mean 43 [38-49] to mean 45 [41-50] g/L, p = 0.04) (Figure 1). No effect on faecal calprotectin, CRP, white cell count, platelet count or albumin was observed amongst patients with inactive colitis or non-IBD controls. Baseline overall dietary pattern, cereal and bread, vegetable or fruit intake did not influence response to faecal calprotectin, or circulating markers of inflammation, of vitamin D replacement (data not shown).

Among patients with active UC, there was no significant correlation between the change in serum 25(OH)D and change in faecal calprotectin (Spearman r = -0.21, p = 0.61). There was no significant alteration in serum calcium, phosphate, or alkaline phosphatase. Serum parathyroid hormone levels declined significantly across the whole cohort (mean 4.0 [range 2.2-5.7] to 3.4 [1.8-6.5] pmol/L, p = 0.02). No patients were hospitalised or required surgery.

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Change in faecal microbiota

All twenty-five patients submitted a faecal sample at baseline, with twenty-three patients also providing a follow-up sample. One patient with active colitis and one non-IBD control did not submit follow-up samples for microbiota analysis.

Between 50228 and 189688 raw sequences were produced per sample following amplicon sequencing. Post filtering and DADA2 analysis, each sample had between 19505 and 98075 sequence variant counts with an average of 45734 counts (Supplementary Table 2).

Diversity analyses

No differences in alpha diversity as assessed by the Simpson, Shannon, Chao or Observed species diversity indices were noted across the three patient groups at baseline. No differences in alpha diversity were noted in samples following vitamin D replacement across the patient groups.

Principal co-ordinate analysis (PCoA) plots using the Bray-Curtis beta diversity metric demonstrated that patients without IBD clustered together distinct from UC patients (Figure 2a; $p = 0.003$, PERMANOVA). When the same analysis approach was applied to both the pre and post Vitamin D supplemented data, no difference between patients with inactive and active UC was noted ($p = 1.0$, Figure 2b).

Taxonomic profiling

Changes in the relative abundance of sequence variants showed statistically significant differences between the three groups at baseline (Table 3). Abundance of the mucus-associated bacterium *Ruminococcus gnavus* was marginally but not significantly higher in

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patients with UC than non-IBD controls (p = 0.068, Kruskall-Wallis, Supplementary Figure 1).

Change in abundance of specific bacteria following vitamin D administration were analysed using LEfSe analysis. Across all participants, an increase in *Clostridium colinae* (p = 0.03; driven by 2 non-IBD controls and 2 patients with inactive UC) and *Enterobacteriacae* (p = 0.03; driven by 5 patients with inactive UC and 3 with active UC) was noted. *Ruminococcus gnavus* marginally but not significantly reduced following vitamin D supplementation across the whole cohort (p = 0.15; Wilcoxon Rank sum, Supplementary Figure 1). No significant change in abundances of other mucus-associated bacteria *Ruminococcus torques* or *Akkermansia muciniphila*, butyrate-producing bacteria from the *Clostridium* Cluster IV or Cluster XIVa groups, or of lactic acid producing bacteria (*Lactobacilli* or *Bifidobacteria*), or of the invasive bacteria *Fusobacterium nucleatum* and *E. coli* were noted (data not shown).

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DISCUSSION

The role of vitamin D as a potential immunomodulator in patients with IBD has been investigated extensively for over a decade. Numerous studies demonstrate involvement of the vitamin D axis in regulation of the epithelial barrier, innate immune cell and T-cell function [20, 21, 23, 24, 40-47]. Though there are some preliminary data suggesting that vitamin D may influence the intestinal microbiota in IBD, this has not been studied in humans. Furthermore, evidence for efficacy at the clinical level remains poor. This study is the first to show that vitamin D replacement in patients with active UC deficient in vitamin D improved objective markers of inflammation. Though this was associated with a significant increase in *Enterobacteriacae* in patients with UC, there was no change in overall diversity or other specific bacteria analysed.

Previous studies have shown that vitamin D supplementation may be associated with reduced rates of relapse in patients with Crohn's disease in remission when given at a dose of 1200 IU daily for 12 months [2], and improved Crohn's disease activity index (CDAI) and quality of life when given at up to 5000 IU daily for 24 weeks [4]. An alternative placebo-controlled RCT showed no significant change in CDAI, quality of life, CRP or faecal calprotectin in patients given 2000 IU vitamin D daily for 3 months [5]. In patients with UC, low vitamin D levels have been associated with greater disease activity, as assessed by symptoms, faecal calprotectin, and endoscopic activity, as well as increased risk of subsequent relapse [7, 8, 48-50]. A small pilot study demonstrated reduction in symptomatic disease activity indices but not intestinal inflammation as measured by faecal calprotectin in patients with UC and Crohn's colitis [51]. No placebo-controlled studies in patients with UC have been reported to date. The current study is the first to show an improvement in objective markers of inflammation (faecal

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calprotectin, albumin, platelet count) following vitamin D replacement, limited to the group of patients with active UC defined by faecal calprotectin ≥ 100 µg/g at baseline.

The reason for the faecal calprotectin reduction in these patients warrants further consideration. Though it has been postulated that a 25(OH)D level higher than 75 nmol/L, or closer to 100-125 nmol/L, may be required for immunomodulatory effect [4, 48, 51-53], such a level was not specifically targeted in this study. Rather, high dose oral weekly supplementation according to local institutional guidelines was administered at the same dose in all patients, as opposed to daily supplementation in most previous studies. Significant inter-individual variation in response to vitamin D supplementation exists, particularly in diseased states like IBD [54], and unsurprisingly the serum 25(OH)D level achieved varied from 75 to 183 nmol/L across the patients with UC. Five of 8 patients with active UC achieved a 25(OH)D of ≥ 100 nmol/L, all of whom had a reduction in faecal calprotectin to varying extents; however, there was no clear correlation between the rise in 25(OH)D and reduction in faecal calprotectin. Therefore, the findings in this study raise the prospect that it may not be the final serum 25(OH)D level, but the administration of a higher dose of vitamin D itself that potentially confers an immunomodulatory effect. This concept requires further investigation in an appropriately powered prospective controlled trial.

The VDR is expressed in colonic intestinal epithelial cells, dendritic cells and macrophages [21, 42]. Vitamin D has been shown to potently stimulate cathelicidin, an anti-microbial peptide produced by macrophages [22] which plays an important role in defence against intracellular organisms such as mycobacteria [21]. VDR expression is significantly increased in inflamed and non-inflamed mucosal biopsies from patients with UC [55]. Vitamin D supplementation suppressed intra-macrophage *E. coli* survival in *in vitro* studies [20], and vitamin D deficient

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and VDR knockout mice had impaired ileal Paneth cell alpha defensin secretion and increased abundance of the colitogenic *Helicobacter hepaticus*, compared with control or wild-type mice [23]. Therefore, there is biological plausibility for an interaction between the vitamin D axis and intestinal microbiota in the pathogenesis and perpetuation of inflammation in patients with IBD, especially UC. In the current study, no overall change in faecal microbial diversity occurred following vitamin D supplementation. Although an increase in the abundance of *Enterobacteriaecae* was noted following vitamin D supplementation in patients with UC, this large family comprises a large proportion of harmless and commensal as well as potentially pathogenic bacteria in the human gut, and therefore the significance of such a change is uncertain.

Ruminococcus gnavus is a Gram-positive anaerobic mucolytic bacterium belonging to Cluster XIVa of the *Clostridia* class, which is increased in abundance in patients with IBD [16]. The intestinal mucus layer provides a protective barrier between the luminal environment and mucosa, comprising dense glycoproteins interspersed with antimicrobial peptides produced by Paneth cells and other epithelial cells [56]. A previous study has shown reduced abundance of *Ruminococcus gnavus* in mucosal biopsies from patients with active UC defined symptomatically [57], and this trend was confirmed in the current study, albeit without statistical significance. Furthermore, abundance of *Ruminococcus gnavus* non-significantly reduced across all patients after vitamin D supplementation. Whether vitamin D supplementation mediates regulation of intestinal mucus antimicrobial composition and therefore susceptibility to specific mucolytic bacteria, warrants further investigation.

Nonetheless, an absence of significant effect on the faecal microbiota across the whole cohort of patients studied is worth noting. It is possible that vitamin D does not alter human

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microbiota, despite laboratory data from mouse studies [23]. Other possible explanations include a differential effect on faecal and mucosa-associated microbiota. Faecal microbiota was assessed during this study rather than mucosal associated microbiota as this is less invasive and is not subject to variation as a result of bowel preparation [58]. However, given the intimate relationship between vitamin D induced anti-microbial peptide secretion and the mucosal microbiota, one may postulate that significant changes in the latter may be more reflective of the effect of vitamin D in this setting. An absence of significant alteration of the faecal microbiota by vitamin D supplementation, however, is not an isolated finding: despite widespread use, there remains a paucity of published data regarding the effect of conventional therapies such as 5-aminosalicylates, thiopurines and anti-TNFα agents on the faecal microbiota independent of changes in mucosal inflammation in patients with IBD [59, 60]. Conversely, the absence of a change in microbiota composition despite reduction in inflammation in the active UC group is also notable, and may be reflective of only a mild reduction in inflammation in these patients. It is important to note that there are few robust data regarding change in microbiota composition in patients with UC in the absence of medical therapy [61]. Furthermore, patients with UC in this study had a relatively long disease duration, with a median of 11-12 years. Data regarding the effect of duration of UC on temporal variability of microbiota are also limited [61]. Longer disease duration has previously been described as a risk factor for vitamin D deficiency [62], but no influence of disease activity on initial 25(OH)D level or response to supplementation was noted in the current study (data not shown).

There are multiple other limitations in this small study. Though no overt toxicity as measured by serum calcium and phosphate was noted, long-term potential effects of the supplementation strategy in this study were not able to be elucidated, particularly the risk of

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hypercalciuria or nephrocalcinosis [51]. Dietary assessment of patients at baseline and follow-up visits showed no clear changes across most patients, but specific effects of change in diet as confounders were difficult to elicit.

In conclusion, vitamin D supplementation at a dose of 40,000 IU weekly for 8 weeks reduced objective circulating and intestinal markers of inflammation in patients with active UC. A significant increase in abundance of *Enterobacteriaceae* in patients with UC, and a trend to reduction in the mucolytic *Ruminococcus gnavus* species, was noted, but overall microbiota diversity was unchanged. Vitamin D may therefore reduce intestinal inflammation, but independently of change in faecal bacterial composition. A larger placebo-controlled clinical trial incorporating immunological and extended microbiota analyses, including functional assessment, will shed further light upon this effect.

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Conflict of interest

The authors declare that they have no conflict of interest with respect to this manuscript.

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FIGURE LEGENDS

Figure 1. Change in clinical and laboratory indices following vitamin D supplementation in participants without IBD, those with inactive and active UC.

Figure 2. Principal Co-ordinate Analysis (PCoA) Plots at (a) baseline and (b) before and following vitamin D supplementation

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Table 1. Baseline participant characteristics. (DM, diabetes mellitus; BMI, body mass index; SCCAI, Simple Clinical Colitis Activity Index; Fitzpatrick skin types: I - pale white skin, blue/hazel eyes, blond/red hair, II - fair skin, blue eyes, III - darker white skin, IV - light brown skin, V - brown skin, VI - dark brown or black skin).

	Non-IBD Controls (n = 8)	**Inactive UC (n = 9)**	**Active UC (n = 8)**	**P value**
Age (mean +/- range)	51 (35-66)	45 (28-72)	45 (30-68)	0.541[a]
Female:Male	3:5	3: 6	4:4	0.793[a]
Ethnicity, n (%)				
British White	3	1	1	
Southern European	0	0	2	
Indian Subcontinental	5	7	5	
Arab & Middle Eastern	0	1	0	
Fitzpatrick Skin Type, n (%)				
I	0	0	0	
II	1	1	3	
III	2	1	0	
IV	0	1	0	
V	5	6	5	
VI	0	0	0	
Co-morbid illnesses, n (%)				
Hypertension	3	1	3	
Hyperlipidaemia	2	1	1	
Type 2 DM	1	0	1	

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Asthma	0	3	1	
Congestive cardiac failure	1	0	0	
Smoking status, n (%)				
Never smoked	5	6	6	
Ex-smokers	2	3	1	
Current smokers	1	0	1	
BMI (mean +/- range)	28.9 (23.5-36.9)	25.8 (20.5-29.7)	24.6 (21.5-28.4)	0.077[a]
Waist circumference, cm (mean +/- range)	104 (93-119)	92 (84-99)	91 (70-109)	0.052[a]
Vitamin D supplementation, n (%)	1	4	3	0.205[b]
Montreal Classification				
Disease extent: E1:E2:E3	NA	0:3:6	1:2:4	
UC Disease Duration, y (range)	NA	11 (0.8-36)	12 (1-40)	
SCCAI (median, range)	NA	2 (0-6)	3 (0-5)	
Partial Mayo Score (median, range)	NA	0 (0-3)	1 (0-4)	
Medical therapy for UC	NA			
Nil		1	1	
Mesalazine only		6	3	
Thiopurine +/- mesalazine		1	1	
Anti-TNF +/- mesalazine		1	0	
Vedolizumab +/- mesalazine		0	1	
Anti-TNF +/- thiopurine		0	2	

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[a] ANOVA

[b] Chi-square, UC vs non-IBD controls

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Table 2. Routine laboratory indices and components of the vitamin D axis in the patient groups and healthy controls.

	Non-IBD Controls (n = 8)	Inactive UC (n = 9)	Active UC (n = 8)	P value
Haemoglobin (g/L, mean, range)	140 (118-155)	145 (122-167)	124 (87-154)	0.07[a]
White cell count (X 10^9/L, mean, range)	7.4 (4.9-10.0)	6.5 (4.9-8.0)	7.2 (5.3-10.6)	0.53[a]
Platelet count (x 10^9/L, mean, range)	266 (200-321)	241 (160-313)	375 (255-509)	**0.001**[a]
Serum albumin (g/L, mean, range)	45 (42-50)	46 (43-51)	43 (38-49)	0.09[a]
Serum C-reactive protein (mg/L, median, range)	1.0 (< 1.0-5.0)	1.0 (< 1.0-8.0)	4.0 (1.0-28.0)	0.054[b]
Faecal calprotectin (µg/g, median, range)	16.4 (12.2-50.9)	34.2 (< 5.3-87.1)	257 (110->2000)	**0.002**[b]
25(OH)D (nmol/L, mean, range)	31 (12-49)	33 (17-49)	34 (16-43)	0.90[a]
Serum calcium (corrected, mmol/L, mean, range)	2.42 (2.29-2.57)	2.44 (2.30-2.55)	2.46 (2.36-2.58)	0.67[a]
Serum phosphate (mmol/L, mean, range)	1.06 (0.83-1.42)	1.02 (0.73-1.65)	0.99 (0.55-1.33)	0.85[a]
Serum PTH (pmol/L, mean, range)	3.3 (2.2-4.7)	4.6 (3.7-5.7)	4.5 (2.7-5.6)	0.20[a]

[a] ANOVA

[b] Kruskall-Wallis test

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Table 3. Significant DESeq2 results comparing the relative abundance of sequence variants across sample types. All values given to three significant figures.

Sequence Variant Taxonomy	Log Fold Change	Adjusted P Value
Inactive UC > Non-IBD controls		
E. coli	5.34	0.00448
Non-IBD Controls > Active UC		
Prevotella copri	8.48	0.0442
Coprococcus genus	8.76	0.0294
Inactive > Active UC		
Prevotella copri	8.46	0.00640
Bacteroides plebeius	9.08	0.0131
Bacteroides fragilis	8.79	0.0158
Bacteroides genus	7.83	0.0116
Ruminococcaceae family	8.73	0.00927
Bacteroides caccae	8.30	0.00640
Coprococcus genus	6.62	0.0284
Active > Inactive UC		
Lachnospira genus	6.03	0.00927

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Sutterella genus	8.75	0.0158
Coprococcus genus	6.62	0.0284

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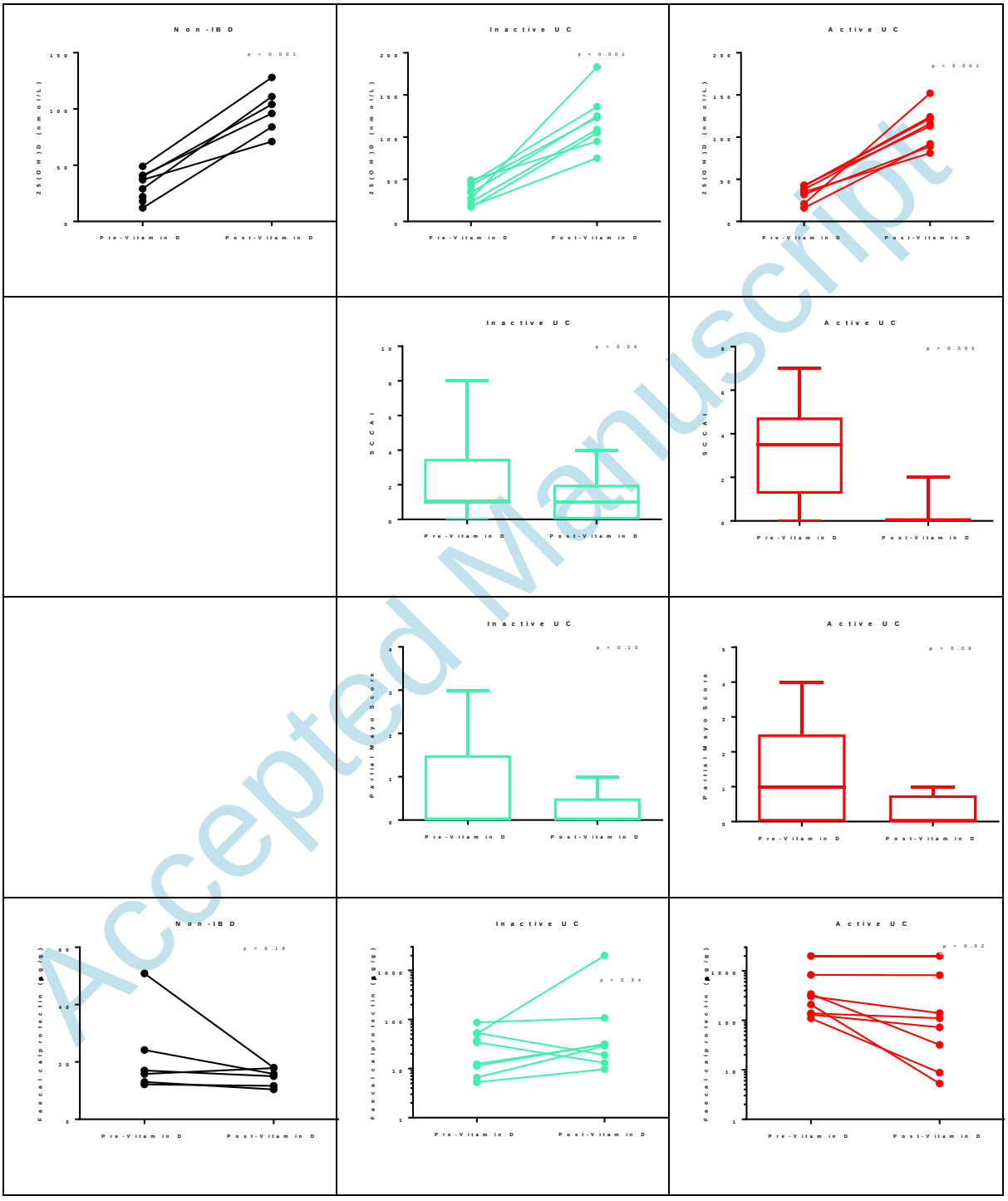
Figure 1. Change in clinical and laboratory indices following vitamin D supplementation in participants without IBD, those with inactive and active UC.



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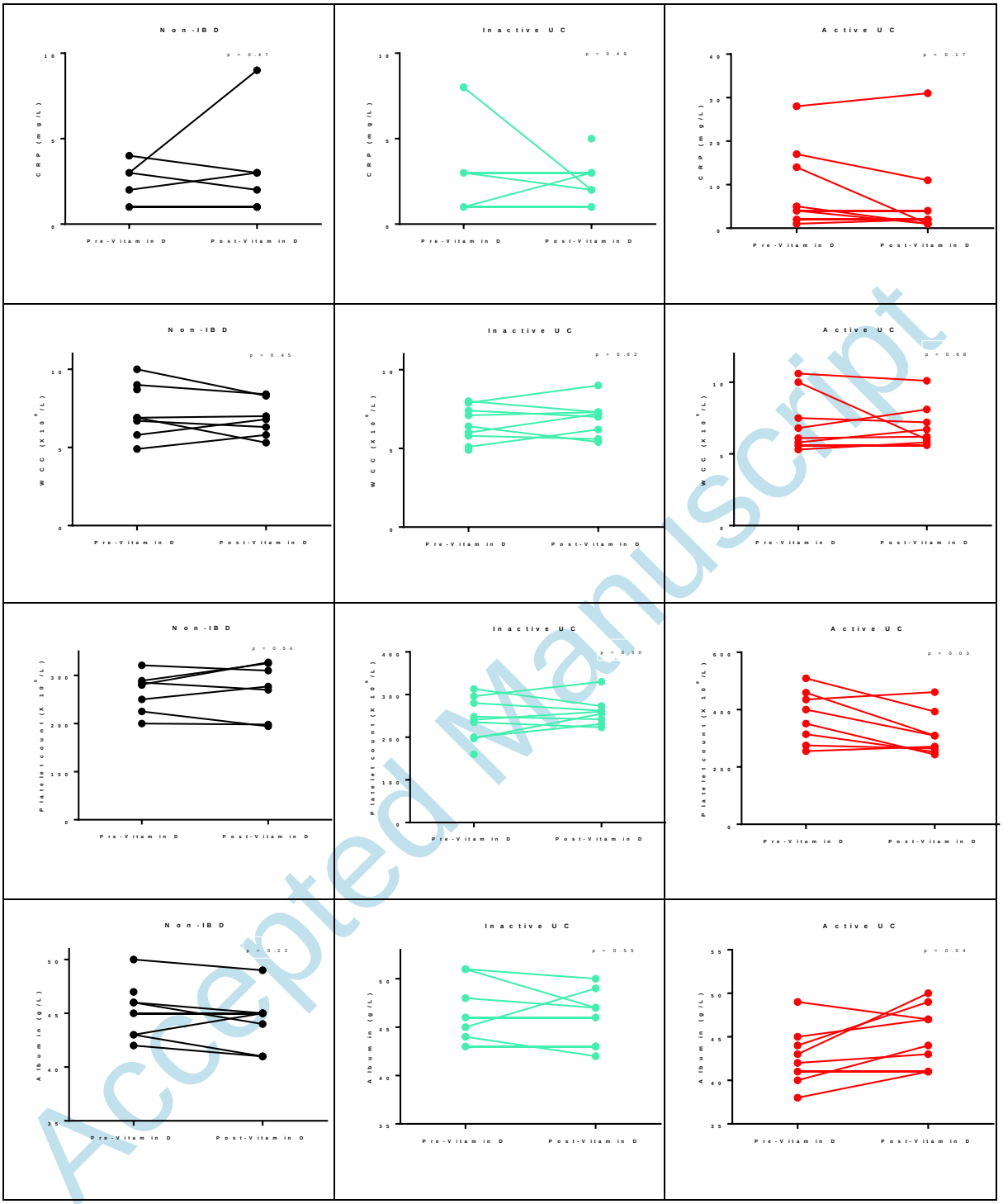

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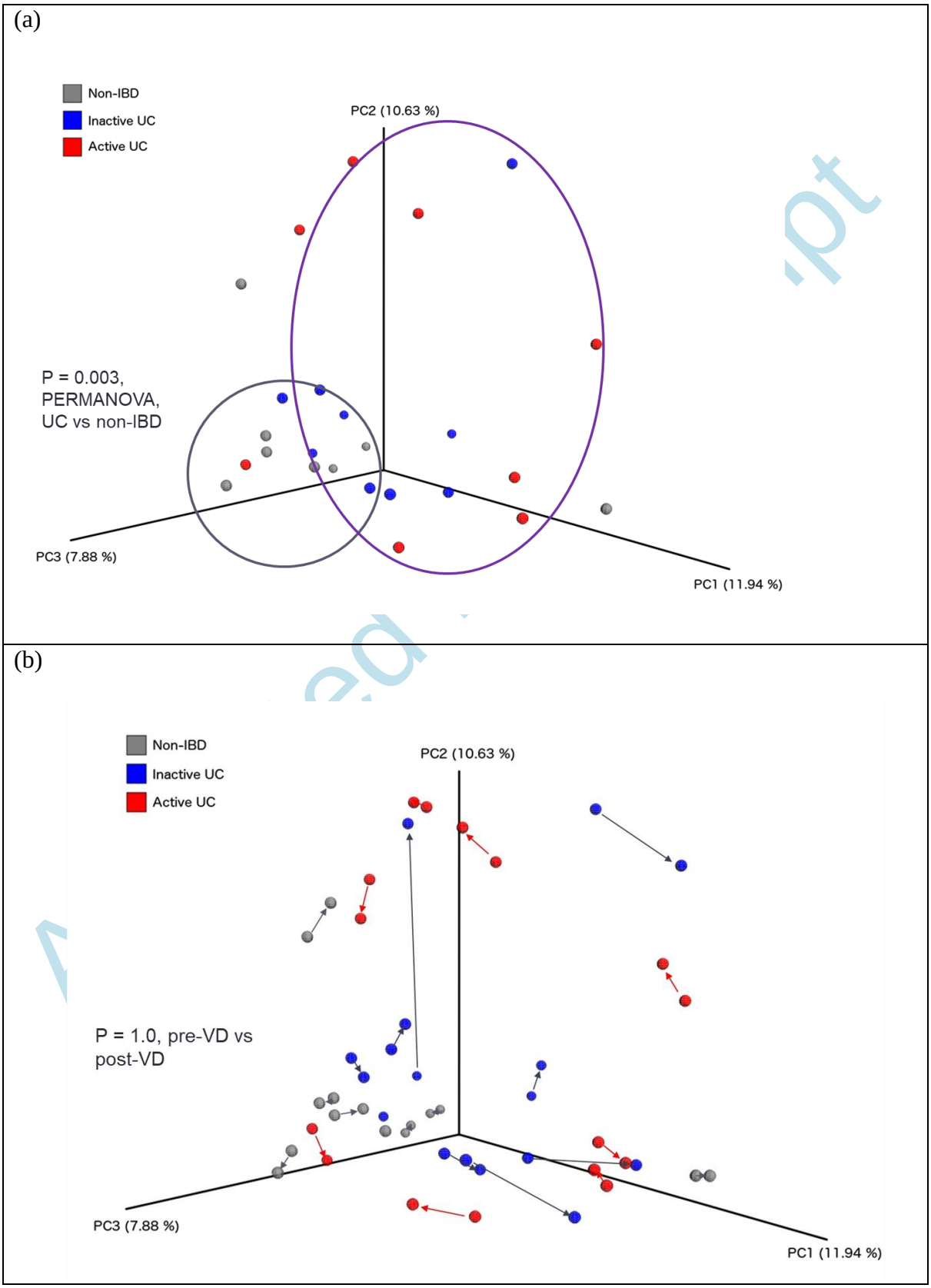
Figure 2. Principal Co-ordinate Analysis (PCoA) Plots at (a) baseline and (b) before and following vitamin D supplementation



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