



# BMJ Open Effects of ileocolonic delivered vitamin B<sub>2</sub>, B<sub>3</sub> and C (ColoVit) or the Groningen anti-inflammatory diet on disease course and microbiome of patients with Crohn's disease (VITA-GrAID study): a protocol for a randomised and partially blinded trial

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## ABSTRACT

**Background** Diet plays a pivotal role in the onset and progression of Crohn's disease (CD). Nutritional interventions revealed effects on intestinal inflammation and gut microbial composition. However, data from well-designed and controlled dietary trials are lacking. Therefore, evidence-based dietary recommendations are still unavailable to patients and physicians. Here, we aim to investigate the effects of an evidence-based anti-inflammatory diet, and an ileocolonic-targeted capsule containing vitamin B<sub>2</sub>, B<sub>3</sub> and C (ColoVit) on patients with CD and their healthy household members.

**Methods and analysis** In this multicentre, randomised, placebo-controlled, partially blinded nutritional intervention trial, we aim to recruit 255 CD patients with Harvey-Bradshaw Index <8 and a faecal calprotectin (FCal) cut-off of ≥100 µg/g at baseline. Participants will be randomised into two experimental intervention groups and one placebo group. In the experimental groups, participants will either adhere to the Groningen anti-inflammatory diet (GrAID) or ingest an ileocolonic-delivered oral vitamin B<sub>2</sub>/B<sub>3</sub>/C capsule (ColoVit). The study consists of a 12-week controlled interventional phase, which proceeds to a 9-month observational follow-up phase in which patients allocated to the GrAID group will be requested to continue the intervention on their own accord. Household members of participating patients will be asked to participate in the trial as healthy subjects and are allocated to the same group as their peer. The primary study outcome for patients is the change in FCal level from baseline. The primary outcome for household members is the change in gut microbial composition, which is set as secondary outcome for patients.

**Ethics and dissemination** The protocol has been approved by the Institutional Review Board of the Stichting Beoordeling Ethiek Biomedisch Onderzoek in Assen, the Netherlands. Written informed consent will be obtained from all participants. Results will be

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The randomised, placebo-controlled, partially blinded study design allows for stringent assessment of the effects of dietary interventions on disease outcomes in patients with mildly active Crohn's disease (CD).
- ⇒ The trial investigates a combination of nutritional, microbial, inflammatory, immunological and antioxidant parameters which will provide in-depth knowledge on the effects, associations and working mechanisms of proposed interventions.
- ⇒ Inclusion of adult household members of patients with CD as healthy volunteers could potentially increase dietary adherence and enables exploration of the effects of the intervention in healthy subjects, who are also prone to pro-inflammatory and anti-inflammatory effects of diets.
- ⇒ This study combines a strictly controlled interventional phase of 12 weeks, with a prospective follow-up phase of 9 months, accommodating insights into the degree of dietary adherence associated with disease outcomes.
- ⇒ Complete blinding of placebo-controlled diet interventions remains an important challenge.

disseminated through peer-reviewed journals and conference presentations.

**Trial registration number** NCT04913467.

## INTRODUCTION

Crohn's disease (CD) is a subtype of inflammatory bowel disease (IBD), and is characterised by relapsing inflammation which may occur throughout the entire gastrointestinal (GI) tract.<sup>1</sup> The exact pathogenesis of chronic

inflammation is multifactorial and yet to be elucidated. A prevailing hypothesis constitutes a complex interaction between host, commensal intestinal microbiota, and environmental factors trigger an aberrant and overactive immune response primarily against gut bacteria in genetically susceptible individuals.<sup>2</sup>

Diet has been recognised as one of the most pronounced environmental factors affecting the onset and course of CD.<sup>3–7</sup> A westernised diet, characterised by an elevated intake of fats and sugars and low intake of vegetables and fruits, is suspected to contribute to the development of IBD.<sup>8,9</sup> Western diet is associated with reduced diversity of bacterial strains colonising the intestines.<sup>10–11</sup> This phenomenon is often referred to as ‘dysbiosis’ which is marked by a disrupted composition and functionality of the gut microbiome, which is strongly correlated with disease severity in patients with IBD.<sup>12–13</sup>

Nutritional interventions and specific micronutrients have demonstrated prominent effects on intestinal inflammation and gut microbial composition.<sup>4–13–15</sup> Evidence regarding the pro-inflammatory and anti-inflammatory properties of specific food groups and food components are well described,<sup>16–17</sup> yet data from structured and controlled trials are lacking.<sup>18–20</sup> Patients with IBD experience an increased and underdiagnosed risk of developing malnutrition and nutritional deficiencies.<sup>21–22</sup> An inadequate nutritional status is associated with poor disease outcomes in IBD.<sup>23</sup> Thus, patients with CD and their treating physicians are in urgent need of evidence-based dietary recommendations. The VITA-GrAID study has been designed with the purpose of working towards such evidence-based dietary recommendations.

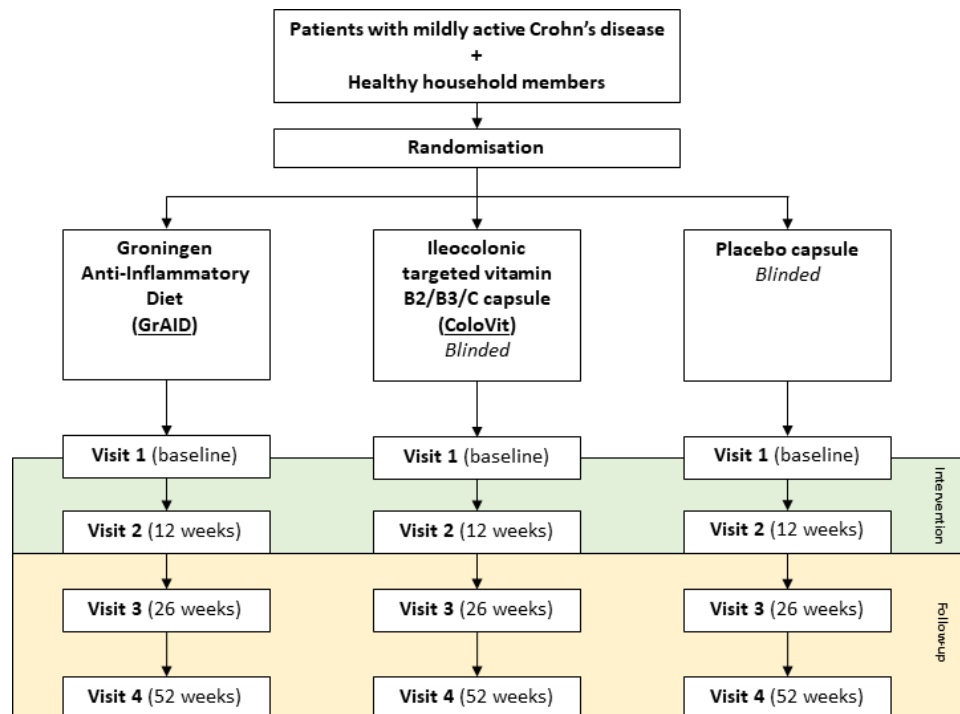
The VITA-GrAID study will investigate the effects of the Groningen anti-inflammatory diet (GrAID), a diet especially designed for the management of IBD.<sup>17</sup> The GrAID consists of three components. First, the GrAID aims to encourage and increase the intake of food items with anti-inflammatory properties and/or are expected to promote the growth of commensal gut bacteria beneficial to the host. Examples of these foods include fruits, vegetables, fish oil, nuts, fermented foods and fibre-rich products. Second, the GrAID aims to deprive possible pathogenic bacteria by reducing the intake of certain food items associated with pro-inflammatory responses. For this purpose, products with an overabundance of sodium and saccharose, red and processed meat, food additives, and alcohol will be excluded or minimised from a patient’s diet. Third, the adequacy of the diet will be assessed. Patients with IBD tend to experiment with their diets, since they often believe that diet contributes to their GI complaints. However, this unguided experimentation often leads to poor dietary quality.<sup>24–26</sup> Assessment of nutritional status, detection of deficiencies and identification of absence of potentially favourable food groups in a participant’s diet, are essential for proper implementation of dietary advice. Special attention is required for the intake of vitamin B<sub>12</sub>, folate and iron.<sup>18</sup> Furthermore, energy and protein needs might be increased due to elevated levels of inflammation

and disruption of the gut homeostasis. Therefore, a research-dietitian specialised in IBD will evaluate all meals on nutritional composition and their anti-inflammatory and pro-inflammatory features. A comprehensive review of the evidence that led to the development of the GrAID and a compositional comparison to other existing IBD diets can be found elsewhere.<sup>17</sup>

Furthermore, the effects of an ileocolonic-targeted vitamin-mixture containing riboflavin (vitamin B<sub>2</sub>), niacin (vitamin B<sub>3</sub>) and ascorbic acid (vitamin C) will be explored. Vitamins, in particular B-vitamins and C-vitamins, are identified as nutrients that have positive effects on gut microbial composition.<sup>27</sup> Mechanisms through which ileocolonic-targeted vitamins can impact gut health and modulate the gut microbiota are suspected to be both direct, by influencing metabolic processes of commensal gut bacteria, and indirect by introducing improvements in luminal ileocolonic conditions which allow for increased growth and activity of specific microbial clusters.<sup>27</sup> B-vitamins and C-vitamins are regarded as potent antioxidants, and both ascorbic acid and riboflavin supplementation in patients with CD have demonstrated significant reductions in oxidative stress markers.<sup>28–29</sup> On a local intestinal level, active scavenging of luminal reactive oxygen species promotes conditions that favour the selective growth of anaerobic gut bacteria that are vulnerable to oxidative conditions, whereas the presence of oxygen in the intestinal lumen favours the growth of potentially pathogenic bacterial strains.<sup>30–31</sup> Clinical studies involving subjects taking vitamins have shown an increase in microbial diversity and an increased abundance of bacteria belonging to short chain fatty acids (SCFA)-producing genera, which is accompanied by an increased presence of SCFA in faecal samples.<sup>32–36</sup> SCFA, for example, butyrate, have anti-inflammatory and barrier-protective effects.<sup>37</sup> Both riboflavin and ascorbic acid supplementation have demonstrated favourable changes in microbial composition through reduction of *Enterobacteriaceae*,<sup>29–32</sup> a bacterial family that has been associated with enhanced inflammatory responses.<sup>38</sup> Additionally, vitamin B<sub>2</sub>, B<sub>3</sub> and C have demonstrated supportive roles for immune and barrier function and have demonstrated anti-inflammatory properties. For example, ascorbic acid supports epithelial barrier function<sup>39</sup> and gut microbiota-derived niacin is implicated as a mediator of intestinal inflammation,<sup>40</sup> the latter of which is further supported by findings that addition of niacin to retention enemas used in the treatment of ulcerative colitis promoted mucosal healing.<sup>41</sup> Riboflavin supplementation in patients with CD introduced decreases in oxidative stress markers and inflammatory markers C reactive protein and interleukin 2.<sup>29</sup>

### Study aims and objectives

The aim of the VITA-GrAID trial is to investigate the effects of a 12-week dietary intervention with either the GrAID or an ileocolonic-targeted vitamin B<sub>2</sub>/B<sub>3</sub>/C oral capsule (ColoVit), divided into three research areas:



**Figure 1** Study design of the VITA-GrAID trial. Patients with Crohn's disease are randomised. Healthy household members are allocated in the same group as their peer.

1. Clinical aim: to investigate whether the proposed dietary interventions can lower intestinal inflammation, defined by faecal calprotectin (FCal), and favourably influence disease course and quality of life in patients with mildly active CD.
2. Biological aim: to investigate the effect of the proposed dietary intervention on gut microbial composition and immunological and oxidative stress parameters in patients and healthy participants.
3. Societal aim: to assess the adherence to the dietary intervention, during and postintervention in patients and healthy participants.

## METHODS AND ANALYSIS

### Study design

The VITA-GrAID study is a multicentre, randomised, placebo-controlled, partially blinded, clinical, nutritional intervention trial. In the context of a parallel study design, adult patients with low-to-mildly active CD will be randomised into three different study groups in a ratio of 1:1:1. Healthy household members of patients with CD will be asked to participate in this study as healthy subjects and will be allocated in the same study arm as their peer. The study consists of a 12-week controlled interventional phase, which proceeds to a 9-month observational follow-up phase. See [figure 1](#) for a schematic overview of the study design.

### Study setting

Patients will be recruited from the University Medical Centre Groningen (UMCG), and three non-academic top-clinical hospitals (Isala Zwolle, Medical Centre

Leeuwarden, Martini Hospital Groningen) in the northern part of the Netherlands.

### Eligibility criteria

Eligible patients have an established diagnosis of CD, experiencing low-to-mildly active CD with signs of intestinal inflammation. This is defined as a Harvey-Bradshaw Index (HBI) score <8 and an FCal  $\geq 100$   $\mu\text{g/g}$ . Household members of patients with CD are eligible if they are willing to participate as study subjects and have no chronic inflammatory condition. See [table 1](#) for a complete overview of inclusion and exclusion criteria.

Concomitant medication use (ie, biologics, immunomodulators) is allowed, if medication is given as maintenance therapy for at least 12 weeks. Patients are not eligible if a switch in medication regimen is planned during the 12-week intervention period. Usage of methotrexate, antacids and antibiotics is not allowed in any form during the study.

### Interventions

The study is divided into two experimental intervention groups, the GrAID group and the ColoVit group, and one comparative placebo group.

### GrAID

The GrAID is an evidence-based dietary advice aimed at patients with IBD.<sup>17</sup> Participants following the GrAID will increase their intake of foods with beneficial, anti-inflammatory properties, while avoiding intake of pro-inflammatory food components. Subjects will be asked to complete a 3-day food diary and a food frequency questionnaire regarding the past month before start of the

**Table 1** Inclusion and exclusion criteria of the VITA-GrAID trial

Patients with Crohn's disease	Household members of patients with Crohn's disease
<p>Inclusion criteria</p> <ul style="list-style-type: none"> <li>▶ ≥18 years of age.</li> <li>▶ Diagnosis of CD (Montreal L1, L2 or L3) existing for at least 12 weeks, confirmed by radiographic, histological and/or endoscopic criteria.</li> <li>▶ Low-mild disease activity with evidence of mucosal inflammation, defined as HBI score &lt;8 and faecal calprotectin ≥100 µg/g.</li> <li>▶ Willing and able to participate in both dietary intervention (adhere to the GrAID OR use the ColoVit/placebo capsule).</li> <li>▶ Able to attend an outpatient clinic.</li> <li>▶ If placed in ColoVit/placebo group, participant is willing to continue his/her normal habitual diet throughout the study period.</li> <li>▶ Willing to maintain his/her habitual physical activity patterns throughout the study period.</li> <li>▶ Understanding of the Dutch language.</li> </ul>	<ul style="list-style-type: none"> <li>▶ ≥18 years of age.</li> <li>▶ No diagnosis of IBD or any other chronic inflammatory condition.</li> <li>▶ Participant has no health conditions that would prevent him/her from fulfilling the study requirements as judged by the investigator on the basis of medical history and routine laboratory test results.</li> <li>▶ Able to attend an outpatient clinic.</li> <li>▶ If placed in ColoVit/placebo group, participant is willing to continue his/her normal habitual diet throughout the study period.</li> <li>▶ Willing to maintain his/her habitual physical activity patterns throughout the study period.</li> <li>▶ Understanding of the Dutch language.</li> </ul>
<p>Exclusion criteria</p> <ul style="list-style-type: none"> <li>▶ Pregnant or breast feeding.</li> <li>▶ Expecting drug intervention or therapy switch within 12 weeks.*</li> <li>▶ Symptomatic stenosis.</li> <li>▶ Use of methotrexate (due to possible riboflavin-methotrexate interaction<sup>49</sup>).</li> <li>▶ Use of antacids, H2-antagonist or proton-pump inhibitors that cannot be stopped for this trial (due to possible interference with pH-sensitive ColoPulse coating of the ColoVit).</li> <li>▶ Use of antibiotics, or probiotic and prebiotic supplements 4 weeks prior to the start of the intervention (due to effects on gut microbiome).</li> <li>▶ Use of oral vitamin/mineral supplements 1 week prior to the start of the intervention.†</li> <li>▶ GI resection or a history of GI conditions that by the judgement of the investigator could interfere with the working mechanism of the pH-sensitive ColoPulse coating.‡</li> <li>▶ Current pouch or stoma.</li> <li>▶ Colonoscopy and colon cleansing in the past 4 weeks (due to effects on gut microbiome).</li> <li>▶ Currently following a vegetarian or gluten free diet.</li> <li>▶ Swallowing disorders, not able to tolerate oral food, use of tube feeding.</li> <li>▶ Previously proven anaphylactic reaction to foods included in the food boxes.</li> <li>▶ Recent history of (within 12 months of screening visit) or strong potential for alcohol or substance abuse.</li> </ul>	<p>Same exclusion criteria for healthy household members as for patients with Crohn's disease.</p>
<p>*Medication use (ie, biologics, immunomodulators) is allowed, provided that medication is given as maintenance therapy for at least 12 weeks.          †Except for vitamin D, B12, magnesium and calcium.          ‡Ileocecal resections and partial colectomy allowed.          CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; GI, gastrointestinal; GrAID, Groningen anti-inflammatory diet; HBI, Harvey-Bradshaw Index; IBD, inflammatory bowel disease.</p>	

GrAID, allowing our dietitian to establish a personalised dietary advice based on current dietary habits of subjects. At baseline visit, a trained dietitian will provide extensive instructions regarding the GrAID and give advice on how to adhere to the anti-inflammatory diet. They will receive written information, a collection of recipes and

an extended list of food items that fit within the GrAID. During the 12-week intervention period, GrAID participants can choose weekly from GrAID-proof recipes and order their groceries from a list of preferred products through an online ordering service. These groceries will be weekly delivered in food boxes at their homes. This

delivery in food boxes enables a strict control on food choices of the participants and attributes to dietary adherence. Participants will have contact with an IBD dietitian at least five times during the intervention period, or more if alleged necessary by the dietitian. The research team will be available daily to answer questions. If a participant is unable to use food from the food boxes (eg, due to a dinner or a holiday), participants are instructed to adhere to the diet as adequately as possible, using the written information provided.

When the intervention period is completed, participants will be asked to follow the GrAID on their own accord during the follow-up phase.

### ColoVit

The ColoVit is a capsule containing 37.5 mg riboflavin (vitamin B<sub>2</sub>), 2.5 mg niacin (vitamin B<sub>3</sub>) and 250 mg ascorbic acid (vitamin C). The ColoVit capsules are encased with the ColoPulse coating, creating a pH-sensitive coating allowing pulsatile ileocolonic-targeted delivery of its contents.<sup>42–44</sup> Leveraging this technology, efficient uptake of vitamins in the upper GI tract can be circumvented<sup>45</sup> and thus the luminal environment of the terminal ileum and colon shall be exposed to the vitamin mixture in a controlled and site-specific manner. Participants in the ColoVit group will be instructed to adhere to their usual diet and exercise habits, while taking one ColoVit capsule two times a day for 12 weeks. Adherence of participants to their own pre-existing dietary habits will be assessed using dietary compliance questionnaires, 3-day food diaries and food frequency questionnaires.

### Control

The control group receives a ColoPulse-coated placebo capsule containing microcrystalline cellulose two times a day. Participants and researchers will be unable to distinguish ColoVit from placebo capsules. In both the ColoVit and placebo group, the intake of capsules will cease after the 12-week interventional period and participants will be instructed to follow their usual diet and eating habits during the 9-month follow-up period.

Once the follow-up phase is completed, participants in the capsule groups will be offered an appointment with the dietitian, where they will receive the written information belonging to the GrAID, which they can follow on their own accord if desired.

## Study outcomes

### Primary outcome for patients with CD

The primary outcome for patients with CD is the change in FCal from baseline till after the 12-week intervention period, compared with changes in control CD subjects.

### Primary outcome for healthy subjects

The primary outcome for healthy subject is changes in gut microbial composition from baseline till after the 12-week intervention period.

### Secondary outcome for patients with CD

The secondary outcomes assess the following changes in parameters in the experimental intervention group compared with the control group. Measurements are carried out at baseline, after 12 weeks, 26 weeks and 52 weeks:

1. Changes in gut microbial composition, using metagenomic sequencing-based profiles.
2. Number of flares. A flare is defined as FCal >200 µg/g and Crohn's Disease Activity Index (CDAI) ≥220.
3. Changes in clinical parameters, such as CDAI, medication use, surgery, and extra-intestinal manifestations of CD.
4. Changes in quality of life scores as assessed by Inflammatory Bowel Disease Questionnaire (IBDQ) and Food-Related Quality of Life (FR-QoL).
5. Changes in nutritional status, measured through anthropometric assessments and bioelectrical impedance analysis (BIA).
6. Changes in faecal SCFA, faecal pH, and faecal redox potential.
7. Changes in inflammatory, immunological, oxidative stress and nutritional parameters measured in blood.
8. Mineral and vitamin concentrations measured in urine.

Furthermore, as an important societal outcome, adherence to the dietary intervention will be assessed using a dietary compliance questionnaire, 3-day food diaries and a food frequency questionnaire (Groningen IBD specific Nutritional Questionnaires (GINQ)) which serves as a proxy for the habitual diet over the past 4 weeks. Adherence to the ColoVit/placebo will be assessed by counting empty capsule strips.

### Secondary outcome for healthy subjects

The secondary outcomes of healthy subjects are similar to the outcomes of patients with CD, with the exception that the 36-Item Short Form Health Survey is used to assess quality of life instead of the IBDQ and FR-QoL questionnaires. Furthermore, healthy subjects do not fill in the GINQ which is specific for IBD.

### Sample size

The number of participants was determined using data from the RISE-UP study<sup>29</sup> in which the effect of riboflavin supplementation was examined in a population of patients with CD (n=70). In this study, a group of patients was analysed which were characterised by an elevated FCal level (>200 µg/g), but with low clinical disease activity (HBI <5: 66.7%; HBI 5–7: 20.0%; HBI 8–12: 13.3%). The mean FCal was lowered in this study from 883.8 µg/g (SD: 885.0) to 669.6 µg/g (SD: 398.8) (~24% reduction). Based on paired t-test at the two-sided 5% significance level, a calculated correlation between groups of 0.6, and an estimated change in FCal as continuous and normally distributed outcome, a total of 64 participants in a 1:1 randomisation would yield at least 80% power. This power analysis is mostly relevant for the

ColoVit intervention. However, for the GrAID group, we expect a larger (50%) reduction in FCal, which is a larger clinically relevant difference  $d$  which would directly imply a lower required  $n$  for this group. Considering the potential non-normally distributed nature of FCal changes and the fact that participants may drop out during the study, we handled an extra inclusion of 30%, resulting in an  $n$  of 85 patients per intervention group.

### Recruitment

All outpatients with CD of the participating centres meeting the inclusion criteria will be identified when a hospital visit has been scheduled. Study information will be sent to these patients by email in the weeks before their scheduled appointment. During the visit to the outpatient clinic, the treating physician will check whether the patient has additional questions and is willing to participate in the study.

When the patient has signed informed consent to participate in the Vita-GrAID study, household members ( $\geq 18$  years of age) of patients will be invited to participate in this study as healthy volunteers. Potential participants will receive a letter with information about the study and will be contacted by the investigators to complete the informed consent procedure.

### Participant timeline

Table 2 depicts a participant's timeline concerning enrolment, interventions and assessments.

### Screening

Two weeks prior to baseline visit, patients will be screened for eligibility. FCal will be assessed if a calprotectin analysis has not been carried out within 4 weeks prior to baseline visit. Reasons for non-participation of eligible patient who decline participation will be recorded.

**Table 2** Schedule of participant's timeline concerning enrolment, interventions and assessments

	Prestudy phase		Allocation	Prebaseline visit	Interventional phase		Follow-up phase	
	$-T_1$	$T_0$		$T_{0.5}$	$T_1$ baseline visit	$T_2$ after 12 weeks	$T_3$ after 26 weeks	$T_4$ after 52 weeks
<b>Enrolment</b>								
Read study information	✓							
Informed consent	✓							
Eligibility screening	✓							
Faecal calprotectin for screening	✓							
Discuss with healthy household members for potential participation	✓							
Randomisation		✓						
<b>Intervention</b>								
Either follow the GrAID or two times daily intake of ColoVit/placebo					■	■		
If allocated in GrAID group, follow diet on own accord							■	■
If allocated in ColoVit/placebo group, stop taking capsules and adhere to own diet							■	■
<b>Assessments</b>								
Visit outpatient clinic					✓	✓		✓
Fill out 3-day food diary				✓		✓		✓
CDAI				✓		✓	✓	✓
Online dietary, lifestyle and Quality of Life questionnaires				✓	✓	✓	✓	✓
Body measurements and nutritional status					✓	✓		✓
Blood samples drawn					✓	✓	✓*	✓*
Collect faecal samples					✓	✓	✓*	✓
Collect 24-hour urine samples					✓	✓		
Adverse events						✓	✓	✓

\*Only patients with Crohn's disease.  
CDAI, Crohn's Disease Activity Index; GrAID, Groningen anti-inflammatory diet.

### Enrolment and baseline visit

After written informed consent is obtained, participants are randomised and a baseline visit is planned. Participants will be requested to fill in a 3-day food diary and return it by email before the baseline visit. This allows the dietitian to assess their dietary habits beforehand. Furthermore, patient will receive a CDAI-form to record their disease activity in the 7 days leading up to the baseline visit. Patients will be sent a container to collect a 24-hour urine sample and requested to bring the container to the baseline visit. At baseline visit, patients will be instructed about their intervention and baseline measurements will be conducted.

### Interventional phase

During the 12-week interventional phase, participants in the GrAID group will have contact with the dietitian at least five times, or more if alleged necessary by the dietitian. Participants in the ColoVit/placebo group will be contacted by phone within 2 weeks after start of the intervention to check adherence and answer potential questions. Participants will be instructed to contact the research team whenever questions arise.

### Second research visit and follow-up phase

After 12 weeks of experimental intervention, participants will be scheduled to visit the hospital for repeated measurements. Adherence, possible effects and adverse events related to the interventions will be assessed by the researcher together with the participant. Furthermore, participants will be asked about their disease course over the past 12 weeks, changes in (non-)drug regimen, doctor visits and alterations in eating and exercise habits. After this second visit, the follow-up phase will start. Participants in the GrAID group will be asked to follow the diet on their own accord. Participants in the ColoVit/placebo group will discontinue the capsules and will be asked to continue their own dietary and exercise habits for the coming 9 months.

### Last study visits and study end

At 26 weeks from baseline, disease course and dietary adherence will be assessed by phone. Participants will be requested to provide blood and faecal samples. At 52 weeks from baseline, the last research visit will take place and measurements will be repeated. The information regarding the GrAID will be made available to participants in the ColoVit/placebo group after their last study visit.

If a patient experiences or suspects a flare-up, the researcher will contact the treating physician and the flare will be objectified using CDAI-scores and FCal measurements. If a patient experiences a flare or has an alteration in drug regimen within the 12-week interventional period, they have the choice to continue the experimental intervention and attend the second research visit after 12 weeks of intervention. If a participant wishes to stop the experimental intervention, an effort will be

made by the researchers to accelerate the second study visit and the scheduled measurements.

### Allocation

All eligible patients who consent to participate will be randomised to either one of the two experimental groups or the control group with a 1:1:1 allocation. The allocation sequence is generated by the clinical pharmacy department of the UMCG, using a computer-generated schedule incorporating permuted blocks of 15. The randomisation list is controlled by the clinical pharmacy department of the UMCG, which serves as an independent party. Using a personalised study ID in combination with a recipe for ColoVit/placebo capsules, the pharmacist can hand out the allocated supplements to the participants, while both the researchers and participants remain blinded throughout the entire duration of the study. Participating household members will be allocated to the same group as their peer. If deemed necessary due to unsafe circumstances, the principal investigator can demand for unblinding of a specific participant.

### Confidentiality and data management

At enrolment, patients will receive a study ID number. All data will be entered and stored linked to this study ID number. All study-related information will be securely stored electronically and at the study site. Patient information will be stored in electronic Case Report Forms in the REDCap data capturing software and in locked file cabinets.

Questionnaires will be completed digitally via a hyperlink sent by the REDCap study website, which will be linked to the patient's study ID number.

Data will be stored during the study period and 25 years thereafter. Residual serum, faecal and urine samples will be stored for a maximum of 15 years at the UMCG for future research.

### Data monitoring

Because of the negligible risk associated with this dietary intervention study, no Data Safety Monitoring Board will be installed. Regular audits will be conducted once a year in all participating study centres by an independent monitor, in accordance with Dutch regulatory affairs.

### Patient and public involvement

The James Lind Alliance Priority Setting Partnership identified the role of diet in the management of mildly active CD as one of the top research priorities.<sup>19</sup> Participants will be actively asked to supply feedback on the experimental dietary interventions and the implementation thereof in their daily lives. Perceived obstacles will be discussed and potentially adjusted during the course of the study. A two monthly newsletter will be distributed among participating subjects and centres, providing updates on study progress.

## Adverse events

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental intervention. All adverse events reported spontaneously by the subject or observed by the investigator, or his staff will be recorded.

No serious adverse effects are expected for participants included in the GrAID arm. The GrAID is specifically designed to prevent nutritional deficiencies and offer patient-tailored dietary advice. Introduction of increased intakes of fibres might temporarily cause abdominal discomfort, bloating or diarrhoea. However, introduction of a semi-vegetarian diet, which has a quantitative fibre intake comparable to the GrAID, did not cause adverse effects such as abdominal discomfort.<sup>46</sup>

The administered daily dosages of the vitamin mixture administered through the use of ColoVit capsules consists of 75 mg riboflavin, 5 mg niacin and 500 mg ascorbic acid. Adverse effects could potentially be observed in subjects taking an excess of vitamin supplements, however, the administered daily dosages remain below the daily safety thresholds at which adverse events occur, as established by food safety authorities such as European Food Safety Authority and the US Council for Responsible Nutrition.<sup>47</sup> The systemic availability of the vitamins after administration is not yet fully understood as the vitamin mixture is given in an oral ileocolonic-targeted manner, which deviates from conventional oral vitamin formulations. Therefore, levels of systemic vitamin uptake will be carefully monitored to further understand the bioavailability after ileocolonic targeted vitamins supplementation. Participants will undergo close monitoring, for example, full blood counts, and adverse events will be carefully monitored and logged to be able to identify potential adverse effects of the vitamin supplement.

## Data collection

Data will be collected at baseline, at the end of the 12-week interventional phase and during the follow-up phase after 26 and 52 weeks. [Table 3](#) depicts a complete overview of measurements and their respective time points.

## Faecal calprotectin

FCal will be determined at baseline, and after 12, 26 and 52 weeks. A quantitative ELISA will be used according to the manufacturer's instructions.

## Microbiome analysis

To assess changes in microbial composition and diversity, faecal samples will be collected at baseline and after 12, 26 and 52 weeks. Participants will be asked to collect and immediately freeze their samples at home. Samples will then be transported on dry ice and stored at  $-80^{\circ}\text{C}$ . Metagenomic shotgun sequencing will be performed using techniques, platforms and statistical methods as previously described elsewhere.<sup>5 48</sup> In summary, DNA extraction will be performed using the AllPrep DNA/RNA Mini kit (Qiagen; cat. #80204). Metagenomic shotgun sequencing

will be performed using Illumina HiSeq platform. A more elaborate description of methods and statistical analysis used to assess microbiome composition can be found in online supplemental material 1.

## Nutritional assessment

Weight, height, midarm circumference, triceps skinfold thickness, waist circumference and handgrip strength will be measured. A BIA (SECA mBCA 525) will be used to measure fat-free mass, skeletal muscle mass, fat mass, total body water, and phase angle.

## Dietary adherence and adherence to the ColoVit

Adherence to the GrAID will be assessed using a dietary compliance questionnaire. Dietary compliance questionnaire consists of self-reported adherence to the dietary intervention, graded by participant from 0 to 10. Adherence to the diet will be further assessed by a trained dietitian using 3 days 24-hour food diaries and GINQ food frequency questionnaire. Adherence to the capsules will be assessed by requesting participants to bring spare capsules and capsules strips, and then count empty capsule strips.

## Measurements in blood, faecal and urine samples

Multiple inflammatory, immunological, oxidative stress and nutritional parameters will be measured in blood, faecal and urine samples. A complete overview of measurements is depicted in [table 3](#).

## Statistical analysis

The primary analyses will be conducted according to the intention-to-treat principle, while secondary analyses will be conducted on a per-protocol base. Baseline descriptive statistics will be calculated for each allocation group and compared between groups using  $\chi^2$  tests or Fisher's exact tests, independent sample t-tests or one-way analysis of variance, and Mann-Whitney U tests or Kruskal-Wallis tests, depending on normality and number of groups considered. In case of significant differences among the three groups, post-hoc comparisons with Bonferroni corrections will be applied to identify the exact significant difference. Correlations between baseline variables will be calculated using Pearson's or Spearman's correlation coefficients. All analyses will be adjusted for multiple comparisons using the Benjamini-Hochberg procedure in which a false discovery rate of 5% will be applied as significance threshold. Two-tailed p values  $<0.05$  will be considered statistically significant.

Short-term differences (baseline vs 12 weeks intervention) will be analysed using multivariate generalised linear models, allowing adjustment for relevant covariables including age, sex, body mass index, medication use, surgical history and baseline disease activity. In case of binary outcome variables (eg, remission vs active disease) or those with count distributions (eg, number of disease flares), the canonical logit and log link functions will be applied (corresponding to logistic and Poisson regression, respectively). Covariate selection will



**Table 3** Overview of measurement and used analysis

Measurement	Description/specification/method	Visits for patients with CD	Visits for healthy subjects
Demographic and health data	Age, gender, social economic status, smoking status, comorbidity, medication use (for comorbidities), surgical history. Collected from electronic patient files and during research visits.	V1, V2, V3, V4	V1, V2, V3, V4
Crohn's disease characteristics	Disease phenotype (Montreal classification), year of diagnosis, medication history, current medication, surgical history, extra-intestinal manifestations, complications of CD. Collected from electronic patient files and during research visits.	V1, V2, V3, V4	
HBI	Harvey-Bradshaw Index assess disease activity over 1-day period. Score <5 was defined as clinical remission, 5–7 as mild disease, 8–16 as moderate disease and >16 as severe disease.	V1, V2, V3, V4	
CDAI	Crohn's Disease Activity Index assess disease activity over a 1-week period. CDAI scores range from 0 to 600, with scores <150 corresponds remission, 150–220 mild activity, 220–450 moderate activity and >450 severe disease.	V1, V2, V3, V4	
Anthropometry	Weight (kg), height (cm), middle arm circumferences (cm) and triceps skinfold thickness (cm), abdominal circumferences (cm).	V1, V2, V4	V1, V2, V4
Handgrip strength	Strength measured in kg using a Jamar dynamometer.	V1, V2, V4	V1, V2, V4
BIA	Bioelectrical impedance analysis using a SECA mBCA 525. Measures fat-free mass (kg, % and kg/m <sup>2</sup> ), skeletal muscle mass (kg, % and kg/m <sup>2</sup> ), fat mass (kg, % and kg/m <sup>2</sup> ), total body water (L and %) and phase angle (°).	V1, V2, V4	V1, V2, V4
3-day food diary	During a period of 3 days, participants will be requested to log their food intake of 2 non-consecutive weekdays and 1 weekend day.	V1, V2, V4	V1, V2, V4
GINQ	Groningen IBD specific Nutritional Questionnaires. Food frequency questionnaire which consists of 105 questions about food items and serves as a proxy for the habitual diet over the past 4 weeks.	V1, V2, V4	
BAECKE	BAECKE physical activity questionnaire. Score range from 0 to 100. A higher score indicates higher habitual physical activity.	V1, V2, V4	V1, V2, V4
SNAQ	Short Nutritional Assessment Questionnaire is used to assess malnutrition. Assessment employs a score range of 0–5, with 0–1 indicates low risk of malnutrition, 2 moderate risk and ≥3 high risk.	V1	
MUST	Malnutrition Universal Screening Tool. Tool employs a score range of 0–6, with 0 indicates low risk of malnutrition, 1 moderate risk and ≥5 high risk.	V1	
SaskIBD-NR	Saskatchewan IBD-Nutrition Risk. Tool employs a score range of 0–9, with 0–2 indicates low risk of malnutrition, 3–4 moderate risk and 2 high risk.	V1	
IBDQ	Inflammatory Bowel Disease Questionnaire. <sup>50</sup> Questionnaire assesses quality of life in patients with IBD. Questionnaire employs a score range of 32–224, the higher the score the higher the Quality of Life.	V1, V2, V3, V4	
FR-QoL	Food Related Quality of Life Questionnaire. <sup>51</sup> Questionnaire assess food related quality of life specifically for CD patients. Questionnaire employs a score range of 29–145, the higher the score the higher the food-related Quality of Life.	V1, V2, V4	
SF-36	36-Item Short Form Survey to assess health-related Quality of Life. Survey employs a 0–100 scale. Higher scores indicate higher Quality of Life.		V1, V2, V4
General serum parameters/inflammatory parameters	White blood cell count, haemoglobin, haematocrit, thrombocyte count, leucocyte count, mean cell volume, erythrocyte sedimentation rate, machine diff, C reactive protein, creatinine, serum iron, total iron-binding capacity, ferritin, transferrin, total bilirubin, albumin, uric acid.	V1, V2, V3, V4	V1, V2
	Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, high-density lipoprotein, low-density lipoprotein, triglyceride.	V1, V2	V1, V2
Serum vitamins/minerals	A, beta-carotene, B1, B2, B3, B6, B11, B12, C, D, E, K1, calcium, chloride, magnesium, phosphorus, potassium, sodium.	V1, V2	V1, V2

Continued



Table 3 Continued

Measurement	Description/specification/method	Visits for patients with CD	Visits for healthy subjects
Serum redox status	Serum albumin-adjusted free thiols are used as a marker for serum redox status. Free thiol groups will be determined by parallel measurement of an L-cysteine (CAS-number 52-90-4, Fluka Biochemika, Buchs, Switzerland) calibration curve (concentration range from 15.6 µM to 1,000 µM) in 0.1 M Tris/10 mM EDTA (pH 8.2).	V1, V2, V3, V4	V1, V2
Serum cytokine panel	Cytokine panel analyses through MSD assay (including interleukin (IL) 6, IL-8, IL-10, IL-12, IL-17a, IL-22, IL-23, tumour necrosis factor-α, interferon-γ, eotaxin-1).	V1, V2	V1, V2
Serum gut permeability markers	Quantitative ELISA according to the manufacturer's instructions (including LPS binding protein, soluble CD14, endotoxin-core antibodies).	V1, V2	V1, V2
Faecal calprotectin	Quantitative ELISA according to the manufacturer's instructions.	V1, V2, V3, V4	
Faecal microbiome analysis	Microbiome analysed using metagenomic sequencing-based profiles. Proposed techniques, platforms and statistical methods are previously described. <sup>5 40</sup>	V1, V2, V4	V1, V2, V4
Faecal short chain fatty acids (SCFAs)	HPLC ion chromatography system (Metrohm AG, Herisau, Switzerland) will be used for fatty acid analysis.	V1, V2	V1, V2
Faecal redox status	A pH and redox metre (PCE-228-R, PCE Instruments, Southampton, UK) is used to measure the pH and the redox potential according to the manufacturer's instructions.	V1, V2	V1, V2
Faecal pH	A pH and redox metre (PCE-228-R, PCE Instruments, Southampton, UK) is used to measure the pH and the redox potential according to the manufacturer's instructions.	V1, V2	V1, V2
24-hour urine	Vitamin B3, creatinine, magnesium, phosphate, sodium, potassium, albumin, urea.	V1, V2	V1, V2
Dietary compliance check	Adherence to the GrAID will be assessed using a dietary compliance questionnaire. Dietary compliance questionnaire consists of self-reported adherence to the dietary intervention, graded by participants from 0 to 10. Adherence to the diet will be further assessed by a trained dietitian using 3-day food diaries and the GINQ.	V2, V3, V4	V2, V3, V4
Capsule count	Adherence to the ColoVit/placebo will be assessed by counting empty capsule strips.	V2	V2
Adverse events	Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental intervention.	V2, V3, V4	V2, V3, V4

\*V1: baseline visit; V2: visit after 12 weeks; V3: visit after 26 weeks; V4: visit after 52 weeks.  
CD, Crohn's disease; HPLC, high performance liquid chromatography; LPS, lipopolysaccharides; MSD, meso scale discovery.

be performed a priori (see above) based on clinical relevance as well as through performing backward selection procedures to identify statistically significant covariates (in which  $p < 0.20$  will be used as significance threshold in univariable assessment and  $p > 0.05$  as exclusion threshold in multivariate analysis).

Long-term differences (longitudinal changes in measured parameters at baseline, 12 weeks, 26 weeks and 52 weeks) will be analysed using general linear mixed models and general additive mixed models. In mixed model analysis, the random slope will be estimated by incorporating patient ID and time-point as random effects, while the allocated intervention group will be incorporated as the between-subject factor to estimate random intercepts.

Statistical analysis will be performed using SPSS Statistics V.28.0 software package (IBM Corp.) and the Python programming language (V.3.9.0, Python Software Foundation, <https://www.python.org>).

#### Ethical and dissemination

This study will be conducted in accordance with the principles of the Declaration of Helsinki (October 2013). The study protocol has been approved by the Institutional Review Board of the Stichting Beoordeling Ethiek Biomedisch Onderzoek Assen (CCMO code: NL66008.056.21). Local ethical approval has been gathered in the investigational sites of UMCG (2021/348), Martini hospital (2021/069), Medical Centre Leeuwarden (2022/00005) and Isala Hospital (2022/0603). Any amendments made to the protocol after METc approval will be submitted

by the investigator to the METc in accordance with local procedures and the clinical trial registry will be updated accordingly. Written informed consent will be obtained by the clinical researchers from all patients prior to study enrolment.

Results of the study will be disseminated in peer-reviewed journals and presented at scientific meetings.

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