

Vitamin D administration leads to a shift of the intestinal bacterial composition in Crohn's Disease patients, but not in healthy controls

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Abstract

Objective: Dysbiosis is a common feature in the pathogenesis of Inflammatory Bowel Diseases (IBD). Environmental factors, e.g. vitamin D deficiency, seem to play a role in the intestinal inflammation of IBD. The aim of the study was to test if vitamin D administration has an impact on the bacterial composition in Crohn's disease (CD) compared to healthy controls (HC).

Methods: We have performed a prospective, longitudinal, controlled interventional analysis in seven patients with CD in clinical remission and ten HC to investigate the effect of vitamin D administration on the intestinal bacterial composition using 16S rRNA gene amplicon sequencing. Clinical parameters were assessed.

Results: In contrast to HC, the microbial communities of the CD patients changed significantly during early vitamin D administration. However, a further increase of the Vitamin D level was associated with a reversal of this effect and additionally with a decrease in the bacterial richness in the CD microbiome. Specific species with a high abundance were found during vitamin D administration in CD, but not in HC, e.g. the abundance of *Alistipes*, *Barnesiella*, unclassified Porphyromonadaceae (both *Actinobacteria*), *Roseburia*, *Anaerotruncus*, *Subdoligranulum* and an unclassified Ruminococaceae (all *Firmicutes*) increased significantly under 1 week of vitamin D administration in CD.

Conclusions: Vitamin D has a specific influence on the bacterial communities in CD, but not in HC. Administration of vitamin D may have a positive effect in CD by modulating the intestinal bacterial composition and also by increasing the abundance of potential beneficial bacterial strains.

Key words: Crohn disease, Inflammatory Bowel Diseases, microbiome, vitamin D

Introduction

Inflammatory bowel diseases (IBD) consist of Crohn's disease (CD) and Ulcerative colitis (UC) which are chronic inflammatory diseases of the alimentary tract [1]. While the pathogenesis is still not completely understood, it is known that an important part of the disease is an inappropriate activation of the mucosal immune system caused by intestinal microbiota in patients with a genetical risk profile [1–4]. Mutations in the nucleotide-binding oligomerization domain-containing protein 2 (*NOD2*) gene, encoding for the NOD2 receptor, are a major risk factor for the development of CD [5–8]. In addition, environmental factors, such as vitamin D deficiency, seem to play a role in the pathogenesis of IBD [8, 9]. Vitamin D is commonly known as an important regulator of calcium and phosphate metabolism and is therefore essential for bone health [10, 11]. However, there is increasing evidence that vitamin D also plays an important role as a regulator of the innate and adaptive immune system [11–13]. In a murine colitis model, the application of 1,25(OH)₂ D₃ was associated with reduced mucosal injury [14]. Vitamin D deficiency has a high prevalence in IBD patients [15, 16]. Interestingly, stimulation with vitamin D increased the expression of the NOD2 receptor in primary monocytic and epithelial cells linking the innate immune system with vitamin D [17]. Vitamin D was shown to have an effect on the dendritic and monocyte-derived macrophages cell function [19–21]. From a clinical point of view, the administration of a TNF- α inhibitor is associated with higher vitamin D levels in IBD [18]. On the other hand, in CD patients where infliximab was initiated, lower vitamin D levels were associated with a higher rate of clinical remission at week 14 [22].

Until now, it is still not clear, whether vitamin D administration has a beneficial effect on the disease course in IBD. In a prospective study, infliximab treatment had a positive effect on bone metabolism [23]. Along the same line in different studies in CD patients an inverse association between vitamin D levels and intestinal inflammation was found [24, 25]. In several interventional studies, vitamin D administration appeared to have beneficial effects on the clinical disease activity and the CRP value in IBD patients [26–29].

However, if vitamin D also has an influence on the intestinal bacterial composition is still not known. In murine models vitamin D and the vitamin D receptor (VDR) are important regulators of the intestinal bacterial composition [30–32]. In a recent genome-wide association study mutations in the *VDR* gene were associated with a different intestinal microbial profile [33].

In order to address the hypothesis, whether administration of vitamin D has an effect on the intestinal microbial communities in CD, we performed a controlled prospective and longitudinal analysis in CD patients in clinical remission and healthy controls (HC). This is the first study which shows a significant shift of the microbial communities in CD during vitamin D administration, while no effect of vitamin D was observed in HC. This result suggests a disease-specific impact of vitamin D on the microbial profile in CD.

Material and Methods

Study Design

The study was approved by the institutional review board of the University Medical Center Rostock (A 2016-0109). The study was registered in the German Clinical Trials Register (Registration number DRKS00013485). Written informed consent was obtained from each participant prior to enrollment.

Seven patients with ileocolonic CD (Montreal classification [34]: L3) and Vitamin D deficiency were recruited from the Outpatient Clinic of the University Medical Center Rostock. The patients were in clinical remission and did not have a change of their CD specific therapy in the previous 6 months before inclusion in this study. Healthy controls (HC) with vitamin D deficiency and no history of IBD were recruited from the Rostock Medical School. In both groups, oral vitamin D administration was performed with Cholecalciferol 20.000 IE daily from day 1 until day 3, then every second day for 4 weeks. In this study, we aimed for a target vitamin D level between 100 and 150 nmol/l. Serum 25-OH vitamin D levels were measured weekly (before administration = week 0). In both groups, 380.000 IU 25-OH vitamin D were administered per patient over the course of the study. In CD, calprotectin levels were measured at week 0 and week 4. Fresh stool was collected weekly (week 0, 1, 2, 3 and 4) for analysis of the intestinal bacterial microbiota. The disease activity in CD patients was assessed using the CDAI [35], other clinical parameters, i.e. localization of the disease, medical therapy, duration of the disease, were recorded. All clinical characteristics are shown in **Table 1**.

DNA extraction

DNA was isolated from EDTA whole blood with the QIAamp DNA blood mini kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany).

NOD2-genotyping

Regarding the *NOD2*-Genotyping we focused on the three major mutations in the *NOD2* gene (SNP 8; R702W, NCBI reference SNP ID: rs2066844, SNP 12; G908R, NCBI reference SNP ID: rs2066845 and SNP 13; 1007fs, NCBI reference SNP ID: rs2066847). For the amplification of the regions of the *NOD2* gene, the Taq PCR Master Mix Kit (Qiagen) and primers/PCR conditions as specified in **Table 1** were employed. Additionally, the PCR products were subjected to Sanger sequencing (Seqlab, Göttingen, Germany). The resulting data were analyzed using with the software Chromas, version 2.6.

Preparation of 16S rRNA gene sequencing libraries, sequencing run and data analysis

The isolated DNA was amplified with the bacterial 16S rRNA gene primers Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC) [36]. The amplicon PCR, the index PCR, a quantity and a quality control and the sequencing of the individual libraries as a pool in one Illumina MiSeq run was performed as described in a previously published study [37]. The raw sequences of the study were deposited at the SRA (Short Sequence Archive) under the accession number "PRJEB21819". For our data analysis, the resulting sequences were assembled using the program QIIME 1.9.1 [38] with the "joins paired-end Illumina reads" function with default settings to merge forward and reverse

sequence with an overlap of at least 30 bp. We discharged sequences without overlap. After converting “fastq” to “fasta” using the "convert_fastaqual_fastq" function, we used the SILVA NGS pipeline for the resulting sequences, using default settings [39]. This pipeline aligns the reads to a database with the SINA aligner [40]. With this program, problematic reads such as PCR artefacts (including potential chimeras) and non-ribosomal reads are filtered out and consecutively discarded. The reads are then quality filtered using the following settings: reads less than 50 aligned nucleotides and reads with more than 2% of ambiguities, 2% of homopolymers or low alignment quality, defined by a 40 alignment score reported by SINA. After the alignment, the sequences were dereplicated by clustering by a 98% sequence identity to each other using CD-HIT [41]. The longest read in each cluster was BLAST searched against SILVA SSU Ref 128 to classify the sequences. The resulting classification of the reference sequence of each cluster was mapped to all the members of the cluster as well as their replicates. The sequences which have an average BLAST alignment coverage and alignment identity of less than 93% were considered as unclassified and we defined them to the virtual taxonomical group “No Relative”.

Statistical analyses

The disease activity, measured by CDAI and the Calprotectin value in the CD cohort from week 0 and week 4 were compared using a paired T-test.

Operational taxonomic unit (OTU) counts based on genus level were rarefied to 3500 reads per sample using the single_rarefaction.py script implemented in QIIME. To compare the dominant taxa in the different time points (week 0 until week 4) during vitamin D administration, the occurrence of the 22 most abundant OTUs was visualized in a heatmap using Explicet [42]. This program was also used in a rarefaction-based analysis, here with

bootstrapping for richness. We visualized the differences in the bacterial community composition through non-metric multidimensional scaling (NMDS) plots using Bray-Curtis dissimilarity indices based on a genus rank classification. We used the software package PAST [43] for non-parametric multivariate analysis of variance (PERMANOVA) to analyze the differences between OTU compositions and a Tukey's pairwise test to calculate significant differences between the number of OTUs between the patient samples. A linear discriminant analysis (LDA) effect size (LEfSe) analysis [44] was performed to determine bacterial groups which are significantly different between samples using the "One against all" strategy for multi-class analysis. The program LEfSe uses a non-parametric test that couples standard tests for statistical significance with additional tests encoding biological consistency and effect relevance.

Results

Effect of Vitamin D administration on clinical parameters in CD and HC

Vitamin D administration led to a significant increase of the 25-OH vitamin D levels in CD and HC (CD: week 0 (39.7 ± 23.0), week 4 (121.4 ± 43.2); $p < 0.001$; HC: week 0 (29.6 ± 6.3), week 4 (143.0 ± 25.2); $p < 0.001$; **Fig 1**). The increase of the vitamin D levels was not significantly different in CD compared to HC at each time point from week 0 to week 4. We identified two of the seven patients to have a mutation in the *NOD2* gene (SNP 8, heterozygous, Table 1) but these did not show a specific response to the Vitamin D treatment (data not shown).

In CD, vitamin D administration was associated with a non-significant decline of the CDAI and the calprotectin levels between week 0 and week 4 (CDAI week 0: 81.6 ± 43 ; CDAI week 4: 57.3 ± 36.2 , **Fig 2A**; Calprotectin week 0: $297.8 \text{ mg/kg} \pm 613.0$; Calprotectin week 4: $178.6 \text{ mg/kg} \pm 305.6$; **Fig 2B**). One patient showed a strong decline of the calprotectin level under vitamin D administration (week 0: 1685 mg/kg ; week 4: 793 mg/kg) while staying in clinical remission for the whole study period.

Vitamin D administration is associated with a temporal shift of the intestinal microbiota in CD, but not in HC

Bacteroidetes and *Clostridia* were among the most abundant phyla/classes in the bacterial community analysis (SFig. 1). This could be observed at all time points (w0 – w4) in CD as well as in HC. However, to assess the effect of vitamin D administration on the different microbial communities, we visualized changes of the bacterial composition on the bacterial

genus levels using NMDS in HC (**Fig 3A**) and CD (**Fig 3B**). In the HC group, there was no significant difference between week 0 and week 4 as well as between the different time points of the vitamin D administration. In the CD group, we observed a shift of the bacterial composition from week 0 to week 1, which reversed in week 2, 3 and 4 (**STable 2**). The shift from week 0 to week 1 was not significant, which might be attributed to the fact that the bacterial composition of two patients at week 0 clustered within the bacterial communities of week 1. The bacterial community at week 1 differed significantly from to the bacterial communities at week 2, week 3, and week 4 in all patients during vitamin D administration (week 1/week 2: $p = 0.007$; week 1/week 3: $p = 0.01$; week 1/week 4: $p=0.011$). In contrast, the bacterial community at the beginning of the study (week 0) did not differ significantly from the bacterial communities at week 2, 3 and 4. The use of a TNF-alpha inhibitor, the disease activity (CDAI) and the presence of a mutation in the *NOD2* gene did not have a significant effect on the bacterial communities during vitamin D administration.

Vitamin D administration is associated with specific abundant bacteria in CD, but not in HC

To further assess if vitamin D has an impact on the abundance of specific strains in CD and HC, we characterized alterations in the bacterial genera using LEfSe [44]. While in CD different specific abundant bacteria before and at different time points during vitamin D administration were found, the analysis of the HC cohort did not detect any abundant strains. The results of the CD group are shown in a heatmap (**Fig 4**). The bacteria were stratified into 5 groups to differentiate the response to the vitamin D administration (highest abundance at week 0, week 1, week 2, week 3 and week 4). Before vitamin D administration (week 0) the typical bacteria (significantly more abundant based on LEfSe analysis) were

Sutterella (*Betaproteobacteria*), next to *Bifidobacterium* (*Actinobacteria*) and an unclassified lineage of the *Lachnospiracea*. After one week of vitamin D administration (week 1), the typical bacteria shifted towards an *Alistipes* (*Bacteroidetes*) dominated bacterial community. *Barnesiella* and unclassified Porphyromonadaceae (both *Actinobacteria*), as well as *Roseburia*, *Anaerotruncus*, *Subdoligranulum* and an unclassified Ruminococaceae (all *Firmicutes*) were also highly prevalent at this time point. After two weeks of vitamin D administration (week 2), the *Bacteroidetes* became less prominent and *Firmicutes*, especially *Faecalibacterium*, and *Veillonella* next to *Blautia*, *Fusicatenibacter* and *Intestinibacter* became the typical part of the bacterial community composition. In the third week of vitamin D administration, *Parabacteroides* (*Bacteroides*) were mainly abundant throughout the study but were significantly less abundant in week 1-2. Other indicator Operational taxonomic units (OTUs) were *Lachnospira* (*Firmicutes*), *Copro bacter* (*Bacteroides*) and *Parasutterella* (*Betaproteobacteria*). At week 4, *Lactobacillus* and *Megasphaera* (both *Firmicutes*) were significantly enriched. However, both had numerically a relatively low abundance.

Vitamin D administration is associated with a decrease of the bacterial taxa in CD, but not in HC

In previous studies, a reduction of the bacterial diversity was found in CD [45, 46]. To address the question, if vitamin D administration also has an effect on the diversity in CD and HC, we analyzed the number of bacterial taxa at the different time points (week 0 until week 4). There was no significant difference between CD and HC in the number of bacterial taxa before vitamin D administration was initiated (Tukey's test $p > 0.05$). While the number of

bacterial taxa in HC did not change significantly (HC; week 0 vs. week 4: Tukey's test $p > 0.05$), the bacterial taxa in CD decreased significantly during vitamin D administration (CD; week 0 vs. week 4: Tukey's test $p = 0.001$). Also, the number of bacterial taxa was significantly lower in CD compared to HC at week 3 and at week 4 of vitamin D administration (Tukey's test CD vs. HC; week 3: $p = 0.007$; week 4: $p = 0.0001$) (**Fig 5**).

Discussion

Dysbiosis is an important feature in the pathogenesis of IBD [45–48]. However, environmental factors such as vitamin D deficiency also seem to play a role in the development and the clinical disease course of IBD. Here we have tested whether there is an interaction of vitamin D and the intestinal microbiota in CD and HC because vitamin D has been shown to have an influence on the intestinal inflammation and therefore possibly also on the microbial communities as well. Vitamin D has been shown to ameliorate intestinal inflammation, but it is not clear whether this is a direct effect on the inflammatory response and the intestinal microbiota is only secondarily altered or whether this is a direct effect on the intestinal microbiota. Therefore, we chose to administer vitamin D in HC and CD patients who were in clinical remission and to analyze the microbial composition in both groups. Vitamin D administration was associated with a significant shift of the intestinal bacterial communities in CD patients at week 1. While the bacterial composition in HC was not significantly affected by vitamin D administration, we observed temporal changes in the bacterial communities during vitamin D administration in CD patients (**Fig. 3**). Vitamin D has therefore a strong effect on the microbial composition in CD, but not in HC, suggesting an important role in the pathogenesis. The strongest effect was observed at week one of Vitamin D administration. However, the increase of the 25-OH vitamin D levels over three additional weeks caused again a shift in the bacterial community that was more similar to the initial bacterial community of week 0. There may be alternative explanations for the temporal nature of the vitamin D effect: First, the effect of vitamin D on the bacterial communities is in itself only temporal and the microbiota reverts after two weeks. Second, there may be an optimal 25-OH vitamin D level (“vitamin D window”) which resembles the

vitamin D levels at week 1 (64.6 ± 29.8 nmol/l) and a further increase of the vitamin D levels might therefore cause reversal of effect on the bacterial composition.

Another important finding of our study is the change of the bacterial diversity during vitamin D administration (**Fig. 5**). Different studies found a lower bacterial species diversity in CD patients compared to healthy controls [49, 50]. However, in our study, the number of bacterial taxa was not significantly different between HC and CD before the administration of Vitamin D. This might be attributed to the fact that our patients were in stable clinical remission. After two weeks of Vitamin D administration, the number of bacterial taxa declined in CD patients but did not change in HC. In general, a higher diversity is thought to be associated with beneficial effects for the host. Our results suggest that an increased vitamin D concentration causes a loss of OTUs that are potentially beneficial and supports the “vitamin D window” hypothesis. However, it can be speculated that week 4 is too early to see a long-term effect of the vitamin D administration on the composition of the bacterial communities. Further, prospective studies will be needed to test the presence of an optimal vitamin D range in IBD.

The mechanisms, how vitamin D administration leads to a shift of the bacterial communities in CD remains speculative. Several studies have shown a correlation between vitamin D status, the mucosal immune system and the microbiota in IBD [51, 52]. Mutations in the Vitamin D receptor (*VDR*) are a risk factor for the development of IBD [8] and vitamin D can induce the *NOD2* pathway [17]. A recent study by Wang et al. showed that variations in the *VDR* have an influence on the gut microbiota [33].

In the treatment of IBD, the role of probiotics is still controversial. While in UC probiotics may have a positive effect in specific, well-defined clinical situations, the efficacy of probiotics in CD remains uncertain [53, 54]. In the present analysis we have identified

specific bacterial species which show an increased abundance during vitamin D administration. In addition to many other abundant strains, we observed a prominent change in the abundance of *Alistipes* and *Parabacteroides* during vitamin D administration in CD but not in HC. Both species appear to be of special importance in the pathogenesis of IBD. In contrast to the increased abundance of *Alistipes*, *Parabacteroides* showed a decreased abundance during vitamin D administration in the first and second week of the study but an increase at week 3 and week 4. In murine DSS-induced colitis, *Alistipes fingoldii* was protective against colitis [55]. Additionally, in a study using VDR knockout mice, *Alistipes* was depleted in coecal stool [31]. Other studies have proposed an important role for *Parabacteroides* in the pathogenesis of intestinal inflammation and found a decrease of *Parabacteroides* at inflamed compared to non-inflamed sites of the intestine [56, 57]. Additionally, the oral administration of *Parabacteroides distasonis* led to decreased severity in a murine model of dextran sulphate sodium (DSS) colitis [58]. Interestingly, in our analysis the abundance of *Parabacteroides* decreased in the first two weeks. As a consequence, *Alistipes* and *Parabacteroides* might therefore have beneficial effects on the host and vitamin D administration may possibly induce the growth of these species. In addition to these highly abundant species, several other OTUs were found to be significantly increased during vitamin D administration in the first week. These included *Roseburia*, of which a decrease of *Roseburia hominis* has been associated with a higher disease activity in UC [59]. Absence of *Roseburia* before colectomy in UC was associated with a higher risk of pouchitis in UC [60]. *Faecalibacterium prausnitzii*, which showed high abundance in week 1 until week 3, is a well-known butyrate-producing strain which is thought to have anti-inflammatory properties [61–63]. A specific microbial community containing *Barnesiella*, also showing a high abundance at week 1, had beneficial effects on the intestinal microbial composition

[64]. We hypothesize from our data that the appearance of several beneficial bacterial strains during vitamin D administration has a protective effect on the disease course in CD. Vitamin D might therefore enhance the probiotic capacity of these strains via an increased abundance. In line with that, vitamin D was required for a positive probiotic effect in a murine colitis model [65]. A combination of probiotics and vitamin D may have a synergistic effect on the disease activity in CD. Further studies will be needed to test this hypothesis.

One aspect of criticism in this study is that the control group was significantly younger than the CD group due to technical reasons, which might also have an effect on the intestinal microbial composition. Although we found a highly significant change of the bacterial communities in CD but not in HC, this effect could also be influenced by the age of the participants of this study. In further studies, age-matched control groups might clarify this aspect.

As a conclusion, the administration of vitamin D has a specific impact on the bacterial profile in CD, shown by the shift of the bacterial composition, the different highly abundant and potentially beneficial bacterial strains and the reduced diversity during vitamin D administration. In contrast to CD, in the HC group, no specific effects of vitamin D administration have been detected. This is the first controlled prospective interventional analysis which shows a specific effect of vitamin D administration on the microbial communities in CD, but not in HC. Therefore, vitamin D administration may be an important additional therapeutic intervention in the management of CD.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

Abbreviations

BP: base pair; CD: Crohn's disease; CDAI: Crohn's Disease Activity Index; CRP: C reactive protein; DSS: dextran sulphate sodium; DNA: deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; HC: Healthy Controls; IBD: Inflammatory Bowel Diseases; IU: international units; LDA: linear discriminant analysis; NGS: next generation sequencing; NMDS: non-metric multidimensional scaling; NOD2: nucleotide-binding oligomerization domain-containing protein 2; OTU: operational taxonomic unit; PCR: polymerase chain reaction; RNA: ribonucleic acid; SNP: single nucleotide polymorphism; SRA: Short Sequence Archive; TNF: Tumor necrosis factor; UC: Ulcerative Colitis; VDR: vitamin D receptor

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Supplements

Table S1:

SNP	Primer	PCR conditions
8	Forward: 5'-CCTCTTCAATTGTGGCAGGC-3' Reverse: 5'-CTCCTGCATCTCGTACAGGC-3'	1. 5 min, 94 °C 2. 1 min, 94/60/72 °C (45 cycles) 3. 7 min, 72 °C 4. 4 °C
12	Forward: 5'-ATGGAGGCAGGTCCACTTTG-3' Reverse: 5'-TTACCTGAGCCACCTCAAGC-3'	
13	Forward: 5'-GATGGTACTGAGCCTTTGTTGA-3' Reverse: 5'-CAGACTTCCAGGATGGTGTCAT -3'	

Primer and PCR conditions for *NOD2*-genotyping

Table S2:

Statistical analysis of the bacterial composition at the different weeks in CD using a Bonferoni corrected PERMANOVA.

Week	0	1	2	3	4
0		0.205	0.439	0.231	0.103
1			0.007	0.01	0.011
2				0.649	1
3					0.038
4					

The similarity or dissimilarity of the different OTUs at each time point under vitamin D administration in CD was calculated using a Bonferoni corrected PERMANOVA. The bacterial composition in the CD cohort at week 1 shows a significant difference compared to the composition at week 2, 3 and 4. In the HC cohort, no significant difference between each time points was found.

Figure S1:

Stack bargraphs of the bacterial community composition on phyla/class level at week 0 (w0), week1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) in Crohn's disease (CD) and healthy control (HC).

Table 1:**Clinical characteristics**

Mean age HC (n=10)	24.8 ± 3.1 years
Mean age CD (n=7)	44.9 ± 12.4 years
Sex HC (male/female)	7/3
Sex CD (male/female)	4/3
Localization of CD (Montreal)	L3
Number of NOD2-mutations (CD)	n = 2 (SNP8 heterozygous)
CD patients treated with TNF-alpha inhibitor (Infliximab / Adalimumab)	n = 5
Total 25-OH vitamin D administered	380.000 IU
CDAI week 0	81.6 ± 43
CDAI week 4	57.3 ± 36.2
Calprotectin week 0	297.8 ± 613
Calprotectin week 4	178.6 ± 305.6

The clinical characteristics of the CD and HC group are depicted in Table 1.

Figures

Fig 1. 25-OH Vitamin D levels in Crohn's disease (CD) and healthy control (HC) from week 0 to week 4

The administration of Vitamin D increases the 25-OH vitamin D levels in CD and HC significantly. However, the increase of the vitamin D levels did not differ between CD and HC at the different time points from week 0 to week 4.

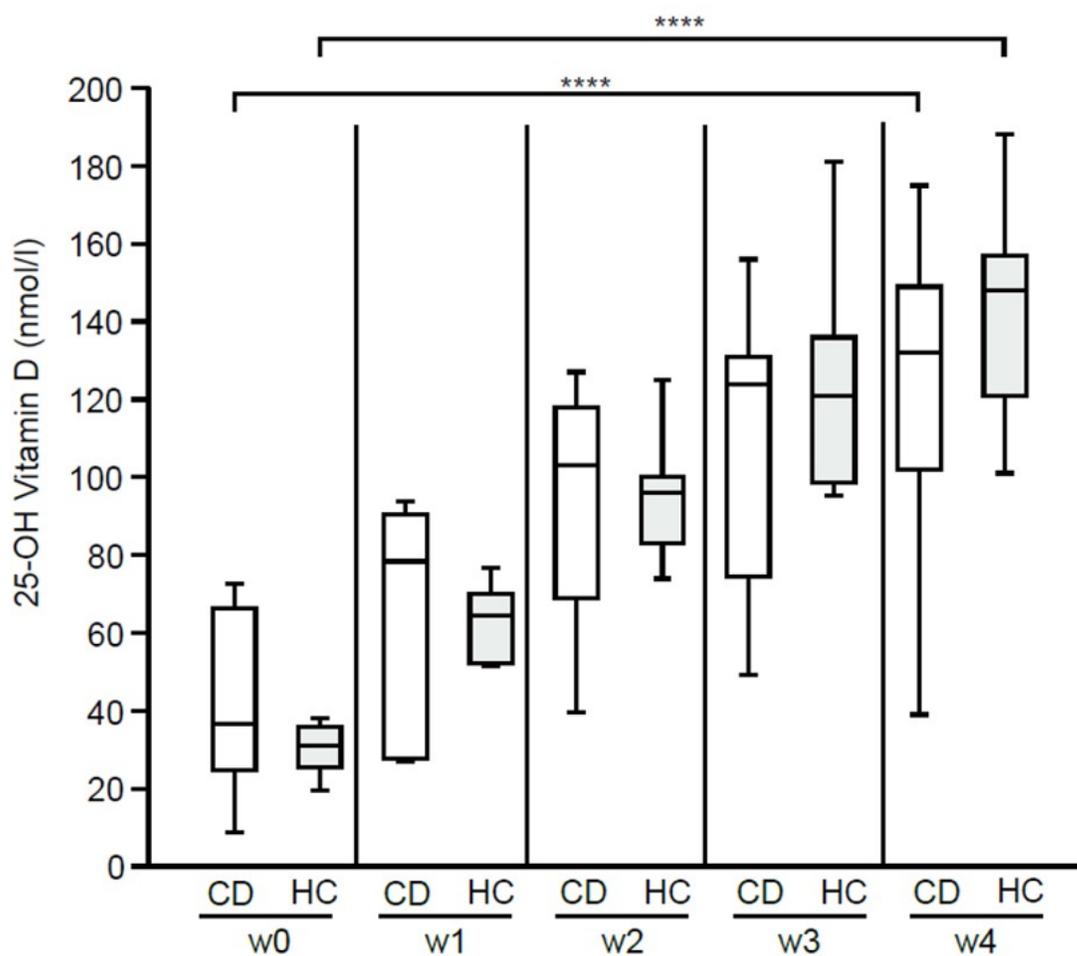


Fig 2. CDAI and Calprotectin during vitamin D administration in CD patients

Vitamin D administration leads to a non-significant decrease of the CDAI (**A**) and the Calprotectin level (**B**) in CD. One patient showed a strong decline of the CDAI and the calprotectin level. Note: logarithmic scale in **Fig 2B**.

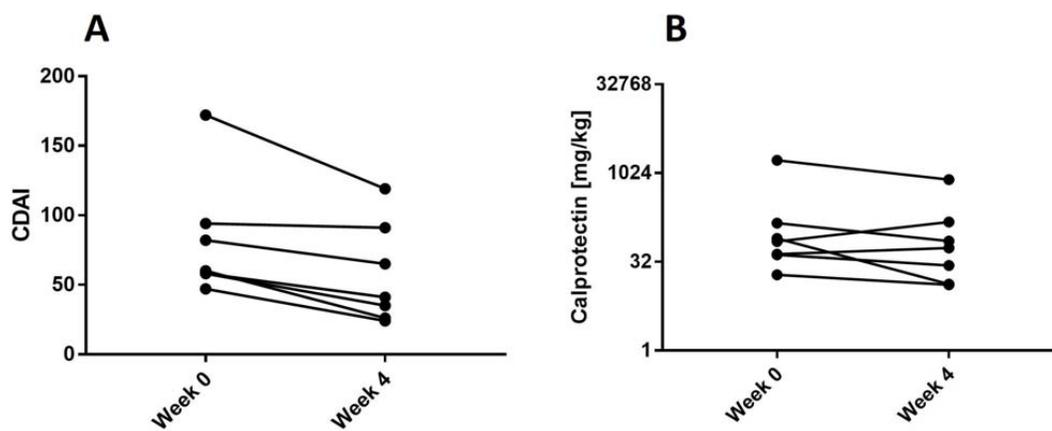
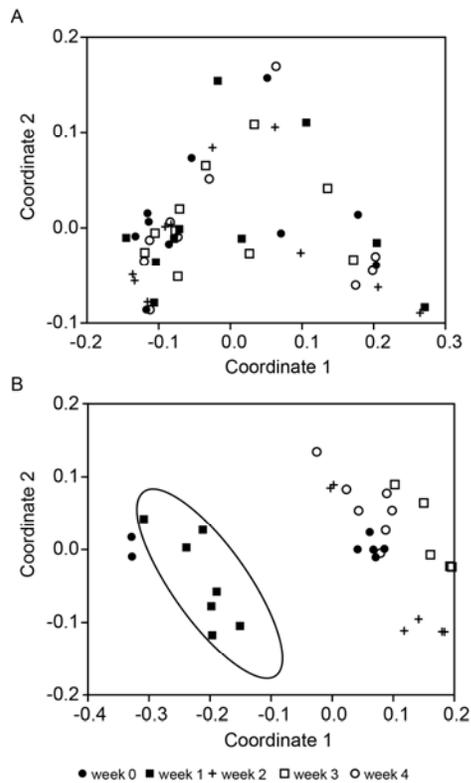
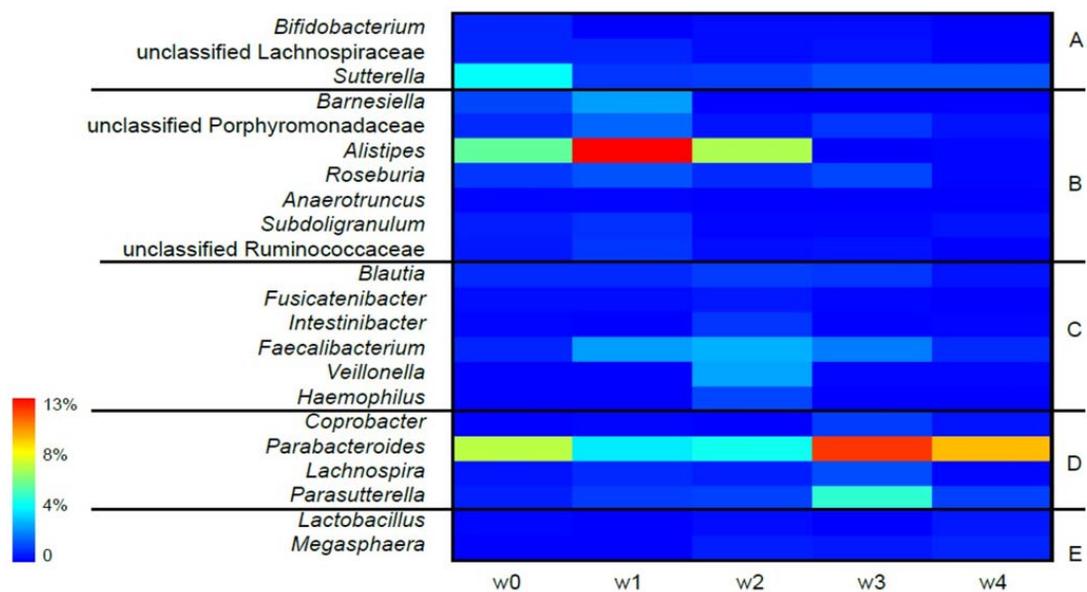


Fig 3. Vitamin D administration leads to a temporal shift of the bacterial communities in CD, but not in HC.



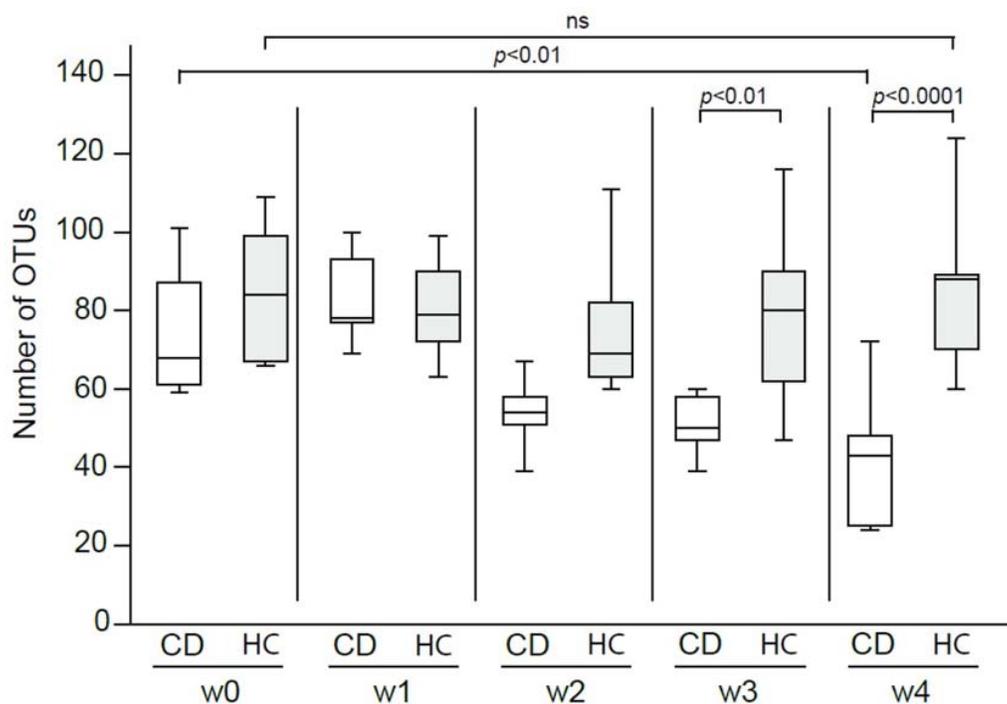
Non-metric multidimensional scaling plot (NMDS) based on Bray-Curtis dissimilarity of the bacterial communities from HC (Fig 3A) and CD (Fig 3B) at different time points during vitamin D administration (NMDS 0.092) without sample CD6 on week 4 and CD1 on week 3. 10 HC and 7 CD received vitamin D for 4 weeks. While there is a shift of the bacterial composition in the CD group from week 0 to week 1, no such effect is found in HC.

Fig 4. Heatmap of the bacterial communities during vitamin D administration in the CD cohort.



Group A shows the bacteria with a high abundance in week 0 (w0), group B in week 1 (w1), group C in week 2 (w2), group D in week 3 (w3) and group E in week 4 (w4). This figure shows the abundant species in the CD group. No abundant species were found during vitamin D administration in the HC group (data not shown).

Fig 5. Number of bacterial taxa during vitamin D administration for four weeks (w0 –w4) in Crohn's disease (CD) and healthy control (HC).



While the number of bacterial taxa, shown in operational taxonomic units (OTU) at week 0 (w0) is not different between CD and HC, the number of taxa decreases significantly in CD compared to HC during vitamin D administration at week 3 (w3) and week 4 (w4).