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Polyphenols and Ulcerative Colitis: An Exploratory Study of the Effects of Red Wine Consumption on Gut and Oral Microbiome in Active-Phase Patients

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Scope: This paper explores the effects of moderate red wine consumption on the clinical status and symptomatology of patients with ulcerative colitis (UC), including the study of the oral and intestinal microbiome.
Methods and results: A case control intervention study in UC patients is designed. Intervention patients ($n = 5$) consume red wine (250 mL day^{-1}) for 4 weeks whereas control patients ($n = 5$) do not. Moderate wine consumption significantly ($p < 0.05$) improves some clinical parameters related to serum iron, and alleviates intestinal symptoms as evaluated by the IBDQ-32 questionnaire. 16S rRNA gene sequencing indicate a non-significant ($p > 0.05$) increase in bacterial alpha diversity after wine intervention in both saliva and fecal microbiota. Additional comparison of taxonomic data between UC patients ($n = 10$) and healthy subjects ($n = 8$) confirm intestinal dysbiosis for the UC patients. Finally, analysis of fecal metabolites (i.e., phenolic acids and SCFAs) indicates a non-significant increase ($p > 0.05$) for the UC patients that consumed wine.
Conclusions: Moderate and regular red wine intake seems to improve the clinical status and symptoms of UC patients in the active phase of the disease. However, studies with a greater sample size are required to achieve conclusive results.

1. Introduction

Inflammatory bowel diseases (IBD) are types of immune-mediated chronic disorders characterized by a dysregulated immune response to the commensal gut microbiota in the gastrointestinal tract in a genetically susceptible host.[1] The main symptom of IBD is inflammation of the intestinal mucosa, accompanied by chronic abdominal pain and altered bowel habits, in the form of diarrhea, constipation or a mix of the two.[2,3] IBD is the most common condition encountered by gastroenterologists, with a global pooled prevalence of 11.2% and a prevalence of around 20% in the Western world.[4] Ulcerative colitis (UC) is one of the diseases that comprise this pathology, characterized by relapsing and remitting mucosal inflammation, starting in the rectum and extending to proximal segments of the colon.[5] This pathology has been related to a dysbiotic state in the gut microbiome, mainly driven by a decrease in bacterial biodiversity, as well as an increase in several opportunistic pathogens.[6–8] Also, and in spite of the few studies covering this matter, a certain dysbiosis in the oral microbiota has been reported to occur in UC patients.[9] Although the causal pathways between oral dysbacteriosis and gut dysbacteriosis remain to be completely clarified, evidences for a shared immunological pattern between oral diseases (i.e., periodontitis) and IBD have been reported.[10–12] Altered levels of cytokines, immunoglobulin A and lysozyme have been found in the oral cavity of IBD patients, which has been related to the abundance of genera commonly dominant in the oral microbiota: *Streptococcus*, *Prevotella*, *Veillonella*, and *Haempohilus*.[12] Bacteria from these genera may migrate into the colon via the digestive tract causing the onset of IBD, although it may also occur that, after the onset of the disease, certain of the mentioned inflammatory biomarkers reach the mouth via the bloodstream and stimulate oral dysbiosis.[9] In any case, it also remains to be clarified if there is a decrease in gut/oral microbial diversity as a consequence of intestinal changes, or if this could play a role in the pathogenesis of UC and its oral location.[13]

Fundamental evidence in cell-based and animal models has confirmed the benefits of dietary polyphenols in controlling cytokine-mediated inflammation, immune signaling, and free radical activity that are implicated in IBD pathogenesis.[14] However, clinical evidence of the impact of polyphenols on

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inflammatory markers in IBD is still scarce and inconclusive.[15,16] For example, in the intervention with red fruits in UC patients with mild/moderate symptoms,[17] it was found that their continued consumption (840 mg of anthocyanins per day) significantly decreased the disease activity index after 1 week, and the fecal calprotectin value—as a clinical parameter of IBD—after 2 weeks. In contrast, supplementation with curcumin (450 mg per day) for 8 weeks was ineffective at inducing remission in mild to moderate cases of UC.[18] Therefore, at present, understanding of clinical and mechanistic effects of polyphenols in IBD requires both more exploratory and long-term trials.[15]

Wine is considered a dietary source of high-content and diverse-structure polyphenols, including flavonoids such as flavan-3-ols, flavonols, flavones, and anthocyanins (only in red wine), and non-flavonoid compounds such as hydroxybenzoic and hydroxycinnamic acids, stilbenes, and condensed tannins. In vitro and in vivo UC models have shown the ability of wine polyphenols to attenuate intestinal inflammation and oxidative markers and to ameliorate clinical symptoms.[19] However, and possibly due to the controversial role of alcohol consumption in the development of IBD,[20] in the literature, there is only one intervention study with wine in IBD/UC patients and they all were in the inactive phase of the disease.[21] After consumption of one to three glasses of red wine daily for 1 week, the authors found no significant change in either clinical disease activity scores or C-reactive protein, but they did find a significant decrease in fecal calprotectin and an increase in intestinal permeability.[21] Nevertheless, they also suggest that patients with inactive IBD who drink red wine daily may be at an increased long-term risk for disease relapse. Moreover, this study did not address the possible relationship with intestinal microbiota, which nowadays is considered one of the most relevant etiopathogenic factors of IBD. In fact, it has been suggested that synergistic interactions between polyphenols and intestinal microbiota might contribute to the alleviation and mitigation of IBD.[22]

In order to provide new evidence on this subject, we carried out a comprehensive study of the effects of moderate wine consumption in UC patients in the active phase of the disease. Patients were selected from a previously clinically diagnosed cohort and were divided into a red wine intervention (250 mL day^{-1}) group and a control (no wine intake) group. Assessments of clinical parameters, symptomatology, and quality of life in both groups before and after the intervention period (4 weeks) were carried out. Fecal samples were collected to assess the impact of wine intervention on the gut microbiome of UC patients, and also in comparison with healthy subjects. Gut microbiota metabolic functionality was assessed by means of concentration of short-chain fatty acids (SCFAs) and phenolic metabolites in feces. Additionally, microbiota from saliva samples were subjected to taxonomic analysis in an attempt to evaluate the effect of moderate wine consumption on oral microbiome.

2. Results

2.1. Effects of Moderate Wine Consumption on Clinical Parameters and QoL Questionnaire

Serum biochemical parameters of the UC patients before and after the intervention period are reported in Table S2, Supporting Information. Differential trends between the control and intervention groups were observed for some serum parameters (**Figure 1**A–E). Of special interest was the total circulating iron that was found to increase after moderate wine consumption (from 63.4 ± 29.2 to 102 ± 40 μg dL^{-1}, as mean ± DS values), a fact that was not found for the control group (Figure 1A). Accordingly, all the patients that consumed wine increased their transferrin level and transferrin saturation index after the intervention period (Figure 1C,D), although changes were only statistically significant ($p < 0.05$) for the transferrin saturation index (Table S2, Supporting Information). In relation to ferritin, the main iron storage protein, its levels significantly decreased in both groups during the intervention time (Figure 1B). Vitamin B12 also decreased after the intervention period for both groups, although it was only statistically significant ($p < 0.05$) for the intervention group (Table S2, Supporting Information), mainly due to the behavior of patient W4 (Figure 1E). Finally, slight changes in the proportions of blood cells after the intervention period were observed; control patients showed reductions in platelets and the percentage of neutrophils, accompanied by an increment in the percentage of lymphocytes, whereas patients consuming wine experienced increases in the percentage of LUC after wine intervention (Table S2, Supporting Information).

Calprotectin, the most important marker of ulcerative colitis in feces, was reduced from 1964 ± 2686–91 ± 104 μg g^{-1} in patients who consumed moderate amounts of wine whereas a slight increase was observed in the control group (from 1400 ± 1510 to 1808 ± 2754 μg g^{-1}), although differences were not significant ($p > 0.05$) in any case (Table S2, Supporting Information). In general, a similar steady trend was observed for patients from both groups, with the exception of patient C3 in the control group that experimented a notable increase, and patients W2 and W4 from the intervention group that exhibited a drastic decrease after wine consumption (Figure 1F).

The overall scores for the IBDQ-32 questionnaire completed by patients from both the control and the intervention group are reported in Table S3, Supporting Information. Among the four categories evaluated (bowel symptoms, systemic symptoms, emotional involvement, and social involvement), the score for bowel symptoms registered a significant ($p < 0.05$) improvement (33% increase) in the final sampling for the intervention group, whereas no change was observed for the control group (Table S3, Supporting Information, **Figure 2**A). These trends after the intervention period in the bowel category were reflected in the total QoL score (Table S3, Supporting Information, Figure 2B).

2.2. Main Events Underlying Gut Environment

2.2.1. Changes in the Gut Microbiome after Moderate Wine Consumption

Taxonomic analysis of fecal samples was carried out as an approach to studying the effect of moderate red wine consumption on gut microbiome. A total of 1856 ASVs were sequenced from the fecal samples ($n = 5$ control initial, 5 control final, 5 intervention initial and 5 intervention final). For all of the identified genera, non-statistically significant differences ($p > 0.05$) in their relative abundance (%) after the intervention period for both

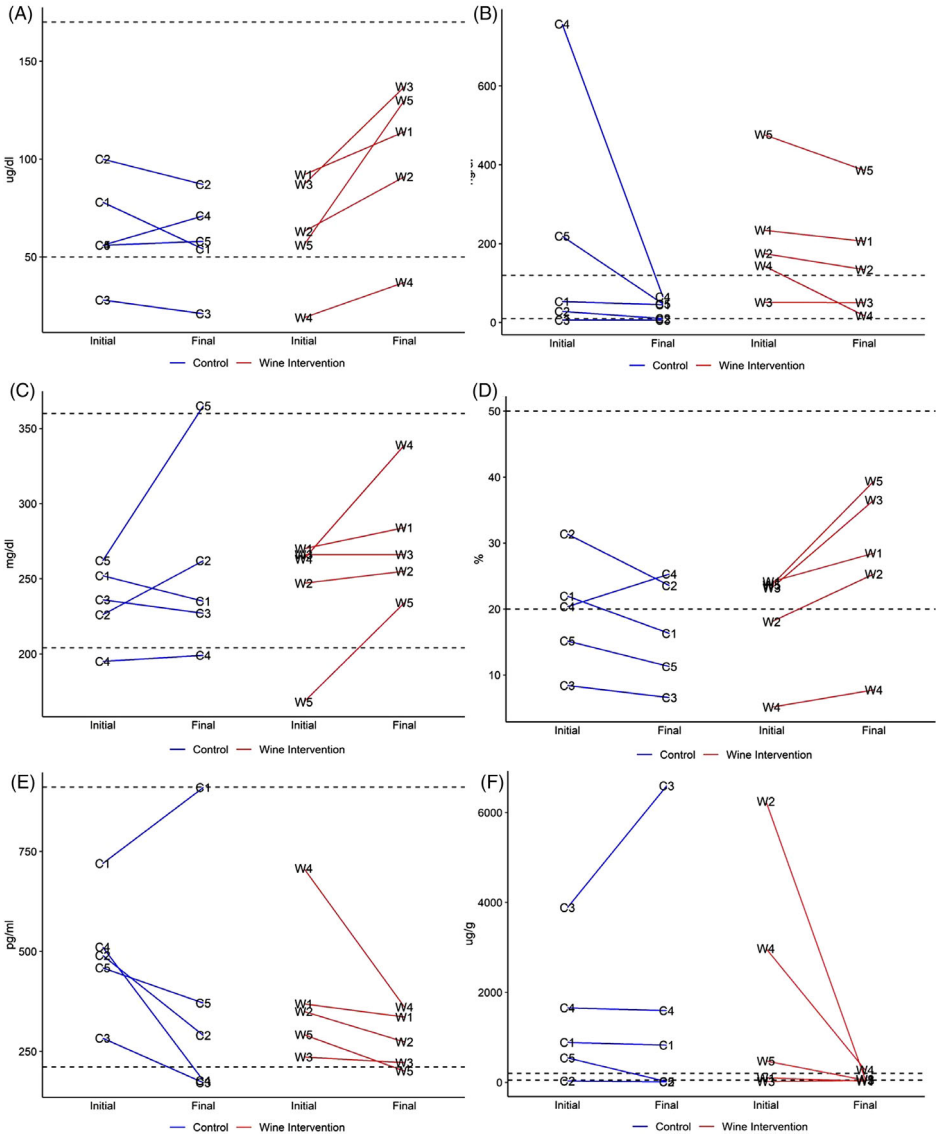


Figure 1. Individual data for iron A), ferritin B), transferrin C), transferrin saturation index D), and vitamin B12 E) for the control and intervention groups before (initial) and after (final) the intervention period. The dotted line indicates the recommended values for each clinical parameter.

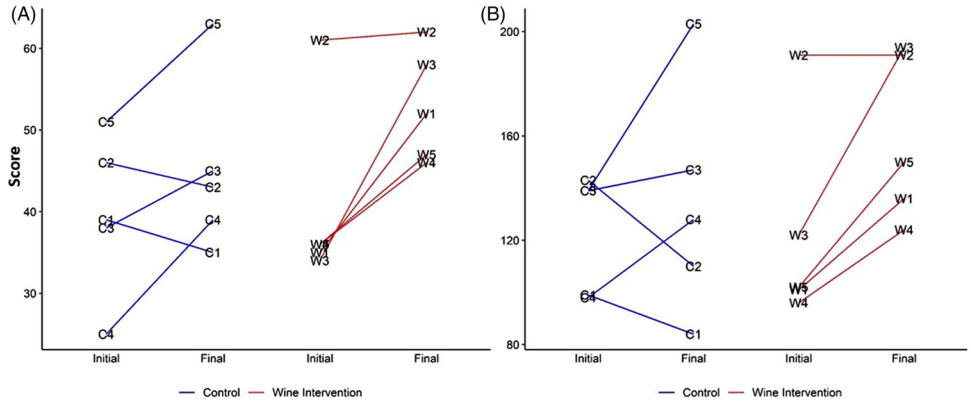


Figure 2. Individual data regarding the IBDQ-32 bowel symptoms A) and total B) scores for the control and intervention groups before (initial) and after (final) the intervention period.

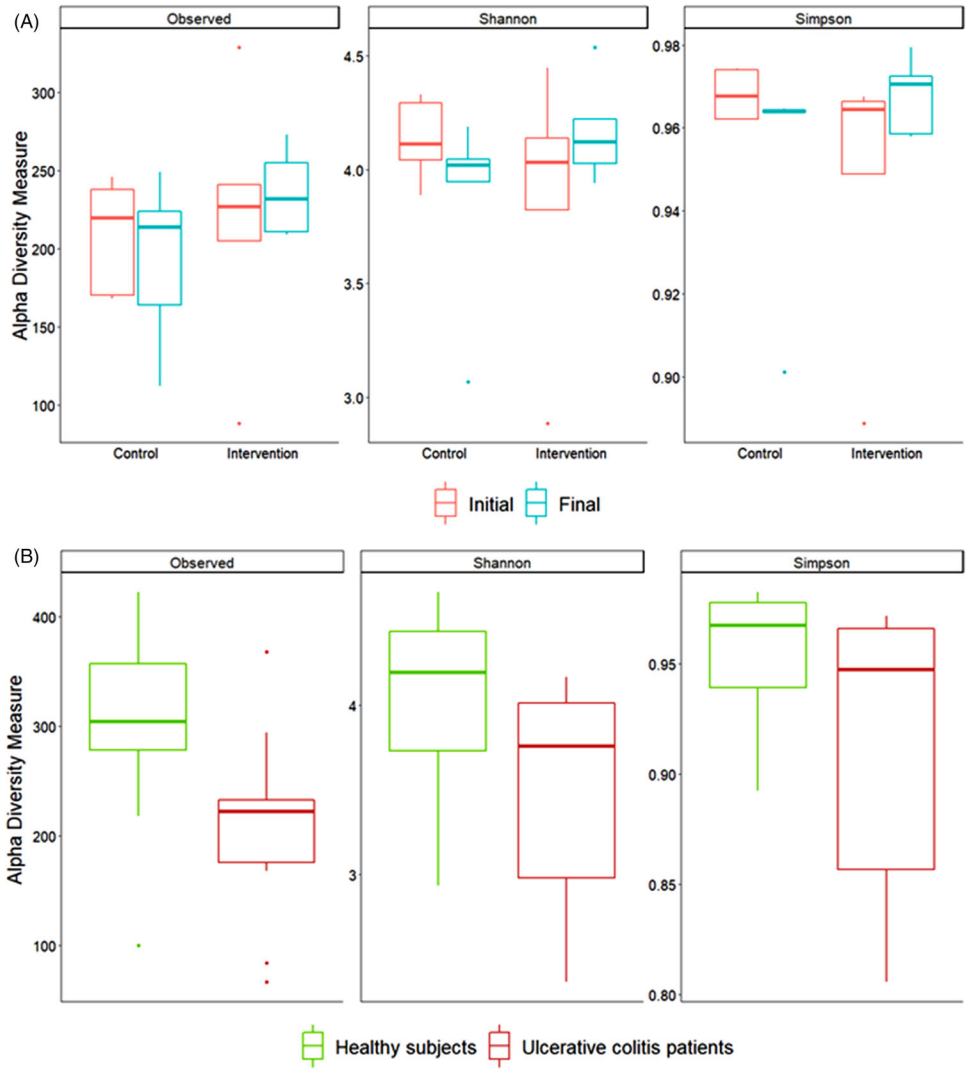
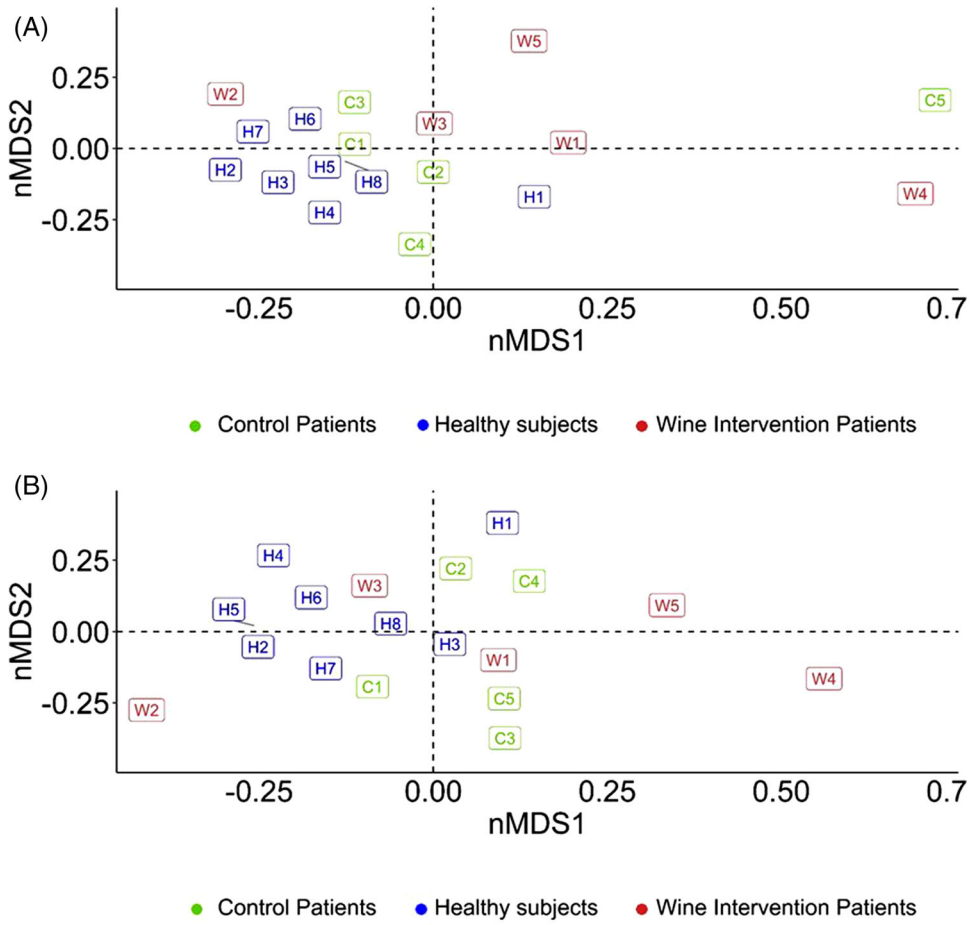


Figure 3. Alpha diversity analysis (Observed, Shannon and Simpson indices) of fecal samples from the control and intervention groups at initial and final sampling A) and from healthy subjects and UC patients before the intervention period B). The Mann–Whitney *U* test confirmed no significant differences between groups.

control and intervention groups were found (Table S4, Supporting Information). However, the results revealed some interesting trends, including a decrease in the relative abundance of *Streptococcus*, *Escherichia/Shigella*, and *Granulicatella* and an increase in *Faecalibacterium* and *Dialister* after the intervention period (Table S4, Supporting Information). Comparison of alpha diversity indices did not show significant differences ($p < 0.05$) after the intervention period for either the control or the intervention group (**Figure 3**A), although a slight improvement of the Shannon and Simpson indices was observed in the case of wine consumption (Table S4, Supporting Information).

To explore the degree of dysbiosis in the gut microbiota of UC patients, taxonomic data of UC patients ($n = 10$) were compared to those of healthy subjects ($n = 8$) after the washout period of 2 weeks (initial sampling). Several statistical differences in the proportions of some taxa were revealed (Table S5, Supporting Information). The most remarkable event at family level was a significantly lower relative abundance, almost to undetectable levels, for the UC participants in Akkermansiaceae ($p = 0.022$), which exclusively consists of the genus *Akkermansia*. A significant alteration was also detected in the proportions of other healthy-associated families such as *Christensenellaceae*, *Eggerthellaceae*, and *Ruminococcaceae*, amongst others, in UC patients. This was directly related to altered populations of the genera *Christensenellaceae*_R-7, *Ruminococcaceae*_NK4A214, *Ruminococcaceae*_UCG-003, *Ruminococcaceae*_UCG-005, and *Subdoligranulum*, which belong to those families. Further, other notorious genera like *Dialister*, *Fusicatenibacter*, and *Parabacteroides* turned out to be damaged by the UC pathology, at the same time as *Streptococcus* was significantly most abundant in the feces of these individuals (Table S5, Supporting Information). In regard to microbial alpha diversity, microbiota from UC patients exhibited lower values for the three indices (Observed, Shannon and Simpson) than those from healthy subjects (Figure 3B), although the differences were not statistically significant ($p > 0.05$).



(C)

	p value	
	Initial	Final
Healthy subjects vs Control Patients	0.228	0.351
Healthy subjects vs Wine Intervention Patients	0.027	0.126
Control Patients vs Wine Intervention Patients	0.919	0.921

Figure 4. Beta diversity analysis of fecal samples from healthy subjects, control patients, and intervention patients before A) and after B) the intervention period. A PERMANOVA was carried out to check statistical differences between the three groups both at initial and final sampling and the *p* values are shown in the table C).

The dysbiosis status of the two groups of UC patients (control and intervention) in comparison to healthy subjects was also measured as means of beta diversity (**Figure 4**). Before the intervention period, samples corresponding to healthy subjects were located quite close, whereas samples corresponding to UC patients (both control and intervention groups) were widely distributed, thereby indicating greater inter-individual variability (Figure 4A). After the intervention period, samples were situated closer and equidistant, especially for those corresponding to the UC patients consuming wine (Figure 4B). Curiously, the two most outlier points corresponded to control patient C5, who exhibited the highest improvement in QoL scores after the intervention period, and intervention patient W4, who showed very low values for blood iron, transferrin saturation index, and vitamin B12 that did not improve much after wine intervention (Table S2, Supporting Information Figures 1 and 2). *P* values confirmed that the greater dispersion exhibited by UC intervention patients at the initial sampling ($p < 0.05$) was corrected after moderate wine consumption, bringing them closer to healthy subjects at the final sampling ($p > 0.05$) (Figure 4C).

2.2.2. Changes in Bacterial Metabolites after Moderate Wine Consumption

The phenolic profiles detected in the feces of patients with UC before and after wine intake are shown in **Table 1**. After intervention with wine, no significant changes were observed in phenolic profiles, probably due to the great variability amongst patients. However, increased levels of several microbial-derived

Table 1. Mean values ± standard deviations of polyphenol and short-chain fatty acids (SCFA) concentrations in fecal samples for the control and intervention groups before (initial) and after (final) the intervention period.

	Control				Wine intervention			
	n	Initial	Final	*p* value	n	Initial	Final	*p* value
Polyphenols [nmol g^{-1}]								
Gallic acid	nd	–	–		1	8.06 ± 18.01	0	1.000
Protocatechuic acid	3	1.2 ± 1.24	3.22 ± 4.96	0.423	5	10.65 ± 14.75	8.17 ± 11.33	0.813
3,4-dihydroxyphenylacetic acid	nd	nd	nd		4	2.48 ± 5.56	12.92 ± 9.04	0.201
3-O-methylgallic acid	1	0.16 ± 0.35	0.2 ± 0.45	1.000	2	0 ± 0	0.99 ± 1.35	0.371
4-hydroxybenzoic acid	3	12.03 ± 24.19	2.77 ± 2.8	1.000	5	7.55 ± 3.96	9.87 ± 7.48	0.813
Mandelic acid	nd	–	–		1	0.43 ± 0.97	1.44 ± 3.23	1.000
4-hydroxyphenylacetic acid	5	4.7 ± 3.26	4.35 ± 2.41	1.000	4	5.56 ± 4.72	5.22 ± 8.05	0.855
3-(3,4-dihydroxyphenyl)-propionic acid	nd	–	–		1	6.08 ± 13.6	1.56 ± 3.49	1.000
3-hydroxybenzoic	3	1.77 ± 2.42	1.17 ± 2.04	1.000	nd	–	–	1.000
Hippuric acid	2	1.16 ± 2.37	0.07 ± 0.15	0.371	nd	–	–	1.000
Caffeic acid	4	0.77 ± 1.52	0.27 ± 0.37	1.000	nd	–	–	1.000
3-hydroxyphenylacetic acid	4	45.57 ± 86.77	22.89 ± 38.57	0.584	3	13.91 ± 14.1	26.12 ± 39.43	0.789
p-coumaric acid	nd	–	–		1	0.64 ± 1.43	1.45 ± 3.24	1.000
3-(3-hydroxyphenylpropionic) acid	5	42.03 ± 57.85	149.19 ± 224.66	0.313	5	82.36 ± 113.61	220.14 ± 161.68	0.188
Phenylacetic acid	2	39.97 ± 57.9	47.55 ± 66.06	0.371	4	91.32 ± 90.31	73.78 ± 57.56	1.000
Short-chain fatty acids [nmol g^{-1}]								
Propionic acid	5	34.14 ± 2.71	34.28 ± 2.81	0.813	5	35.2 ± 2.96	36.95 ± 4.04	0.625
Butiric acid	5	5.68 ± 1.89	6.75 ± 3.37	1.000	5	8.07 ± 3.12	11.69 ± 3.52	0.313
Acetic acid	5	32.38 ± 9.25	38.69 ± 21.22	1.000	5	48.12 ± 9.06	58.58 ± 19.9	0.313

n = number of cases considered for each compound.

metabolites such as 3-O-methygallic acid, 4-hydroxybenzoic, 3,4-dihydroxyphenylacetic, and 3-hydroxyphenylacetic were observed (Table 1). UC participants consuming wine also experienced non-significant ($p > 0.05$) rises in propionic acid, butyric acid and especially acetic acid levels (Table 1).

2.3. Main Events Underlying Oral Environment

A total of 1257 ASVs were sequenced from the saliva samples ($n = 20$). From a taxonomic point of view, the results did not show statistical significance ($p > 0.05$) after the intervention period for any of the taxa evaluated (Table S6, Supporting Information). However, bacterial diversity analysis revealed a slight non-significant increase ($p > 0.05$) in alpha diversity measurements after the wine intervention, which was more evident in the case of Simpson indices of dominance, contrary to what was observed for the control group, in which a decrease can be seen in the three alpha diversity measurements (**Figure 5**).

Joint beta diversity analysis of fecal and saliva samples from the control and intervention groups ($n = 20$) at both sampling times (Initial and Final) clearly separated them according to their microbial environment (**Figure 6**). The analysis also showed greater dispersion amongst patients for the fecal samples than for the saliva samples at both sampling times (Figure 6), indicating a richer and more complex microbial niche for the gut environment. After the intervention period, both oral and gut environments tended to reduce their dispersion in parallel, leading to greater differentiation between them (Figure 6).

3. Discussion

Diet is one of the most influential factors in the appearance and course of IBD, especially because dietary imbalances can exacerbate the disease. In addition, diet conditions the composition of the gut microbiota, an aspect that is increasingly associated with the development and evolution of IBD.[23,24] At first glance, wine consumption should be examined as a complex nutritional intervention in UC pathology because of the presence of ethanol, which has been associated with intestinal dysmotility and local inflammation and oxidative stress, amongst other effects.[25] In addition, the only wine intervention in UC patients reported in the bibliography revealed controversial results, since moderate wine consumption increased gut permeability at the same time that reduced intestinal inflammatory markers.[21] This finding was attributed to the presence of a relatively high concentration of bioactive compounds, such as polyphenols.[26] From this background, this paper sought new evidence about the effects of wine polyphenols on ulcerative colitis, bearing in mind the human microbiota (oral and intestinal) as an active actor in this disease.

The most frequent manifestations of the disease are related to chronic diarrhea, abdominal pain, rectal bleeding, and/or fatigue, leading to an impaired quality of life. To mathematically quantify the degree of disturbance, the IBDQ-32 contains several questions grouped in four domains covering bowel and systemic symptoms and emotional and social functions.[27] Our outcomes revealed an improvement of the bowel and systemic symptoms in patients who had consumed moderate wine. These findings agree with those of previous works evaluating supplementation

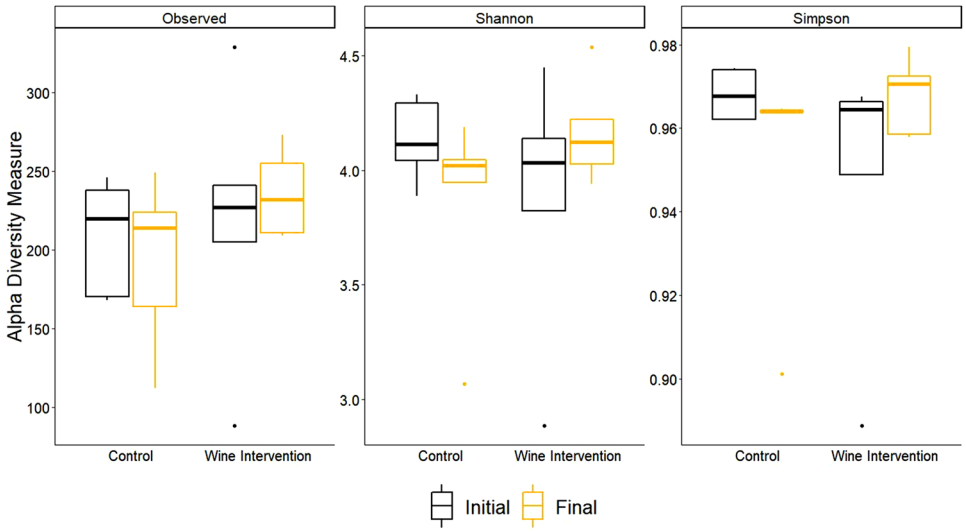


Figure 5. Alpha diversity analysis (Observed, Shannon and Simpson indices) of fecal samples from the control and intervention groups before (initial) and after (final) the intervention period. The Wilcoxon signed-rank test confirmed no significant differences between sampling times for both groups and the three indices.

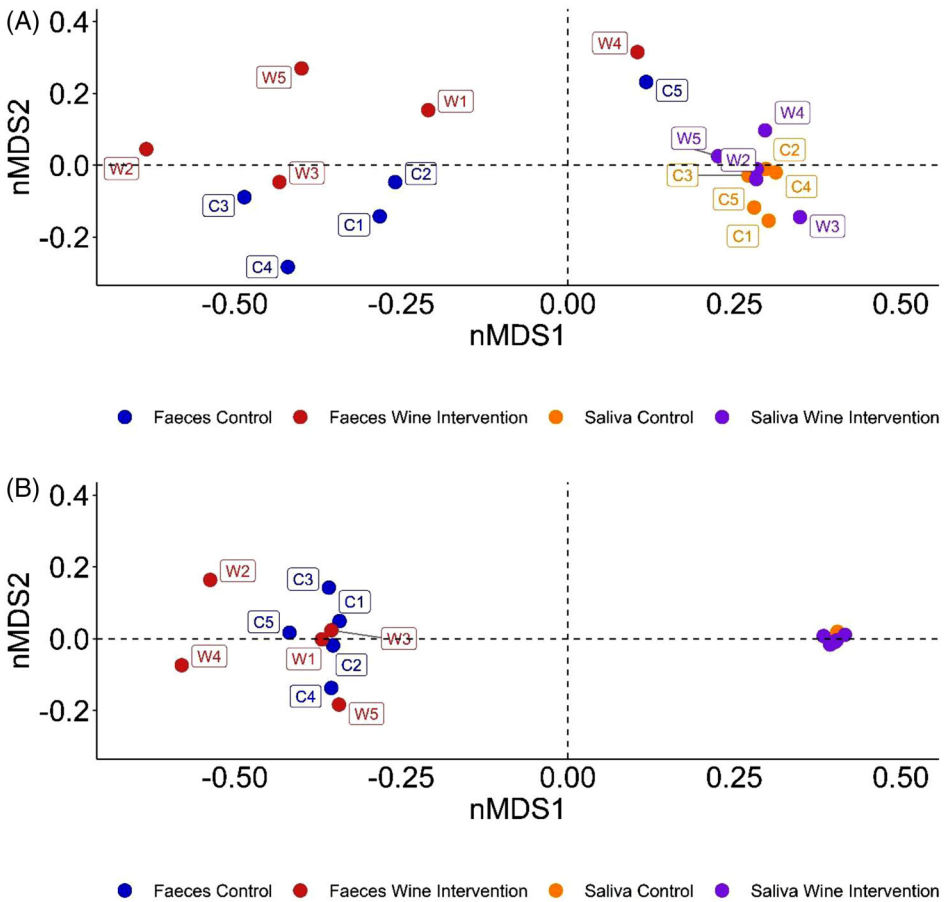


Figure 6. Beta diversity analysis of fecal and saliva samples from the control and intervention groups before A) and after B) the intervention period.

with resveratrol, one of the most noticeable polyphenols in wine, in subjects with mild or moderate UC.[28,29] Specifically, a wide variety of systemic symptoms may be a consequence of impaired markers of anemia, which are extremely frequent in UC patients.[30] In fact, previous investigations have reported improved health-related answers in QoL questionnaires after the correction of anemia in patients suffering from inflammatory bowel disease.[31–33] This condition arises from blood loss after mucosal ulceration, reduced iron release from enterocytes as a consequence of ferroportin degradation, reduced dietary intake due to digestive discomfort and decreased absorption of iron.[30] Under the conditions of this study, moderate wine consumption led to higher levels of iron, transferrin and transferrin saturation index in serum collected from the intervention group while a different pattern was noticed for controls. These outcomes suggest an ameliorative effect of wine in UC-related anemia that explains the increased score in the systemic symptoms of the IBDQ-32. Nevertheless, it should not be forgotten that the subjective nature of this type of test means that there is a great deal of variability.

Some biomarkers of gut impairment are used for non-invasive monitoring of UC activity. One of the most extended is calprotectin, a protein mainly liberated from monocytes and thus directly related to neutrophil migration, a key process of inflammation.[34] In our study, calprotectin levels showed great interindividual variability, with some patients exceeding the initially estimated range (see Experimental Section), although other authors have also reported extremely high values (above 3000 μg g^{-1}) for outlier individuals.[35] Among its functions, calprotectin sequestrates iron from the diet to manage bacterial growth via a mechanism known as ''nutritional immunity,''[36] which also implies iron malabsorption. Bearing all this in mind, in the development of the disease, there will come a time when an exacerbated migration of neutrophils occurs, therefore the release of calprotectin greatly increases, leading to reduced iron availability and unspecific inhibition of bacterial growth. In addition, the immune system is overactivated, consuming a lot of iron, so that iron stores are considerably reduced. In short, UC development gives rise to two negative consequences, iron deficiency and loss of microbial biodiversity, the latter also being aggravated by other processes that will be explained below. What previous investigations have revealed in this context, studied amongst people suffering from UC, is that calprotectin levels were reduced after moderate wine consumption,[21] in line with our results. Others also described the same pattern when UC patients consumed another polyphenol-rich food,[37] which supports the idea that polyphenols improve intestinal function. This activity of wine polyphenols may be an indirect consequence of their ability to reduce inflammatory and oxidative markers such as interleukins (TNF, IL-6, IL-1), adhesion molecules (ICAM-1, VCAM-1), oxidative enzymes (iNOS, COX-2) and inflammation-related transcriptional factors (NF-$\kappa\beta$), both in vitro[38] in murine models[39–41] and ex vivo in human colonic tissue,[42] as well as the capacity of polyphenols to improve gut barrier integrity, enhancing tight junction expression and assembling.[43,42,44,45] This potential improvement in intestinal function by wine polyphenols might also explain, at least partially, the increase observed in the bowel symptoms scores (IBDQ-32 questionnaire) after the wine intervention. In relation to vitamin B12, the reduction observed after the intervention period was in agreement with previous studies showing that the development of inflammatory bowel disease can lead to a reduction in certain micronutrients.[46] Also, reductions in vitamin B12 have also been reported with alcohol consumption.[47]

Regarding wine polyphenols-derived metabolites, our results revealed that UC patients showed a poorer profile of phenolic metabolites in comparison to healthy volunteers,[48,39] probably due to the gut dysbiosis associated with the disease. However, increased levels of some phenolic acids derived from bacterial metabolism were detected after moderate wine consumption, in line with previous studies with healthy volunteers.[49,50] In this regard, some species of *Bacteroides* and *Parabacteroides*, which increased in a non-significant manner after wine intervention in our study, have been reported to metabolize flavonoids into other small compounds.[51] On the other hand, inflammation derived from IBD is closely associated with impaired SCFA fermentative pathways,[52] and/or blocking of the absorption and oxidation of SCFAs by monocytes.[53,54] In our study, low contents in fecal SCFAs were observed in comparison to previous studies.[55,56] Nevertheless, after wine consumption an increase was observed in acetic, propionic, and butyric acid levels, in line with previous works evaluating polpyphenols in animal models suffering from UC.[57–59] This, in turn, could contribute to the anti-inflammatory effect and the protection of gut barrier integrity associated with wine consumption.[60] Among SCFA producers, *Roseburia*, *Faecalibacterium*, and *Bacteroides* showed a tendency to increase after wine consumption, which could be responsible for the higher proportions in SCFAs.

Another way of polyphenol action in IBDs is through the regulation of gut microbiome, which with UC patients, with biodiversity reduced by about 25% compared to healthy subjects, there is no room for discussion about the existence of dysbiosis.[6,61,62] There is still no consensus as to whether dysbiosis is a consequence or a cause, but it is a fact that it is related to overstimulation of the mucosal immune response.[6] In our study, in UC patients, we observed a tendency for Actinobacteria and Proteobacteria, which generally encompass pathogen species, to increase at phylum level, in line with previous investigations.[6,62] Regarding some bacteria associated with good health, the phylum Verrucomicrobia showed a drastic reduction in UC patients in comparison to healthy subjects, which was directly related to a lower relative abundance of *Akkermansia*, in line with the findings of other authors.[63–65] Focusing on the genus level, the lower proportions of *Dialister*, *Fusicatenibacter* and *Parabacteroides* in UC patients, together with higher levels of *Streptococcus*, is noteworthy, a pattern previously described in literature.[6,66] Given these results, we also evaluated the effect of moderate wine in microbial populations. We are aware that the low sample size in the intervention study (n = 10), although it has been big enough to relate clinical data to questionnaire answers, is a limitation in explaining the management of the disease through the modulation of gut microbiome; however, we observed trends of interest. Some harmful bacteria, like *Streptococcus* or that belonging to Enterobacteriaceae (i.e., *Eschericchia*, *Shigella*, *Enterococcus*, etc.), experienced a reduction trend after wine consumption, in line with previous works evaluating in mice the effect of kaki procyanidins, similar to those present in wine.[67] The same authors also reported increases in the relative abundance of *Bacteroides*,[67] usually more predominant in healthy subjects

than in UC patients.[6,62] Other genera susceptible to improvement following polyphenol consumption include *Akkermansia*, *Faecalibacterium*, and *Bifidobacterium*,[68] however none of them showed a clear trend in our study.

One of the most notorious effects of the wine intervention was the restoration of intestinal microbial dysbiosis as means of alpha diversity indices. They seemed to be more stable after wine consumption compared with controls, something described previously in CU-induced rodents subjected to dietary interventions with polyphenols that are also abundant in wine, like quercetin.[69,70] Interestingly, this pattern could be also extrapolated to oral microbiome, in which wine intervention increased the three alpha diversity indices after four weeks of consumption. Moreover, the great dispersion in beta diversity at the initial sampling tended to diminish in feces and saliva in parallel. This was interpreted as the existence of an interrelationship between the two ecosystems in the context of UC, something previously described as the gum-gut axis.[71] In fact, it has been hypothesized that, once established, IBD can be driven by microbiomial and inflammatory changes originating specifically from the gingival niche through saliva, thereby worsening IBD outcomes and thus perpetuating a vicious cycle.[71]

4. Concluding Remarks

To the best of our knowledge, this is the first study in which the effect of moderate wine consumption in UC patients has been evaluated, focusing not only on symptoms and quality of life of the participants but also on evaluating changes in blood parameters, polyphenol metabolism, and gut microbiome. Wine intervention resulted in an improvement of anemia-related biomarkers, such as iron and transferrin levels. Also, moderate wine consumption seemed to reduce levels of fecal calprotectin, that was related to iron malabsorption and with an exacerbated UC state, indicating a possible anti-inflammatory effect of wine polyphenols in UC patients. As regards microbiome, our study confirmed the presence of dysbiosis in UC patients as reported in previous investigations. Although the small sample size of the intervention limited the findings of our study, there is a significant amount of evidence suggesting a putative effect of wine in the gut and oral microbiome, including stabilisation of biodiversity and increases in some key bacteria and their metabolites. All these outcomes led to an improvement in the quality of life of the participants of this exploratory study as revealed by the IBDQ-32 questionnaire. Nevertheless, a greater number of participants are required to confirm the results, especially in the case of microbiota-related data.

5. Experimental Section

Wine: Red wine was produced with the Cabernet Sauvignon and Cabernet Franc grape varieties (vintage 2006), from the Penedès appellation (Spain). The ethanol content in the wine was 13.5% and the total phenolic content reached 2500 mg of gallic acid equivalents per L as determined by the Folin–Ciocalteau method. Main components of the wine used in this study together with its phenolic profile analyzed by UPLC-ESI-MS/MS[43] were presented in Table S1, Supporting Information.

Intervention Study in UC Patients: The participants in this study were selected from the patient cohort of the Digestive System Department of the "Infanta Sofía Hospital'' (San Sebastián de los Reyes, Madrid, Spain). As exclusion criteria, participants should not have received antibiotics for at least 6 months before the study, or suffer from type I diabetes, had severe cardiac, endocrine, or other disorders, had a previous history of alcohol or drug abuse, or followed exclusive diets (vegan or vegetarian). A case control intervention study was designed comprising two consecutive periods: 1) an initial washout period of 2 weeks during which all patients did not consume wine or any other alcoholic beverages and followed a diet low in polyphenols (excluding excessive intakes of vegetables and fruits); and 2) a period of 4 weeks during which all patients maintained a low-polyphenol diet, but patients from the intervention group also consumed a daily intake of red wine (250 mL per two doses). The study was approved by the Ethics Committee of the Hospital Infanta Sofia and Hospital La Paz (Madrid, Spain) (reference HULP-PI2908), and incorporated into the ISRCTN register (reference ISRCTN39987). It was conducted according to the guidelines laid down in the Declaration of Helsinki.

The minimum sample size was calculated according to the following formula,[72] taking fecal calprotectin as the calculation biomarker:

$$n = \frac{2(Z\alpha + Z\beta)^2 \times S^2}{d^2} \quad (1)$$

where $Z\alpha$ represented a p value of less than 0.05 ($Z\alpha = 1.96$) and $Z\beta$ a power of 80% ($Z\beta = 0.842$), while d and S referred, respectively, to a mean reduction of 700 µg of calprotectin per g of feces[21] and a standard deviation of 400 µg g^{-1} (own experimental data). Following this formula and these criteria, the minimum n to carry out the intervention study with a statistical power of 80% would be 5.12 participants per group (control and intervention), that is, 10.24 participants in total.

Initially, a total of 20 patients in the active phase of UC, categorized as mild or moderate (according to the partial Mayo index), agreed to participate. But unfortunately, 10 of them dropped out for different reasons. Therefore, the study finally involved 10 patients of both sexes and aged between 18 and 42 years. All participants signed an informed consent form. Then, participants were randomly allocated to either the control ($n = 5$) or intervention ($n = 5$) group. Both groups showed comparable disease state, and received identical medical prescriptions during the study. After the washing (Initial) and intervention periods (Final), patients from both groups were questioned about their disease symptoms and quality of life (QoL) using the IBDQ-32 questionnaire. Blood, fecal and saliva samples were collected at both times (Initial and Final).

Serum biochemical parameters were measured in plasma using an automated biochemical auto-analyser. The blood tests included the measure of glucose levels, lipids, hepatic enzymes, immunological cell profile, vitamins, micronutrients, and hematology (Table S2, Supporting Information). Fecal calprotectin was determined by quantitative enzyme-linked immunosorbent assay (ELISA) immediately after collection. All determinations were carried out at least in duplicate.

Fecal solutions were prepared with 1 g of fresh samples diluted in 10 mL of PBS. After a vigorous homogenization, samples were centrifuged at 10 000 rpm for 10 min at 4 °C. The pellet was stored at −80 °C until 16S rRNA gene sequencing, and the supernatant was filtered with a 0.22 µm filter and stored at −80 °C until metabolite analysis. Saliva samples were frozen and kept at −80 °C until 16S rRNA gene sequencing.

Samples from Healthy Subjects: Healthy subjects ($n = 8$) from the same area and social environment as the UC patients were recruited from the Primary Care Centre "Paseo de la Chopera'' (Madrid, Spain), taking into consideration the same exclusion criteria as the UC patients. They were asked to follow the same washout period of 2 weeks. After this time, fecal samples were collected, and subjected to the same sample preparation as that indicated above for the UC patients.

DNA Extraction and Sequencing: Saliva samples were subjected to DNA extraction using the MasterPure Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA) following the manufacturer's instructions with a prior lysis with lysostaphin from *Staphylococcus staphylolyticus* (5000 U mL^{-1}), mutanolysin from *Streptomyces globisporus* ATCC 21553 (2500 U mL^{-1}) and lysozyme from chicken egg white (50 000 U mL^{-1}) (Sigma–Aldrich, San Louis, CA, USA). The V3–V4 region

of the 16S ribosomal RNA gene was amplified using the primers from Klindword et al.,[48] which produce a PCR product of 460 bp. Fecal pellets were also subjected to DNA extraction using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The V3–V4 region of the 16S ribosomal RNA gene was amplified using the following primers: forward 5'-CCTACGGGNBGCASCAG-3' and reverse 5'-GACTACNVGGGTATCTAATCC-3'.

The two-step Illumina PCR protocol was followed to prepare the libraries and both kinds of samples were submitted to 2×500 bp paired-end sequencing by means of an Illumina MiSeq instrument (Illumina, San Diego, CA, USA). Raw files were available at the National Center for Biotechnology (NCBI) repository under the project code PRJNA749914 for fecal samples and PRJNA749643 for saliva samples.

Sequence Processing: To process the files with raw reads from the Illumina instrument, RStudio v.4.03 software was used. The FastQC files were filtered for reads of low-quality and with the presence of alien DNA using DADA2. The DADA2 algorithm was also employed to denoise, join paired-end reads, and filter out chimeras in the raw data.[73,74] This algorithm allowed the differentiation of even a single nucleotide, leading to the formation of amplicon sequence variants (ASVs). The taxonomic assignment was performed using the naïve Bayesian classifier implemented in DADA2 using Silva v.138 as the reference database,[75] with a bootstrap cut-off of 80%. A total of 3 152 891 complete good-quality reads for feces and 1 283 321 for saliva were used for the analysis.

Analysis of Phenolic Metabolites by UPLC-ESI-MS/MS: Phenolic metabolites were analyzed in fecal supernatants in triplicate using an UPLC-ESI-MS/MS following a previously reported method.[76] For quantification purposes, was were collected through the multiple reaction monitoring (MRM) and cone voltage, collision energy, and MRM transition were reported previously.[49] All metabolites were quantified using the calibration curves of their corresponding standards, except for 4-hydroxy-5-(3′,4′-dihydroxyphenyl)valeric, 4-hydroxy-5-(3′-hydroxyphenyl)valeric, and 4-hydroxy-5-phenylvaleric acids, which were quantified using the calibration curves of 3-(3,4-dihydroxyphenyl)propionic, 3-(3- hydroxyphenyl)propionic, and propionic acids, respectively. 5-(3′-Hydroxyphenyl)-γ-valerolactone was quantified using the calibration curve of 5-(4′-hydroxyphenyl)-γ-valerolactone. Data acquisition and processing were realized with MassLynx 4.1 software.

Analysis of Short-Chain Fatty Acids (SCFAs): Analysis of SCFAs in feces was carried out by duplicate following the SPME-GCMS method described previously.[77] Quantitative data were obtained using calibration curves of each of their corresponding standards compared to the internal standard (2-methylvaleric acid).

Statistical Analysis: Main changes in blood markers, fecal metabolites, the IBDQ-32 answers, relative abundances of the different taxa, and alpha diversity indices between the initial and the final sampling times were evaluated using the non-parametric Wilcoxon signed-rank test through the ''stats'' package. The Mann–Whitney *U* test was chosen to check non-related samples (healthy subjects vs UC patients). Biodiversity, expressed in terms of alpha diversity, was estimated by calculating the Shannon and Simpson indices through the ''phyloseq'' package. Differences between samples (beta diversity) were obtained employing a Bray-Curtis dissimilarity matrix represented by non-metric multidimensional scaling (NMDS). Permutational multivariate analysis (PERMANOVA) belonging to the ''vegan'' package was conducted to find statistically significant differences between experimental groups. All tests were conducted with RStudio v.4.03.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

D.T. and I.Z.-P. contributed equally to this work. Conceptualization, M.V.M.-A., B.B., and R.P.; methodology, I.Z.-P., D.T.M.S., N.M., and R.P.; statistics, D.T. and N.M.; validation, D.T. and N.M.; formal analysis and resources, N.M., I.Z-P., and D.T.; data curation, N.M.; writing original draft preparation, D.T., N.M., I.Z-P., B.B., and M.V.M.-A.; writing review and editing, D.T., N.M., B.B., and M.V.M.-A.; visualization, D.T., N.M., B.B., and M.V.M.-A.; supervision, B.B. and M.V.M.-A.; funding acquisition, M.V.M.-A. and B.B. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords

clinical parameters, gut microbiome, moderate wine consumption, oral microbiome, phenolic metabolites, polyphenols, ulcerative colitis

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