



Review

Role of vitamin D on gut microbiota in cystic fibrosis

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ABSTRACT

This review explores the potential for vitamin D to favorably alter the gut microbiota, given emerging evidence of the role of vitamin D in controlling mucosal inflammation in the gut. It will focus on cystic fibrosis (CF) patients, a population with both vitamin D deficiency due to gut malabsorption and an altered gut microbiota composition. Recent evidence shows that vitamin D acts to maintain the integrity of the gut mucosal barrier by enhancement of intercellular junctions that control mucosal permeability and reduction of pro-inflammatory cytokines such as IL-8. In addition, vitamin D receptor-mediated signaling has been shown to inhibit inflammation-induced apoptosis of intestinal epithelial cells. As a result of these effects on the intestinal mucosa, maintenance of sufficient vitamin D status may be essential for the development of a healthy gut microbiota, particularly in conditions defined by chronic mucosal inflammation such as CF. We hypothesize here that high dose vitamin D may be used to favorably manipulate the aberrant mucosa seen in patients with CF. This may result in improved clinical outcomes in association with a low inflammatory environment that allows beneficial bacteria to outcompete opportunistic pathogens. Current evidence is sparse but encouraging, and additional evidence is needed to establish vitamin D as a therapeutic approach for gut microbiota modification.

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1. Introduction

Cystic fibrosis (CF), an autosomal recessive condition, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR is an ion channel important for the

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maintenance of chloride transport, essential for normal functioning of epithelial surfaces of lung and pancreas [1]. CF is characterized by recurrent lung infections, malabsorption and failure to thrive related to abnormal mucosa and chronic inflammation in the respiratory and digestive systems.

A common co-morbidity of CF is vitamin D deficiency, resulting from fat malabsorption secondary to exocrine pancreatic insufficiency, inadequate sun exposure, and inadequate dietary vitamin D intake [2]. An important extra-skeletal function of vitamin D is its role in immune regulation, such as upregulation of antimicrobial peptides expression (cathelicidins and B-defensins) and turning off effector T cells Th1 and Th17 [3–5]. More recently, vitamin D was identified as a factor influencing the gut microbiota, through the vitamin D receptor (VDR) [6,7]. Mechanisms underlying this association involve the role of VDR in maintaining the integrity of the intestinal mucosal barrier [8].

Microbiota refers to the complex community of microorganisms that colonize the human body, including the skin, respiratory, gastrointestinal and urogenital systems [9]. The intestinal microbiota contains 10^4 microorganisms with more than 3 million genes, collectively referred to as the microbiome [10]. This complex and dynamic microbial community plays a central role in human health, including macronutrient metabolism, nutrient capture and immune function. In addition to non-modifiable factors that affect the microbiota, such as maternal microbial composition and host genetics, it is profoundly influenced by modifiable factors such as diet and medications, especially antibiotics [11]. Moreover, disruption of the gut microbiota (or dysbiosis) has been linked to both intestinal inflammation, such as inflammatory bowel disease, and extra-intestinal conditions associated with chronic inflammation and metabolic dysfunction, such as obesity and metabolic syndrome [12–16]. There is also emerging evidence that aberrant intestinal mucosa in CF patients leads to a state of intestinal dysbiosis [17,18], likely due to a combination of abnormal mucus secretion and repetitive antibiotic exposures for treatment of recurrent sino-pulmonary infections.

Given the emerging studies on the influence of vitamin D on gut microbiota, this review explores the current evidence for

modifying the gut microbiota to influence clinical outcomes in CF. Vitamin D supplementation may provide an easy and inexpensive potential option for positive modulation of the gut microbiota that would lead to improvement of CF-related symptoms.

2. Methods

A PubMed search was performed to select relevant publications, using the following search terms: dysbiosis, gut microbiota, cystic fibrosis, vitamin D, probiotics, mucosal inflammation and vitamin D receptor. We examined all studies in humans and in rodent models.

3. Altered gut microbiota in CF

Emerging research suggests that intestinal microbiota may be disrupted in individuals with CF [17], with multiple independent groups showing a significant decrease in species richness and enrichment of potentially pathogenic species in individuals with CF compared to healthy controls [18–22]. Key findings of these studies have been reviewed in Table 1.

3.1. Review of murine studies

Lynch et al. studied the gut microbiota composition in CFTR knock out (KO) mice compared to wild type mice (WT) and found that CFTR KO mice displayed significantly less bacterial richness and diversity compared to WT animals. Moreover, they found that the loss of functional CFTR is associated with an increase in pathogenic bacteria, such as *Mycobacteria* and *Bacteroides fragilis*, and a decrease in bacteria that provide protection at mucosal surfaces, such as *Acinetobacter lwoffii* and members of the order *Lactobacilliales* [23]. Similarly, Bazett et al. looked at the intestinal microbiome in CFTR knockout mice with three different phenotypes and found alterations in intestinal structure, bacterial overgrowth, and dysbiosis as compared to wild type mice [24].

Table 1
Studies examining alteration of gut microbiota in patients with CF.

Type of study	Study design (n)	Effect on microbiota	Clinical significance	Reference
Cross-sectional and Longitudinal	Compared fecal microbiota between patients with CF(21) and healthy siblings(24)	Increased <i>Enterobacteriaceae</i> <i>Gammaproteobacteria</i> Decreased <i>Bacteroides</i> <i>Prevotella</i>	Not assessed	[18]
Prospective longitudinal analysis	Gut microbiota samples collected from birth to 34 months (13)	Increased Not applicable Decreased <i>Parabacteroides</i> <i>Bacteroides Bifidobacterium</i>	Decline in the abundance of Parabacteroides prior to initial <i>Pseudomonas aeruginosa</i> colonization ($p < 0.001$)	[22]
Prospective longitudinal analysis	Comparison of gut microbiota between children with CF (23) aged from 0 to 18 years old and healthy controls(35)	Increased Proteobacteria <i>Enterococcus</i> , <i>Veillonella</i> <i>Megasphaera</i> <i>Clostridium</i> group XI <i>Blautia</i> Decreased <i>Ruminococcaceae</i> <i>Alistipes</i>	Not assessed	[19]
Prospective longitudinal analysis	Analysis of gut microbiota from patients with CF and healthy siblings (21)	Decreased <i>Bifidobacterium</i> <i>Clostridium</i> cluster XIVa	Not assessed	[21]

3.2. Review of human studies

Cross-sectional and longitudinal observation studies looking specifically at the gut microbiota in CF have indicated a trend of suppression in the potentially beneficial *Bifidobacterium* species accompanied by a trend of increase in potentially pathogenic organisms – *Fusobacterium* and *Enterobacteriaceae* species [18,21]. Members of the *Bifidobacterium* species are considered a marker of intestinal health and a decrease in abundance has been associated with intestinal diseases such as inflammatory bowel disease and colorectal cancer and extra-intestinal diseases, such as obesity and asthma [25]. In a recent study examining gut microbial communities in children with CF compared to age matched healthy controls, Nielsen et al. found that gut dysbiosis in CF patients was present in early childhood with further digression with advancing age. They observed an increase in the relative abundance of *Ruminococcaceae* family and *Alistipes* genus (potentially important beneficial strains for mucosal health) with increasing age in healthy children, but not in CF. In contrast, this analysis revealed that potential pathogenic organisms, such as *Escherichia* and *Shigella* (Proteobacteria), *Enterococcus*, *Veillonella*, *Megasphaera*, *Clostridium* group XI and *Blautia* were more abundant in CF compared to healthy controls [19].

3.3. Potential interest in gut microbiota manipulation for CF management

The gut microbiota is involved in both regulatory and pro-inflammatory immune pathways, via interaction with intestinal mucosal receptors [26,27]. Although direct evidence is limited, recent studies imply a potential role of the gut microbiota in CF management, via affecting inflammatory and immune response. It is well established that repeated exacerbations of pulmonary symptoms in persons with CF are associated with a progressive decline in lung function. Carmody et al. described that the onset of symptoms of exacerbation may be preceded by marked shifts in the baseline microbial communities [28]. The suppressed species richness found by Duytschaever et al. [18] suggest that biodiversity of gut microbiota remains lower in children with CF compared to healthy siblings. A growing body of evidence suggests that microbial diversity is an important marker of gut health, with a low diversity associated with intestinal inflammation and several diseases, from inflammatory bowel disease to obesity [16,29–31]. Further, in patients with CF, Madan et al. have shown that a decrease in gut microbiota diversity and loss of beneficial microbial species were associated with the first CF pulmonary exacerbation,

as well as with initial *Pseudomonas* colonization. Thus, it may be reasonable to hypothesize that interventions that alter the gut microbiota in a beneficial way may have a potential role in clinical management in CF. Efrati et al. found that probiotic supplementation in patients with CF who had mild-moderate lung disease and were chronically infected with *Pseudomonas aeruginosa* resulted in a significant decline in the rate of pulmonary exacerbations for 6 months post-treatment compared to the prior two years, with none of the patients experiencing pulmonary exacerbation during the probiotic treatment [32]. In a similar study, Guarino et al. assessed the effect of the probiotic *Lactobacillus rhamnosus* strain GG (LGG) on pulmonary outcomes in nineteen children with CF through a prospective, randomized, placebo-controlled, cross-over study. They found a reduction in pulmonary exacerbations and hospital admissions and an improvement in the forced respiratory volume in 1 s (FEV1) in patients treated with LGG [33]. While the exact mechanism is unclear, these studies suggest that probiotics may affect the gut microbiota by locally improving intestinal barrier function [34] and preventing membrane barrier disruption by pathogens [35]. These data suggest that manipulation of gut microbiota has the potential to improve respiratory outcomes in CF.

4. Vitamin D and the gut microbiota

A growing body of research suggests that gut epithelial VDR signaling plays a central role in maintaining the integrity of the intestinal mucosa. The proposed mechanism is through the inhibition of inflammation-induced epithelial cell apoptosis and enhancement of intercellular junctions [8,36].

4.1. Review of murine studies

Studies in murine models report that vitamin D deficiency increased the severity of colitis and bacterial numbers in the colons of mice, with these deleterious effects being reversed by vitamin D supplementation [37,38]. DSS (Dextran sulfate sodium) increases mucosal permeability and causes disruption of the mucosal barrier in intestinal epithelial cells, thus the DSS induced experimental colitis model has been widely used to study the effects of vitamin D on mucosal injury [39]. Table 2 reviews studies that looked at the effects of VDR and 1,25(OH)₂D₃ on protecting mice from DSS induced colitis. These studies elucidate that vitamin D deficiency predisposes mice to severe colitis as compared to their wild type counterparts [38,40,41]. Ooi et al. also demonstrated that treatment of 1,25(OH)₂D₃ deficient mice with 1,25(OH)₂D₃

Table 2
Role of VDR/1,25(OH)₂D₃ in protection from experimental colitis.

Murine model and genotype	Study design	Study findings	Reference
C57BL/6 VDR KO vs. WT mice	-Exposed to DSS ^a	-Increased mortality of the VDR KO mice at doses of DSS that only caused a mild form of colitis in WT mice. -Administration of 1,25(OH) ₂ D ₃ resulted in decreased weight loss and improved colonic injury scores in the DSS-induced inflammation in WT mice compared to controls.	[41]
C57BL6 Cyp27b1 (CYP) KO and WT mice	-Mice were exposed to DSS to induce colitis - 1,25(OH) ₂ D ₃ was supplemented to some of the mice in the CYP KO group.	- CYP KO mice were more susceptible to DSS colitis and had greater intestinal permeability after exposure to DSS. - CYP KO mice treated with 1,25(OH) ₂ D ₃ showed decreased severity of colitis as compared to their untreated counterparts.	[40]
C57BL/6 mice	Mice were fed on vitamin D deficient or vitamin D sufficient diets and then treated with DSS to induce colitis	- DSS induced weight loss and colitis was more prominent in vitamin D deficient mice - Vitamin D deficient mice showed an inability to contain colonic enteric bacteria even in the absence of experimental colitis	[38]

Abbreviations: VDR KO- Vitamin D receptor knockout, WT – wild type, Cyp KO- Cyp27B1 knock out (unable to produce 1,25(OH)₂D₃ due to defective 1 α -hydroxylase enzyme).
^a DSS colitis model refers to mice with experimental colitis produced by administration of 2.5% dextran sodium sulfate (DSS) in drinking water.

decreased the severity of colitis [40]. Further, Zhang et al. showed that $1,25(\text{OH})_2\text{D}_3$ prevented DSS induced disruption of tight junctions in colonic mucosa [42]. This data provides compelling evidence that vitamin D has an important role in maintaining epithelial integrity and regulating mucosal injury. It can thus be postulated that vitamin D deficiency leads to a disruption of the intestinal epithelial barrier and thus predisposes to dysbiosis. This was demonstrated in a recent study by Jin et al. who found that the lack of VDR in a VDR KO mice model is associated with intestinal dysbiosis and that VDR regulates the composition and functions of the gut microbiota. Precisely, they found a dramatic reduction of lactic acid bacteria in VDR knockout mice, associated with an increased abundance of *Bacteroides* and *Clostridium* [6]. *Clostridium* has been associated with intestinal virulence and enteritis and *Bacteroides*, may predispose the mice colon to cancer [43,44].

Further support of the role of vitamin D in intestinal dysbiosis is provided by the work of Sun et al. who showed that decreased expression of intestinal epithelial VDR disturbs microbial homeostasis, leading to fewer butyrate producing bacteria and predisposing VDR knock out mice to chemically-induced colitis [7]. Butyrate, a by-product of carbohydrate breakdown by microbiota, has a well established role in preventing mucosal inflammation and inhibiting colorectal cancer [45,46]. Moreover, Manor et al. found an increased capacity for catabolism of butyrate in the gut microbiota of children with CF, which may lead to increased mucosal inflammation [47]. In addition, Sun et al. found that administration of the bacterial product butyrate increased intestinal VDR expression and suppressed inflammation in a colitis model. They propose that lack of VDR causes dysbiosis by impairing ATG16L1, an important regulator for autophagy [7].

4.2. Review of human studies

While human studies are limited, similar observations have been made, whereby high dose vitamin D_3 supplementation

showed a beneficial effect on the human gut microbiota with a marked reduction in typical opportunistic pathogens and an increase in phylotype richness [48]. Healthy volunteers given vitamin D_3 supplementation experienced changes in the gut microbiota in the upper GI tract; decreased relative abundance of pathogenic species including *Pseudomonas*, *Escherichia* and *Shigella* and increased bacterial richness [48]. The possible mechanisms by which vitamin D may serve as a therapeutic intervention to decrease intestinal mucosal inflammation and thus influence gut microbiota are illustrated in Fig. 1.

4.3. Potential role for vitamin D to modify gut microbiota and pulmonary outcomes in CF

Crites et al. showed that loss of functional CFTR in intestinal epithelial cells leads to a pro-inflammatory status specifically increased IL-6 and IL-8 secretion [49]. Studies reviewed above establish that an underlying state of chronic inflammation in CF is associated with an altered intestinal microbiota. It can thus be postulated that vitamin D may attenuate intestinal inflammation and thus favorably modify the altered gut microbiota seen in the CF gut. This theory is supported by the work of Morin et al. who looked at the effects of vitamin D_3 and its metabolites in CFTR knockdown intestinal epithelial cells. Interestingly, they observed that $1,25(\text{OH})_2\text{D}_3$ leads to an inhibition of IL-8 and reduces cytokine-induced NF- κB nuclear translocation thus resulting in a suppression of inflammatory mediators [50].

The role for vitamin D in affecting pulmonary function was proposed by the Third National Health and Nutrition Survey (NHANES III) data, which showed an association between serum 25-hydroxyvitamin D levels and pulmonary function in healthy adults [51]. Further, Grossman et al. found that in adults hospitalized for a pulmonary exacerbation, a single, oral bolus of cholecalciferol was associated with better antibiotic therapy-free days, hospital-free days and 1 year survival compared to

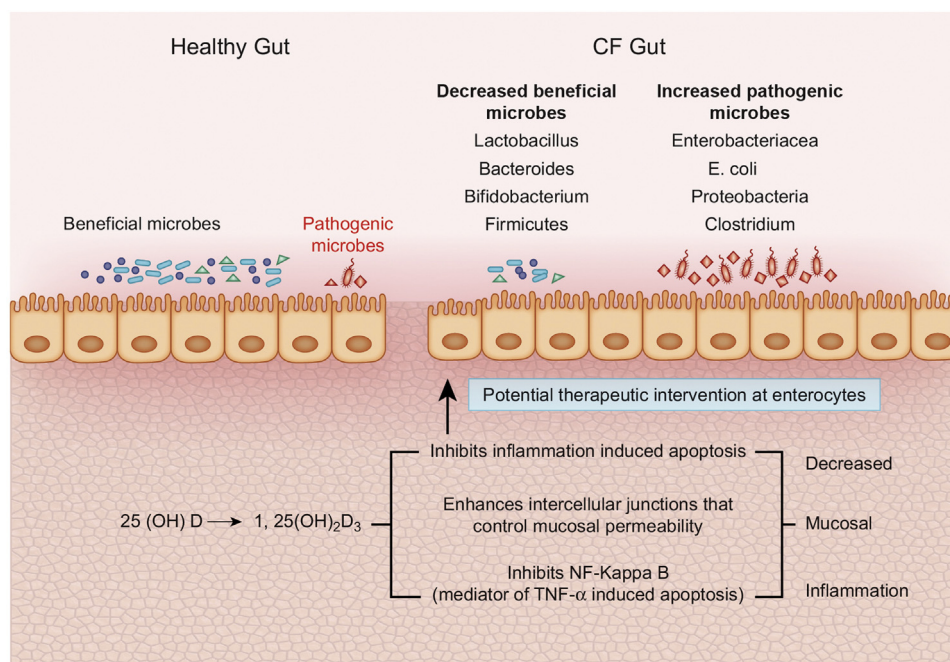


Fig. 1. Schematic representation of mechanisms by which vitamin D may decrease intestinal mucosal inflammation and thus alter dysbiosis in CF.

Description: Microbial dysbiosis is a hallmark of the CF gut underlying to chronic mucosal inflammation. Vitamin D has been proposed to decrease intestinal inflammation through the inhibition of inflammation-induced epithelial cell apoptosis and enhancement of intercellular junctions that are important in maintaining mucosal permeability. In addition it reduces cytokine-induced NF- κB nuclear translocation thus resulting in a suppression of inflammatory mediators. Together, these effects may restore gastrointestinal homeostasis and prevent intestinal dysbiosis in CF.

subjects in the placebo group [52]. In a longitudinal study by McCauley et al. in the pediatric population, higher 25(OH)D levels were associated with lower rates of pulmonary exacerbations [53]. Since these studies show that vitamin D has a role in pulmonary outcomes in the non-CF population it may be reasonable to hypothesize that these effects of vitamin D may be inferred to affect lung function in CF. In fact, recent studies suggest that vitamin D may have a role on pulmonary function and even pulmonary infections in CF. Sexauer et al. found that serum 25(OH)D levels showed a significant correlation with forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) in a group of 597 children and adults with CF [54]. In addition, Vanstone et al. found a significant association between serum 25(OH)D levels and annual frequency of pulmonary exacerbations in pediatric CF patients [55].

5. Conclusion

Review of studies above suggests that intestinal dysbiosis is a hallmark of CF and vitamin D therapy in CF patients with vitamin D deficiency may result in restoration of gut microbial homeostasis, with reduction in opportunistic pathogens and increase in microbiota diversity. Given the potential role of vitamin D on pulmonary function, this in turn may have implications for the improvement of clinical outcomes in patients with CF, with reduction in respiratory infections and pulmonary exacerbations, and ultimately improved survival. Above studies support the hypothesis that intestinal microbial dysbiosis is predominant in CF. However, the clinical significance of dysbiosis in CF are not entirely understood. There are also no studies to date to evaluate if vitamin D deficiency predisposes CF patients to greater dysbiosis and no clinical trials to show an improvement in gut microbial homeostasis with replacement with vitamin D. Further research is required to look at the role of vitamin D deficiency on modifying the gut microbiota in patients with CF and if supplementation with vitamin D can manipulate this altered microbiota.

Conflicts of interest

None.

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