Molecular Nutrition; a metabolic approach for nutritional assessment in inflammatory bowel disease

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Abstract

Background: Around 7 million people worldwide are diagnosed with inflammatory bowel disease (IBD), which is the overarching term used to describe Crohn's disease (CD) and ulcerative colitis (UC). All patients diagnosed with IBD experience periods of active illness followed by periods of remission; suggesting that environmental factors play an important role in the presence of IBD symptoms and symptom severity. Previous studies have identified dietary factors including red meat and emulsifiers to be associated with the onset of IBD and disease severity. While dietary patterns are well studied in this context, limited data is present on the specific mechanisms driving this association.

Methods: To gain more precision in studying dietary factors related to disease, we have implemented a data algorithm calculating dietary compounds from food measured by the Food Frequency Questionnaire (FFQ). This calculation was performed using the web-based food compound database FooDB. Blood and fecal samples from the same participants were used to measure metabolites present in plasma and feces, which were subsequently correlated to the derived dietary compounds.

Results: Derived intake of carbohydrates and fiber correlates well with fecal and serum succinate, whereas tryptophan intake shows a stronger correlation with its metabolites in serum compared to feces. Secondly, dietary compounds; pentanoic acid ($p < .05$) and selenium ($p < .05$) are negatively associated with IBD diagnosis and intestinal inflammation. Amongst others, these anti-inflammatory compounds are found to be more prevalent in the diet of healthy individuals compared to IBD patients ($p < .001$).

Conclusions: By taking a metabolic approach of studying the role of diet in IBD patients, we can gain a better understanding of the role of specific dietary compounds in active inflammation. The main results of this study form a basis for more targeted dietary approaches and patient-tailored advice to prevent intestinal inflammation through integration of newly discovered metabolomic and dietary profiles.

Abbreviations

Abbreviation	Full term
CD	Crohn's disease
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
ESI	Electrospray ionization
Fcal	Fecal calprotectin
FFQ	Food frequency questionnaire
IBD	Inflammatory bowel disease
MUFA	Monounsaturated fatty acid
NMR	Nuclear magnetic resonance
PUFA	Polyunsaturated fatty acid
UC	Ulcerative colitis

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Introduction

There is a new trend ongoing where there is an increasing interest in treating intestinal complaints using non-drug strategies[1]. This is not without reason, as in 2017 around 7 million people worldwide were described to cope with inflammatory bowel disease (IBD)[2]. This is the overarching term used to describe Crohn's disease (CD) and ulcerative colitis (UC). More specifically, CD patients experience inflammation anywhere along the intestine, while in patients with UC inflammation is mainly located in the colon[3]. All patients diagnosed with IBD experience periods of active illness followed by periods of remission. Through reoccurring inflammation, patients experience symptoms such as: abdominal pain, frequent and urgent bowel movements, fatigue, and lack of appetite. The reappearance of IBD related symptoms are also known as 'a flare'. Currently, flares are managed through pharmaceutical suppression; thereby aiming to reduce disease severity and prolong periods of disease-free remission [4–6]. However, there is an increase in knowledge that the environment of a patient can also be altered to lower the risk of IBD development. Some environmental factors that play a role in the development of IBD include smoking, major life stressors, diet, and lifestyle factors (**Figure 1**). Interestingly, none of the defined risk factors alone are sufficient for the development of disease. In fact, complex interactions between different factors are reported to play a pivotal role in the development of IBD[7].

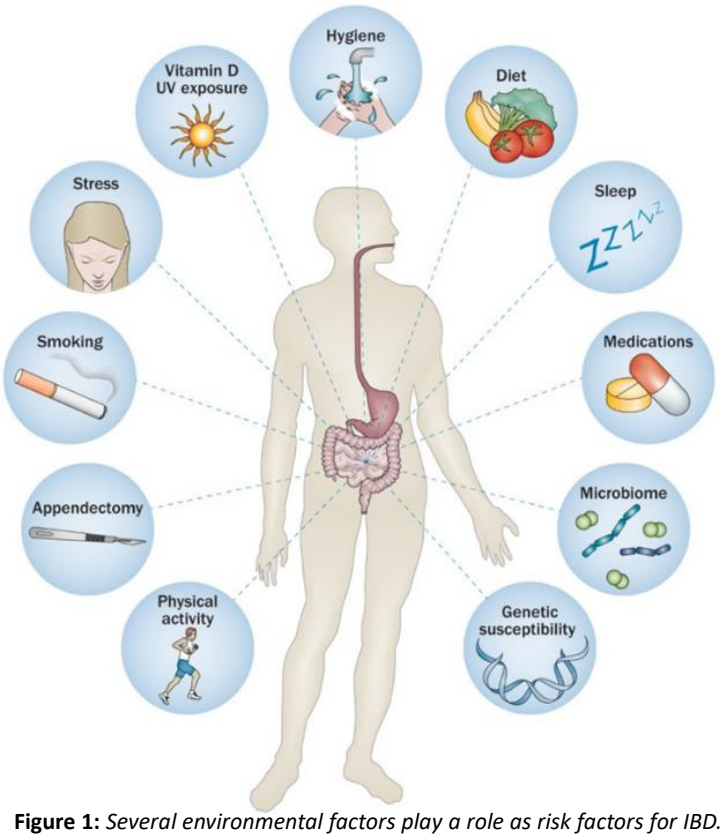

Figure 1: *Several environmental factors play a role as risk factors for IBD. None of the risk factors alone are sufficient for development of disease, complex interactions between several factors takes place; leading to development of IBD*[7]

Food groups and dietary patterns associated with IBD

Prospective cohort studies have already been examining the role of diet in development of IBD, these studies mainly focus on food groups and dietary patterns obtained from the food frequency questionnaire (FFQ)[8–10]. This questionnaire is designed to capture habitual diet and the main results of these studies show that specific food groups and dietary patterns are associated with intestinal inflammation[8]. For instance, a systemic review evaluating the association between pre-illness intake of nutrients and food groups and the risk of IBD diagnosis showed a positive association between high intake of dietary fats, polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), and meat with IBD diagnosis[11]. The same review reports a negative association between diagnosis status and dietary fiber or fruits[11].

Interestingly, recent studies suggest that the gut microbiome and its metabolites could serve as mediators between diet and intestinal inflammation. For example, a plant-based Mediterranean diet; including high intake of legumes, vegetables, fruits, nuts, and fish, has been associated with anti-inflammatory features of the gut microbiome[12]. These results suggest a positive role of healthy and plant-based foods in supporting beneficial gut bacteria, thereby decreasing the risk of experiencing intestinal inflammation.

Mediterranean dietary patterns have also been reported to be benificial in managing IBD flare ups[13,14]. Due to the high amount of fibres, this diet can be unsuitable for patients during flares of the disease, but it is highly recommended during remission, with appropriate adjustments. These adjustments are of most importance since the course and symptoms of IBD vary from person to person, and can change over time. To be able to make the most accurate adjustments for the individual patients it is important to obtain mechanistic insights of dietary intake. These insights provide a better understanding of why certain food groups and dietary patterns are beneficial or detrimental for specific diseased conditions.

Moving towards higher precision dietary assessment

While dietary patterns are well studied in this context, limited data is available on why specific dietary factors lead to the development of disease whilst other factors do not. While food groups and dietary patterns reflect a cumulative effect of different nutrients on intestinal inflammation, the role of specific compounds has not been well studied yet. The limiting factor in gaining more precise results from nutritional assessment is the way that dietary data is being analyzed to date. Currently, FFQ's or dietary recalls are coupled with national food composition databases, resulting in data on food groups and macro nutrient composition. To be able to gain more precision we have implemented a tool in which the data gained from nutritional assessment methods can be transformed to show the molecular composition of an individual's diet.

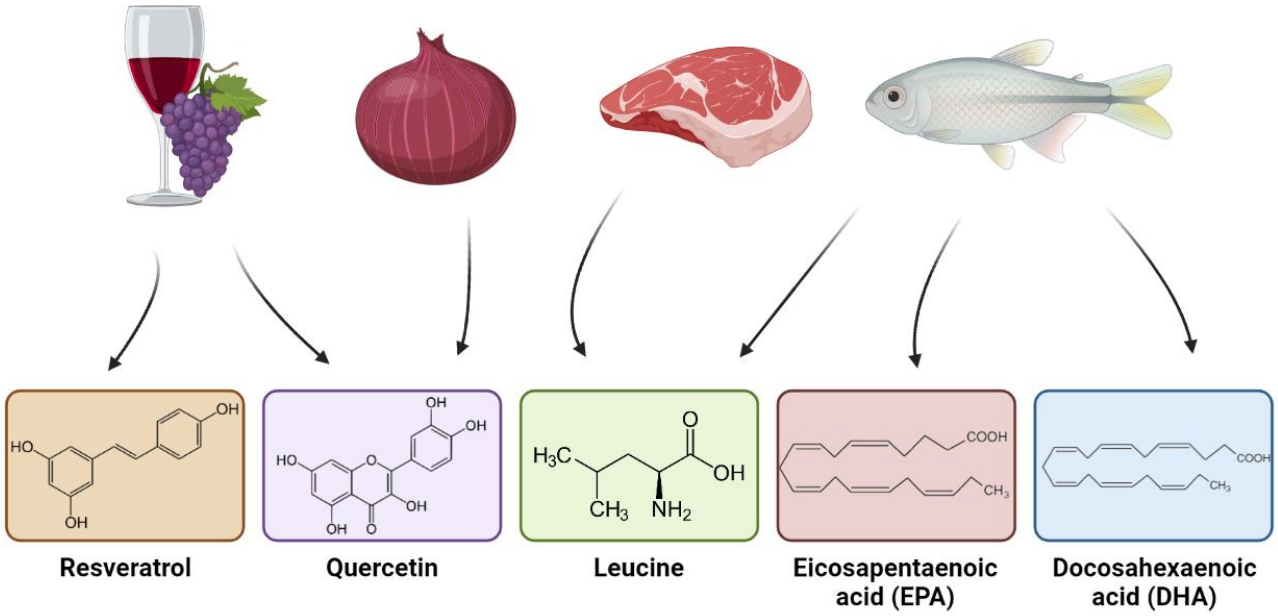

Figure 2: *Similarities between food items on the level of molecular components, giving insights into the specific effects of dietary composition in health and disease*[15–20].

When zooming into the molecular composition of different food items it is revealed that different types of food share similarities on a molecular level (**Figure 2**)[15–20]. Therefore, earlier reported results suggesting a beneficial or detrimental effect of certain food groups or dietary patterns in development of IBD can be revisited using a metabolic approach[4,5,8,11,13,14]. Studying the role of diet in active inflammation in IBD patients using this metabolic approach, it is expected to gain mechanical insights into the effect of dietary composition in health and disease. This knowledge can be applied in for instance treatment of IBD symptoms and management of disease.

From the list of relevant environmental factors in IBD (**Figure 1**), this report will focus on the role of dietary composition for active inflammation in IBD patients. Thereby, going beyond dietary patterns and foods by taking a metabolic approach to study diet. Using this method, we can gain a better understanding of the impact of specific dietary compounds in IBD which ultimately could be used to give more concrete dietary advice for patients suffering from IBD. Secondly, this knowledge can play a pivotal role in lowering the risk for disease development together with the management of active illness; thereby lowering disease severity.

Materials and Methods

Study population

This study includes 1983 participants from two cohorts situated in the northern Netherlands. *Cohort 1* consists of 533 patients with IBD from the 1000IBD cohort of the University Medical Center Groningen (UMCG). Within the 1000IBD project, detailed phenotypic information along with serum and fecal samples has been collected for over 1000 patients with IBD[21]. The diagnosis of IBD was based on accepted radiological, laboratory and endoscopic findings. For a subset of *Cohort 1* (*n = 497*) information on flare occurrence was collected, thereby specifying the timing that a flare occurred[22]. The information on flare timing was divided into a binary distribution in reference to the time the nutritional assessment took place; before a flare and during or after a flare. *Cohort 2* consists of 1450 individuals from the Dutch general-population-based cohort Lifelines DEEP[23]. Detailed phenotypic data has been collected including dietary data using the same Food Frequency Questionnaire (FFQ) as distributed in the 1000IBD cohort, environmental factors, and diseases. Furthermore, phenotypic data indicating gut inflammation has been collected in both cohorts, including a biomarker for inflammation[24,25]; fecal calprotectin. The measured fecal calprotectin (fcal) levels were divided into a binary distribution; above 150 µg/g and below 150 µg/g.

Dietary assessment

Dietary intake was measured using an FFQ. In this questionnaire it is assessed how often a food item was consumed over the previous month on a 7-item scale, along with the usual amount taken. The average daily intake was calculated by multiplying frequencies of consumption by portion size in gram as indicated in the NEVO food composition database (NEVO 2016, RIVM, Bilthoven, the Netherlands). Implausibly low or high values of estimated energy intake were excluded in a sex dependent matter. The plausible intake threshold in males was set between 800 and 4000 kcal/day, in females the threshold was set between 500 and 3500 kcal/day[26].

Calculation of dietary compounds

Dietary compounds were retrieved from FooDB (*https://foodb.ca*), a web-based food compound database. Earlier established FFQ groups (*n = 52*) (**Appendix 1**) were used to derive compound intake per participant based on the average daily intake. Most represented food items from the average Dutch food consumption were selected within the FFQ groups. Different FooDB entries were combined to calculate the dietary compound intake from FFQ groups (*n = 15*) in which more than one food item had to be included. In a few FFQ groups (*n = 4*) were very specific food items included, which could not be matched to a FooDB entry. These specific food items were combined with FFQ groups that contained similar features and then matched to one FooDB entry.

Sample collection and processing

Blood samples were collected at the UMCG, and serum was processed and frozen. Serum samples were stored at -80°C until analysis. Participants collected fecal samples at home and stored the sample at approximately -20°C until collected. Fecal samples were transported on dry ice and stored at -80°C until analysis. All serum and fecal samples were processed at the UMCG according to standardized protocols.

Quantification of serum and fecal metabolites

Metabolites present in plasma were measured using the nuclear magnetic resonance (NMR) method, as described by Kettunen et al. Fecal metabolites were quantified by Metabolon (North Carolina, USA). Quantification started with removing proteins and organic solvents from the sample, whereafter the sample was divided into four fractions for further analysis. Two fractions served in two separate reverse phases (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one fraction was used in RP/UPLC-MS/MS with negative ion mode ESI, and one fraction was used in HILIC/UPLC-MS/MS with negative ion mode ESI. Raw data processing and quality control were performed according to standard protocols of Metabolon.

Statistical analysis

All statistical analyses were performed in R (v.4.1.1), the corresponding scripts are available at the collaborative GitHub page (*https://github.com/EOosterhout/Chem-Food-IBD*). For all analyses correction for multiple testing was performed using a False Discovery Rate (FDR) correction, where FDR < .05 was selected as significance cut-off.

Correlation analysis

Derived dietary compounds were correlated with the original food groups using Pearson correlation. Next, derived dietary compounds were correlated with serum and fecal metabolites, using the same approach.

Linear regression analysis

Associations of dietary compounds with clinical outcomes; diagnosis status (binary, IBD yes/no), fcal (binary, > or < 150 µg/g) and consumption before a flare was tested using linear regression; the confounding factors age, sex and BMI were included in the different models, represented as follows:

$$\text{Predicted dietary compound} \sim \text{IBD diagnosis (yes|no)} + \text{age} + \text{sex} + \text{BMI}$$

$$\text{Predicted dietary compound} \sim \text{fcal} (< 150 | > 150) + \text{age} + \text{sex} + \text{BMI}$$

$$\text{Predicted dietary compound} \sim \text{before a flare (yes|no)} + \text{age} + \text{sex} + \text{BMI}$$

Results

Participants characteristics

The demographic characteristics of the participants separated by diagnosis are shown in **Table 1**. Participants of the general population cohort (*n = 1450*) are on average 43.43 years old, with 58.3% female. Participants of the IBD cohort (*n = 533*) are on average 43.24 years old, with 60.4% female. The primary aspects in which the control and IBD group differ are the following: the use of laxatives is significantly higher in the IBD group compared to the control group ($p < .0001$), this is also the case for the use of antibiotics ($p = .0439$) and PPI's ($p < .0001$). The IBD group had a higher median concentration of fecal calprotectin compared to the control group ($p < .0001$).

Table 1: *Demographic characteristics of the study population, with between-group comparisons. Normally distributed data is represented by the mean value, comparisons were performed using t-tests. Non-normally distributed data is represented by the median value, and comparisons were done using Wilcoxon Rank Sum tests. Proportions were tested using chi-square tests.*

	Control (n = 1450)	**IBD (n = 533)**	**P-value**
Age			
Mean (SD)	43.42 (13.42)	43.24 (14.32)	*P* = 0.7934
Minimum	18	18	
Maximum	86	82	
Gender, n (%)			*P* = 0.4364
male	604 (41.7%)	211 (39.6%)	
female	846 (58.3%)	322 (60.4%)	
BMI			*P* = 0.5957
Median (Q1, Q3)	24.50 (22.30, 27.20)	24.60 (22.02, 27.98)	
Minimum	15.80	15	
Maximum	48.80	57.80	
Smoking status			*P* = 1.0000
Smoker	282/1,440 (19.6%)	102/523 (19.5%)	
Non-smoker	1,158/1,440 (80.4%)	421/523 (80.5%)	
Medication types used, n (%)			
Laxatives	19/1,105 (1.7%)	35 (6.6%)	*P* < 0.0001
Antibiotics	12/1,105 (1.1%)	16 (3.0%)	*P* = 0.0362
PPI	92/1,105 (8.3%)	127/504 (25.2%)	*P* < 0.0001
Caloric intake (kcal/day)			
Mean (SD)	1,946.88 (610.24)	1,954.36 (671.47)	*P* = 0.7135
Minimum	296.77	319	
Maximum	7343.47	5578	
Animal protein intake (g/day)			
Mean (SD)	43.14 (15.00)	39.32 (13.27)	*P* = 0.6734
Minimum	7.21	0	
Maximum	145.90	80.20	
Plant protein intake (g/day)			
Mean (SD)	30.14 (10.13)	29.63 (11.46)	*P* = 0.7256
Minimum	5.35	2	
Maximum	78.24	84.50	
Fecal calprotectin (µg/g)			
Median (Q1, Q3)	40.00 (40.00, 59.88)	150.00 (45.00, 396.25)	*P* < 0.0001
Minimum	10	0.10	
Maximum	1540	15590	

Measured dietary intake of food groups correlates with their derived compounds

Correlation analyses were performed as a quality control, to check whether the derived dietary compounds showed a correlation with the food groups they are expected to be present in (**Figure 2**)[15–20]. The Pearson coefficient was calculated and depicted using a correlation plot (**Figure 3**).

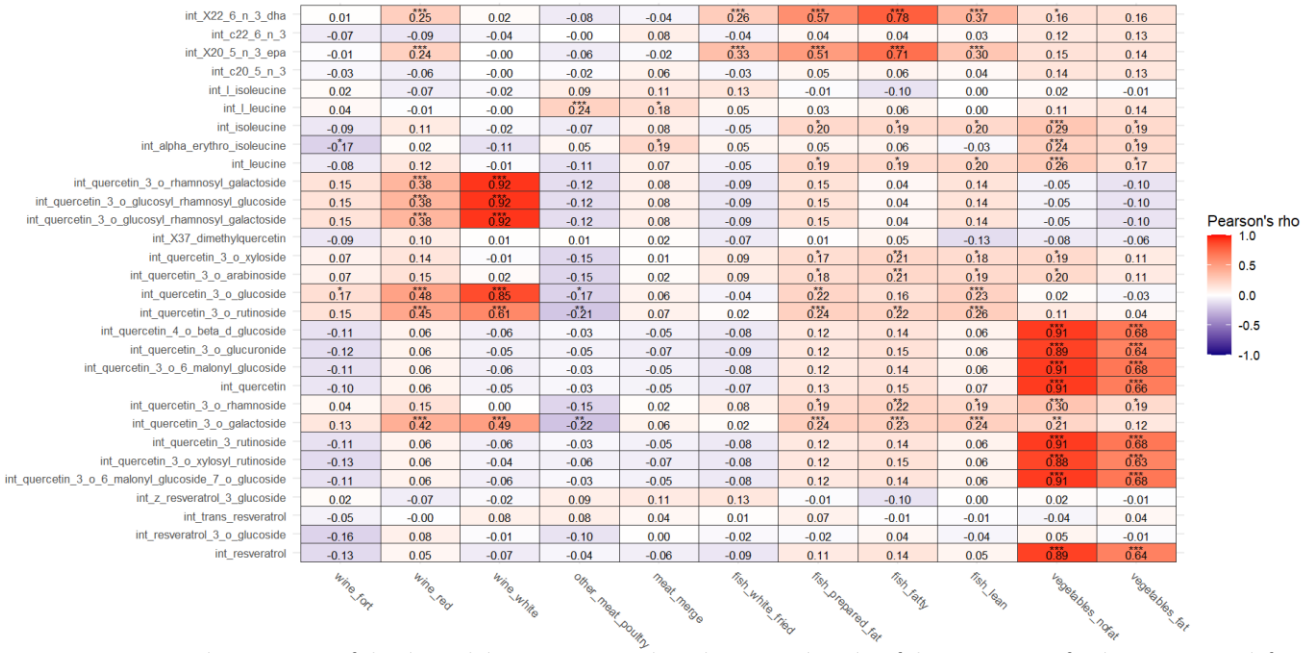

Figure 3: *Pearson correlation matrix of the derived dietary compounds and measured intake of their respective food groups. From left to right the correlation coefficients are shown from the respective dietary compounds and food groups. Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

When using measured dietary intake from food groups and derived dietary compounds in a Pearson correlation, 77 positive correlations and 4 negative correlations are identified between food groups and derived dietary compounds.

As expected, leucine was positively correlated with animal protein sources; fish and meat (**Figure 3**). L-leucine positively correlates with the intake of poultry ($r = .24$, $p < .001$) and meat ($r = .18$, $p < .05$). Intake of meat also shows a positive correlation with α-erythro-leucine ($r = .19$, $p < .05$) Isoleucine shows a positive correlation with intake of fish prepared with fat ($r = .20$, $p < .05$), fatty fish ($r = .19$, $p < .05$) and lean fish ($r = .20$, $p < .05$).

The food groups white wine and red wine positively correlate with several plant flavanols including quercetin-3-O-rhamnosyl galactoside ($r = .92$, $p < .001$) (**Figure 3**). On the contrary, quercetins showed negative correlations with poultry. Other polyphenols positively correlate with vegetables, including resveratrol ($r = .89$, $r = .64$, $p < .001$) and several quercetin glucosides (**Figure 3**).

Expectedly, docosahexaenoic acid (DHA) positively correlates with different types of fish; white fried fish ($r = .26$, $p < .001$), fish prepared with fat ($r = .57$, $p < .001$), fatty fish ($r = .78$, $p < .001$) and lean fish ($r = .37$, $p < .001$). The same pattern is shown between eicosapentaenoic acid (EPA) and fish (**Figure 3**).

Correlations between subsets of derived dietary compounds and their related fecal and serum metabolites

Correlation analysis between intake and fecal or serum metabolites were performed to explore how well the predicted intake of dietary compounds correlates with products of metabolic pathways involved in breaking down the consumed compounds. Therefore, subsets of the database were used based on information from previous studies describing metabolites from food intake found in feces that can be related to the action of the gut microbiome (**Figure 4-7**)[27].

Dietary fibers correlate with carboxylic compounds and fiber degradation products in feces

Correlations between the predicted carbohydrate and fiber intake with carboxylic acids in feces[28,29] were investigated and depicted using a correlation plot (**Figure 4**).

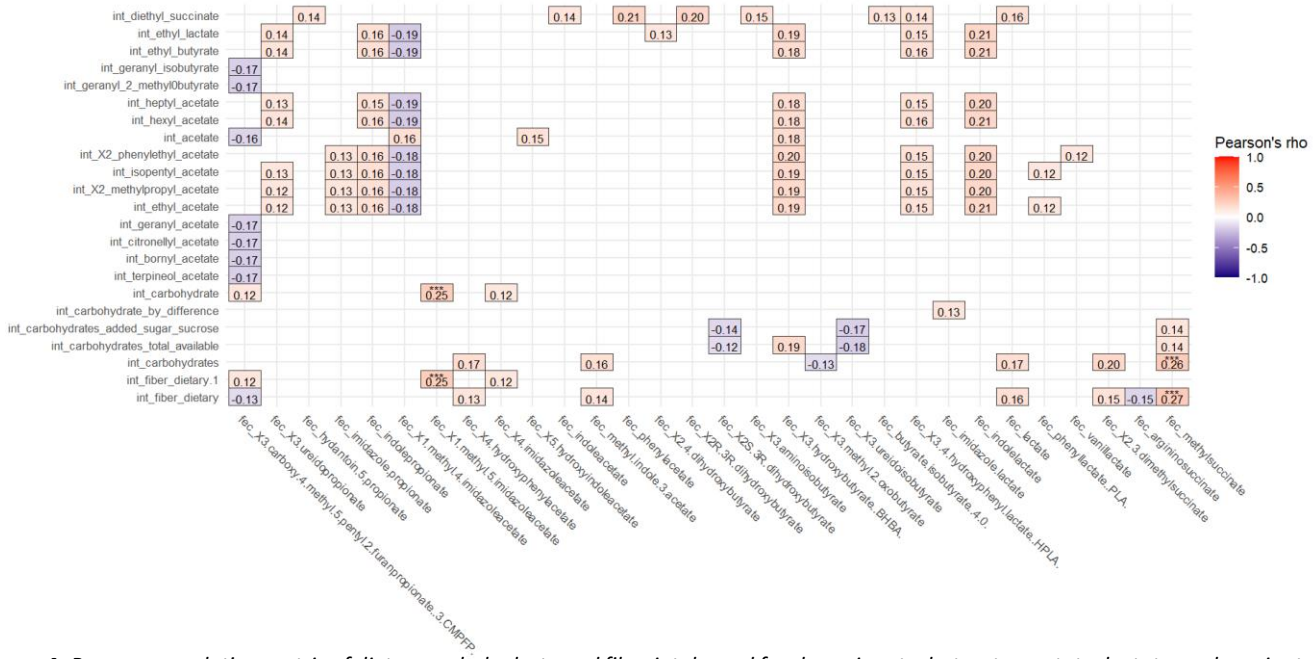

Figure 4: *Pearson correlation matrix of dietary carbohydrate and fiber intake and fecal propionate, butyrate, acetate, lactate, and succinate levels. From left to right the correlation coefficients are shown from the respective dietary compounds and fecal metabolites. Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

Fecal metabolites 1-methyl-5-imidazole acetate (*r = .25, p < .001*) and methyl succinate (*r = .26, p < .001*) showed a positive correlation with the intake of carbohydrates (**Figure 4**). Fecal metabolites 1-methyl-5-imidazole acetate (*r = .25, p < .001*) and methyl succinate (*r = .27, p < .001*) positively correlated with the intake of dietary fiber (**Figure 4**). 1-methyl-5-imidazole acetate belongs to the class of organic compounds known as imidazolyl carboxylic acids and is identified as secondary metabolite[30]. These metabolites are non-essential metabolites that may serve a role as defense or signaling molecules. Furthermore, methyl succinate is known as a tricarboxylic acid (TCA) cycle intermediate[31].

Dietary carbohydrates and fiber correlate with phenyl propanoic acids and dicarboxylate-hydroxybutyrate cycle intermediates in serum

Additionally, correlations between intake of carbohydrates and fiber and serum metabolites were performed (**Figure 5**).

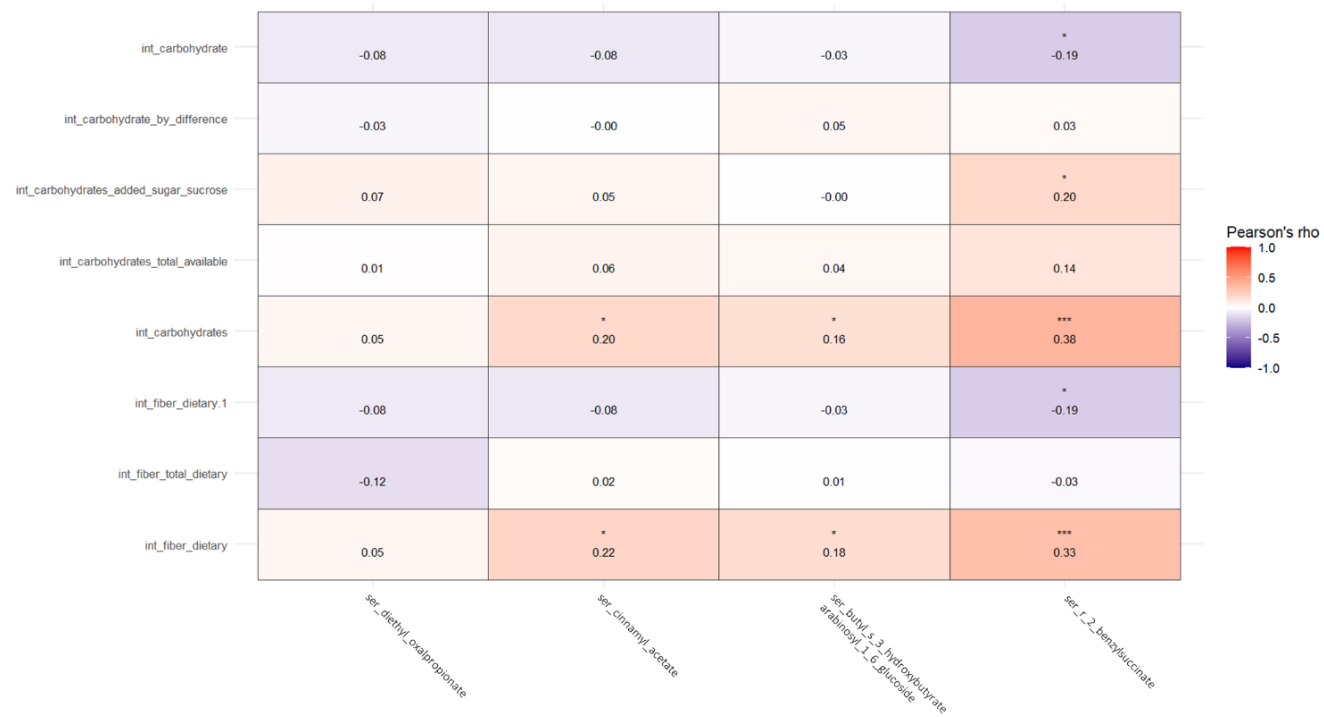

Figure 5: *Pearson correlation matrix of dietary carbohydrate and fiber intake and propionate, butyrate, acetate, lactate, and succinate levels in serum. From left to right the correlation coefficients are shown from the respective dietary compounds and serum metabolites. Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

In the above correlation matrix, a significant positive correlation was found between serum (R)-2-benzylsuccinate (*r = .36, p < .001*) and intake of carbohydrates (**Figure 5**). In a similar way, cinnamyl acetate (*r = .20, p < .05*) and butyl-s-3-hydroxybutyrate-arabinosyl (1,6)-glucoside (*r = .16, p < .05*) positively correlated with intake of carbohydrates (**Figure 5**). This positive correlation pattern is also shown between cinnamyl acetate (*r = .22, p < .05*) and butyl-s-3-hydroxybutyrate-arabinosyl (1,6)-glucoside (*r = .18, p < .05*) and intake of dietary fibers.

Interestingly, these metabolites are phenolic compounds, which in general are reported to exert anti-inflammatory effects. Benzyl succinate is a phenyl propanoic acid involved in anaerobic toluene oxidation to benzoyl-CoA[32]. Furthermore, butyl-s-3-hydroxybutyrate-arabinosyl (1,6)-glucoside belongs to the fatty acyl glycosides of mono- and disaccharides and is involved in the dicarboxylate-hydroxybutyrate cycle in microorganisms[33].

Dietary intake of tryptophan doesn't correlate with indole variants and tryptamine levels in feces

Next, correlations between predicted dietary intake of tryptophan and fecal indole and its derivatives[27,34]; indole-3-aldehyde, indole-3-propionic acid, indoxyl sulfate and tryptamine were performed (**Figure 6**). The selection of fecal metabolites is based on the metabolites of tryptophan that are expected to be produced by the gut microbiome[35].

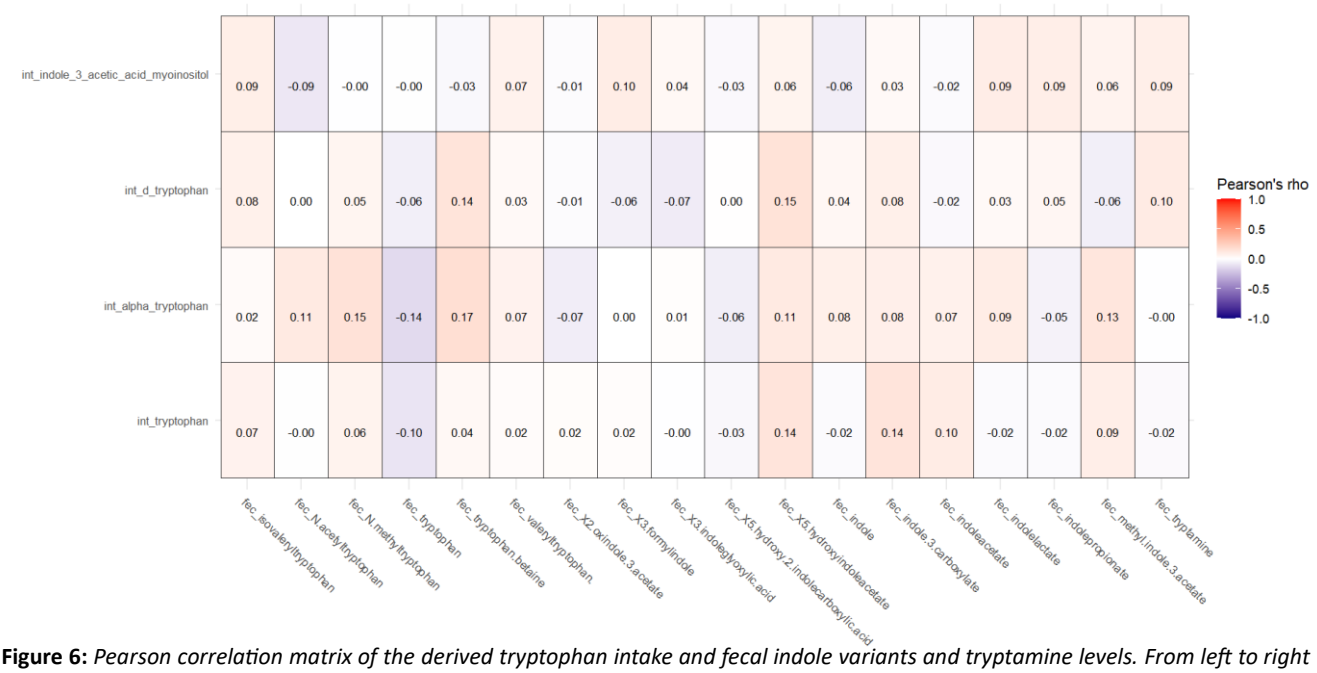

Figure 6: *Pearson correlation matrix of the derived tryptophan intake and fecal indole variants and tryptamine levels. From left to right the correlation coefficients are shown from the respective dietary compounds and fecal metabolites. Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

No significant correlations are found when using predicted dietary tryptophan and measured fecal indole variants and tryptamine in a Pearson correlation matrix (**Figure 6**). However, there were modest positive correlations between fecal metabolites; N-methyl tryptophan (*r = .15, p = .34*), tryptophan betaine (*r = .17, p = .34*) and methyl-indole-3-acetate (*r = .13, p = .38*) with intake of α-tryptophan, none of these were statistically significant (**Figure 6**).

Dietary tryptophan correlates with Indole-3-propionic acid in serum

The same relationship was investigated between dietary tryptophan and serum metabolites (**Figure 7**).

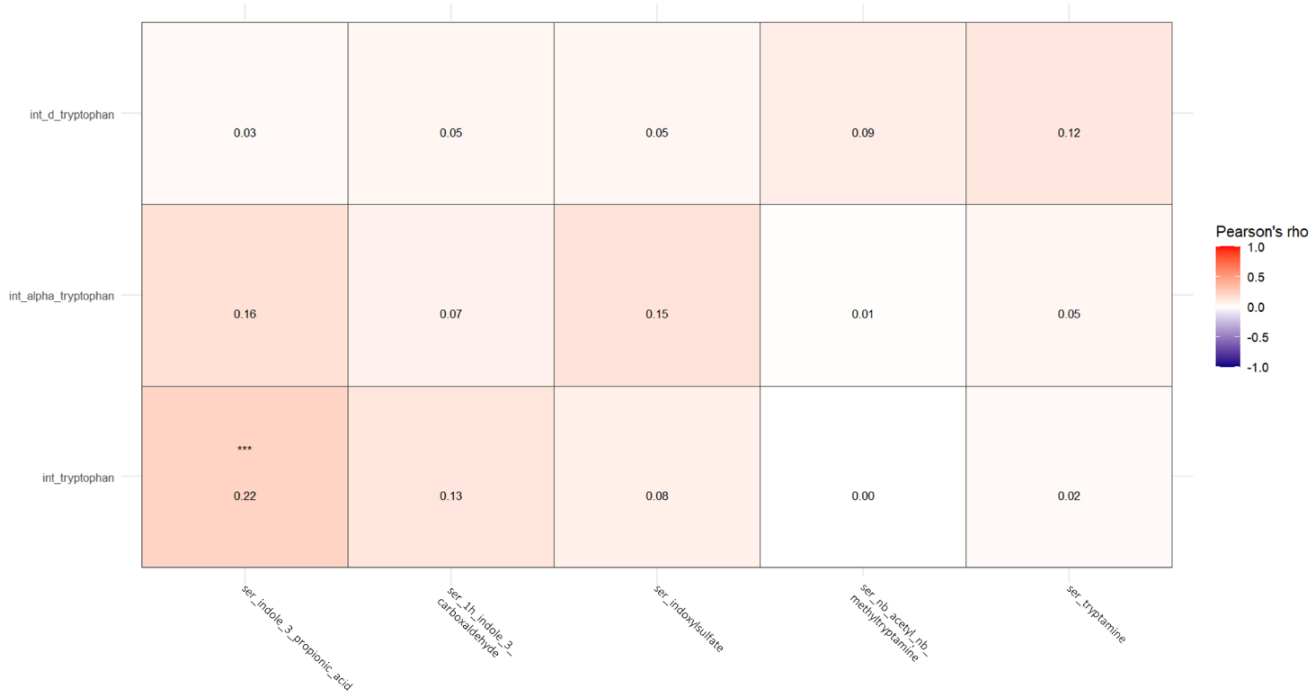

Figure 7: *Pearson correlation matrix of the derived tryptophan intake and indole variants and tryptamine levels measured in serum. From left to right the correlation coefficients are shown from the respective dietary compounds and serum metabolites. Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

In this correlation matrix a significant positive correlation between intake of tryptophan and indole-3-propionic acid ($r = .22$, $p < .001$) measured in serum was found. Serum levels of indole-3-propoinic acid ($r = .16$, $p = .10$) and indoxyl sulfate ($r = .15$, $p = .11$) showed a positive correlation with intake of α-tryptophan, neither correlation was significant.

Associations of dietary compounds with clinical outcomes related to IBD

To analyze whether diagnosis of IBD or clinical outcomes are associated with the intake of dietary compounds, a linear regression analysis was performed in which each dietary compound was defined as dependent variable and the clinical outcome together with relevant covariates, as independent variables (**Figure 8-10**).

Myristic acid and saturated fatty acids are positively associated with IBD diagnosis

First, associations between derived dietary compounds and diagnosis of IBD (binary, IBD yes/no) were tested to determine differences between patients with IBD and controls (**Figure 8**). Thereby, adjusting for age, sex, and BMI.

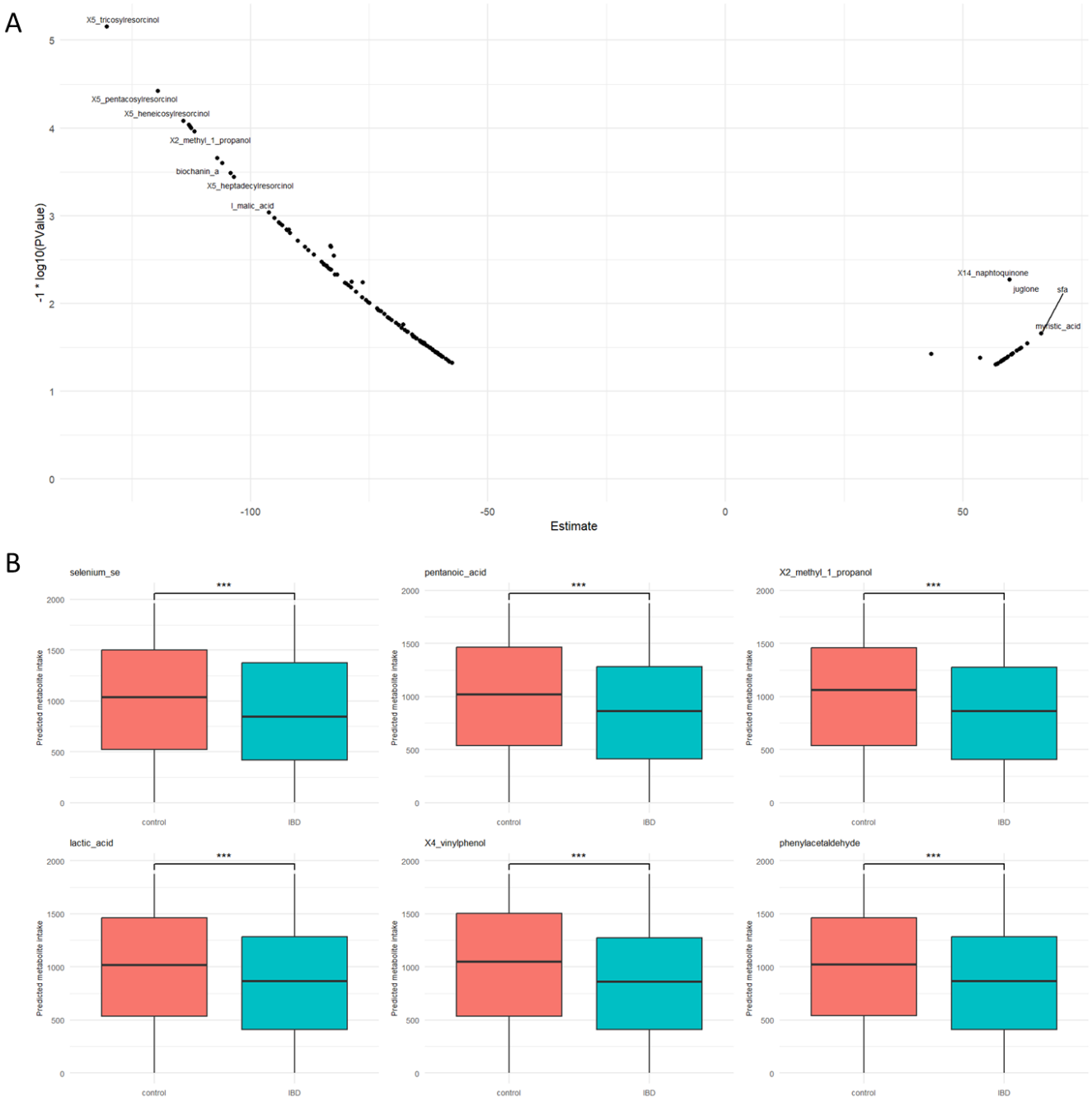

Figure 8: *Linear regression of dietary compounds associated with diagnosis status in IBD.* ***A:*** *volcano plot with -1 * log10(p-value) plotted against the calculated estimate.* ***B:*** *intake of dietary compounds with the highest r-squared is plotted against the compared groups; control and IBD. Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

IBD diagnosis i.e., presence of confirmed IBD was negatively associated with 274 dietary compounds including 5-tricosylresorcinol, 5-pentacosylresoricnol, 5-heneicosylresorcinol, 2-methyl-1-propanol, biochanin a, 5-heptadecylresorcinol and malic acid (**Figure 8A**). Amongst others (*n = 145*), the compounds 1,4-naphtoquinone, juglone, saturated fatty acids and myristic acid were positively associated with IBD diagnosis (**Figure 8A**). A significantly higher intake of dietary metabolites with the highest absolute value of R^2 was identified in the healthy controls compared to participants diagnosed with IBD (**Figure 8B**).

Fecal calprotectin levels can be predicted by intake of phytosterols

Secondly, associations of dietary compounds with clinical outcome fcal (binary, > or < 150 µg/g) were investigated, adjusting for age, sex, and BMI (**Figure 9**).

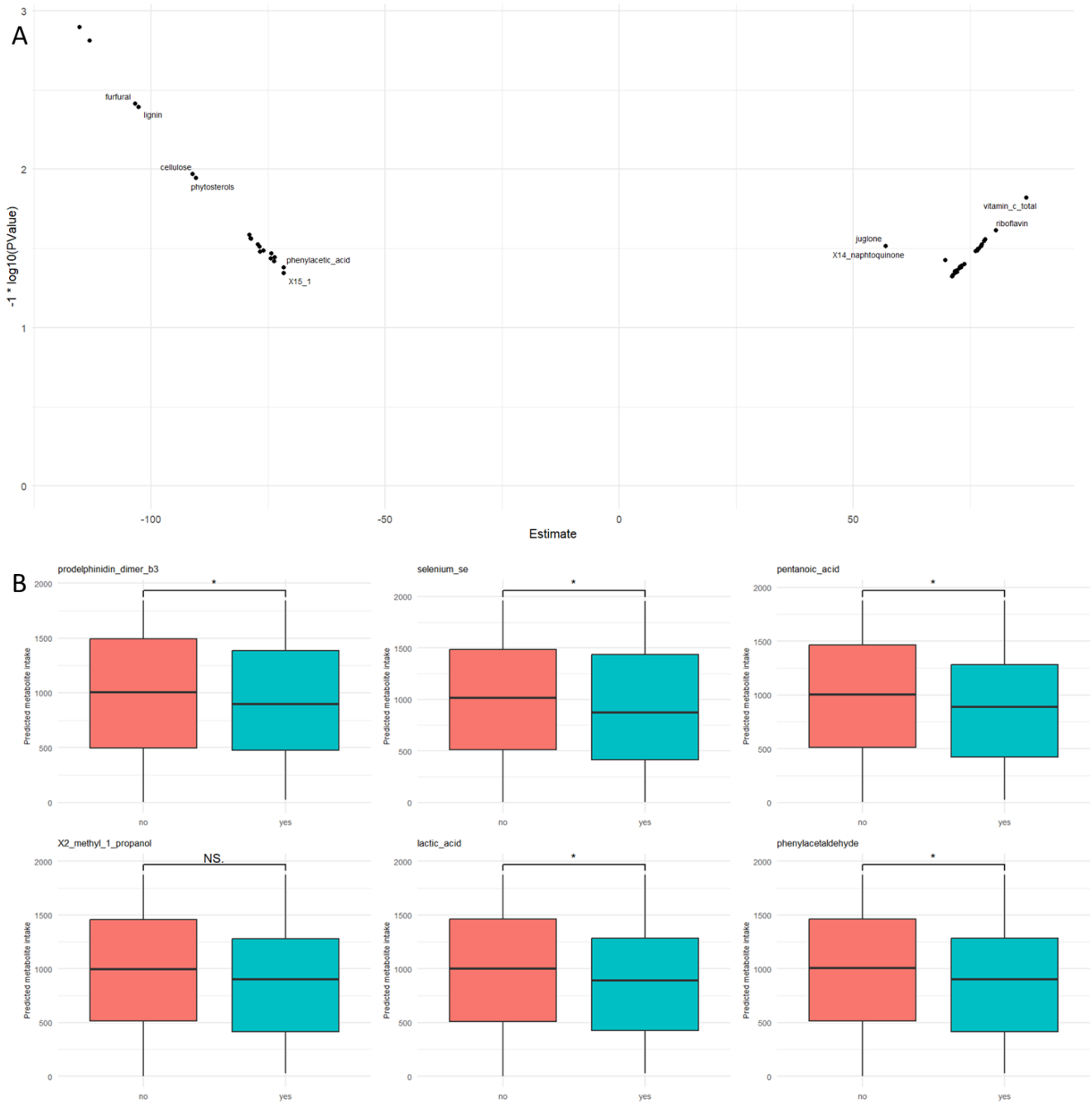

Figure 9: *Linear regression of dietary compounds associated with fecal calprotectin levels.* ***A:*** *volcano plot with -1 * log10(p-value) plotted against the calculated estimate.* ***B:*** *intake of dietary compounds with the highest r-squared is plotted against the compared groups; fecal calprotectin <150 µg/g (no) and fecal calprotectin >150 µg/g (yes). Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

When looking at the associated dietary compounds with fcal; the resulting output showed that abundance of, amongst others (*n = 48*), dietary compounds; furfural, lignin, cellulose, phytosterols, phenylacetic acid and pentadecanoic acid (15:1) is negatively associated with fcal < 150 µg/g, hereinafter referred to as fcal positive cases (**Figure 9A**). Fcal positive cases are positively associated with 57 compounds; of which 1,4-naphtoquinone, juglone, riboflavin and vitamin C are examples (**Figure 9A**). Higher intake of dietary compounds was identified in fcal negative cases compared to fcal positive cases, when subsetting the data based on the highest absolute value of R^2 (**Figure 9B**).

Occurrence of a flare is positively associated with intake of long chain fatty acids

Lastly, the ability of dietary compounds to predict occurrence of a flare was investigated (**Figure 10**).

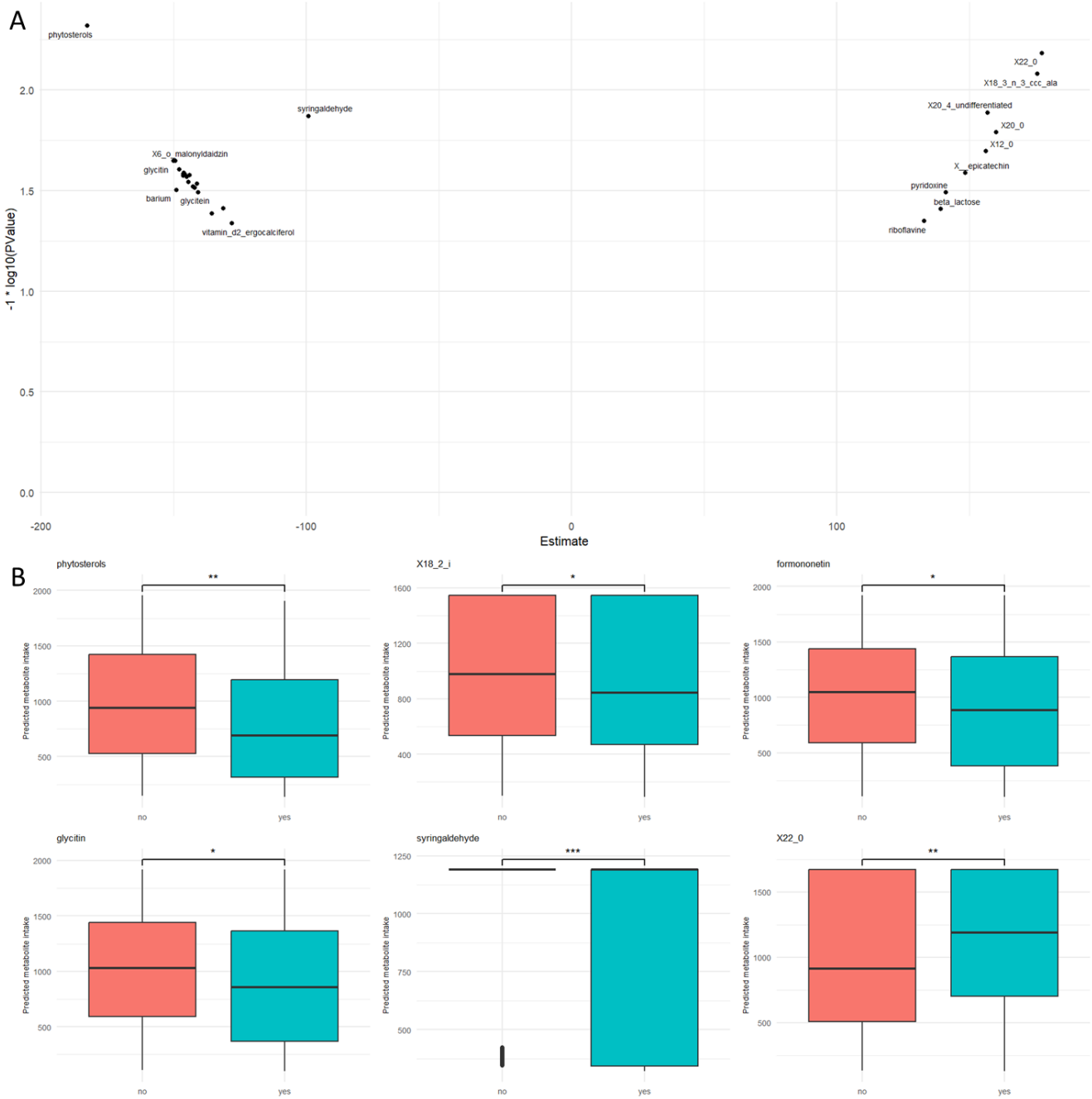

Figure 10: *Linear regression of dietary compounds associated with occurrence of a flare after the dietary assessment period.* ***A:*** *volcano plot with -1 * log10(p-value) plotted against the calculated estimate.* ***B:*** *intake of dietary compounds with the highest r-squared is plotted against the compared groups; during or after a flare (no) and before a flare (yes). Significance levels are adjusted for multiple comparisons using the false discovery rate method. *** padj < .001, ** padj < .01, * padj < .05*

In the model assessing association of dietary compounds with occurrence of a flare after the dietary assessment period; the resulting output showed that abundance of amongst others dietary compounds (*n = 39*); phytosterols, glycitin, barium, glycitein, 6-O-malonyl daidzein, vitamin D2 (ergocalciferol) and syringaldehyde is negatively associated with occurrence of a flare after the dietary assessment period (**Figure 10A**). The compounds; riboflavin, ß-lactose, pyridoxine, epicatechin, lauric acid (12:0), arachidic acid (20:0), arachidonic acid (20:4), α-linolenic acid (18:3 n-3) and behenic acid (22:0) were positively associated with occurrence of a flare after the dietary assessment (**Figure 10A**). A significantly higher intake of a subset of dietary compounds in the group where a flare did not take place after dietary assessment compared to participants where a flare did take place after dietary assessment (**Figure 10B**). One exception is behenic acid (22:0), of which higher intake is reported in the group where a flare took place after the dietary assessment period compared to participants who did not experience a flare (**Figure 10B**).

Conclusion and Discussion

In this thesis a framework to investigate the role of diet in IBD on a level of specific dietary compounds has been developed and employed. Since the course and symptoms of IBD vary from person to person, and can change over time, it important to explore environmental factors influencing the disease course. Previous studies have identified food groups and dietary patterns that are associated with the level of disease severity in IBD. However, this approach of dietary pattern and food analysis remains to reflect the cumulative effect of the various nutrients and compounds contained in the diet. Investigating nutrition on the level of nutrients and metabolic compounds enables us to disentangle why certain food groups and diet patterns are beneficial or detrimental in IBD. Secondly, this approach allows us to derive more targeted dietary approaches in the future, tailored to a patient's needs.

This framework was developed using information obtained from FFQ's and converting this information to dietary compounds using the online database FooDB. The framework was validated through correlations identified between derived dietary compounds and the food groups they can be expected to be found in, as well as expected fecal and serum metabolites based on the involvement of the gut microbiome[27,36].

Dietary compounds correlated with food groups that they are expected to be found in[15–20], aligning with existing literature on food composition. These results validate the data algorithm that was developed during this project. Correlations between intake of quercetin and wine or vegetables together with intake of DHA and EPA and intake of fatty fish are very well represented in the newly acquired data.

Furthermore, correlation analysis between intake and fecal or serum metabolites were performed to explore how well the predicted intake of dietary compounds correlates with products of metabolic pathways involved in breaking down the consumed compounds. Therefore, subsets of the database were used based on information from previous studies describing metabolites found in feces that can be related to the action of the gut microbiome[27].

Main results from these analyses show that intake of carbohydrates and fiber correlates well with fecal and serum succinate and acetate. This is in line with the reported pathways involved in breakdown of carbohydrates and fiber. Interestingly, the fecal metabolites correlating with intake of carbohydrates and fiber are methyl succinate and 1-methyl-5-imidazole acetate, whereas the correlating serum metabolites are (R)-2-benzylsuccinate and cinnamyl acetate. Diving deeper into the pathways involved in synthesis of these compounds it can be found that methyl succinate is an intermediate of the citric acid cycle with fumarate as precursor metabolite[37]. Fumarate is also active in the formation of (R)-2-benzylsuccinate. However, this compound is proposed to be involved in anaerobic toluene oxidation to benzoyl-CoA[32]. The benzyl succinate synthase reaction has been documented in three anaerobic toluene-degrading bacteria: denitrifying strain T, *Thauera aromatica* and sulfate-reducing strain PRTOL1[38–40]. The fact that (R)-2-benzylsuccinate is found in serum and not in feces could be explained by the fact that reabsorption of (R)-2-benzylsuccinate could take place[36]. Methyl succinate enters oxidative phosphorylation or is degraded further to succinyl-CoA[37]. Although it is normally regarded as an intermediate; succinate is observed to accumulate in certain pathophysiological situations, especially in areas of inflammation and metabolic stress[41].

Regarding the carbohydrate and fiber intake correlating metabolites; 1-methyl-5-imidazole acetate and cinnamyl acetate, recent literature hasn't reported a direct link between the intake of carbohydrates and fiber and the production of these compounds in humans. However, cinnamyl acetate has also been detected, but not quantified in, several carbohydrate rich foods, such as figs, fruits, and guavas[42]. Furthermore, 1-methyl-5-imidazole acetate has been linked to fructose consumption in mouse models. In this same study the fecal microbiota, *Rikenellaceae*, *Pseudomonadaceae* and *Pseudomonas* were reported to play a role in the production of 1-methyl-5-imidazole acetate from fructose[43].

Given their importance in maintenance of metabolic health, intake of tryptophan was investigated more closely. Results show a stronger correlation between tryptophan intake and its expected metabolites in serum compared to feces. No significant correlations were found between intake of tryptophan and fecal metabolites. In serum, a strong significant correlation between intake of tryptophan and indole-3-propionic acid was found. Regarding the fact that this is one of the hypothesized metabolites of tryptophan, the results seem to validate the new database of dietary compounds. Although indole-3-propionic acid is a gut microbiota-derived metabolite[44], it has been reported that this compound plays a significant role in cardiovascular health[45]. Therefore, it can be suspected that after production of indole-3-propionic acid by gut bacteria; the compound is reabsorbed and exerts health supporting functions[34].

Lastly the newly acquired layer of nutritional data was tested in a clinical setting using linear regression models of dietary metabolites with clinical outcomes of IBD; diagnosis status, fcal and occurrence of a flare set as an independent variable. Main results show that amongst others; saturated fatty acids as well as myristic acid are associated with IBD diagnosis, suggesting that the diet of IBD patients contains more of these types of fats compared to the controls. Interestingly, a study which investigate the relation between high dietary Intake of specific fatty acids and the risk of flares in UC patients reported to be in remission found that increasing intake of especially myristic acid was associated with increasing odds of relapse[46]. Despite being diagnosed with IBD, patients don't seem to eat healthier, these results align with previous studies of the same cohort[47]. Together these findings suggest that IBD patients consume a diet higher in saturated fats and myristic acid than controls, which may also negatively influence their disease course. Furthermore, phytosterols show a negative association with fcal positive cases and occurrence of a flare, which have been reported in multiple studies to contain anti-inflammatory properties[48–50].

Based on the value of R^2, phytosterols together with other compounds show the strongest negative association with the investigated outcomes related to IBD and are most of these compounds are reported to play a role in lowering inflammation and supporting intestinal barrier function[51–59]. Examples of compounds supporting the intestinal barrier are selenium, pentanoic acid and lactic acid, which were more abundant in the diet of healthy individuals compared to IBD patients. Fcal positive cases compared to fcal negative cases show a similar pattern in terms of abundance of selenium and pentanoic acid and lactic acid in the diet.

In individuals experiencing a flare, phytosterols and glycitin are less abundant compared to the individuals where a flare didn't occur. Interestingly, behenic acid (22:0) tends to be more prevalent in the diet of individuals experiencing a flare after the dietary assessment period than individuals who did not experience a flare. This is a saturated fatty acid, which is poorly absorbed form the diet due to its long chain length, therefore it has been reported as a cholesterol-raising fatty acid in humans[60]. Despite the thorough investigation of the relationship between behenic acid and cholesterol, no direct link between the intake of behenic acid and inflammation has been reported to date.

Limitations of this study include the fact that some food groups are frequently eaten together. This may present an error when referring the derived dietary compounds of the food groups back to the measured content of these food groups reported in literature. Secondly, the role of the microbiome in metabolism of the dietary metabolites can now only be suspected. To be able to measure this a correlation analysis between dietary compounds and the presence of specific bacteria can be performed, this way a preliminary idea regarding how both factors influence each other can be constructed.

In summary, the main findings suggest that by taking a metabolic approach of studying the role of diet in active inflammation in IBD patients, we can gain a better understanding of the impact of dietary compounds in active inflammation. This gives new perspectives in treatment of IBD patients through specifying nutritional protocols and lowering the risk for disease development together with the management of active illness; thereby lowering disease severity.

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Appendices

Appendix 1: Food groups established from the Food Frequency Questionnaire

vegetables_nofat
vegetables_fat
peanutbutter
snack_nut
porridge
nut_d
legumes
coffee
soup_legumes
applesauce
fruit
rice
potato_baked_fries
potato_cooked_mashed
beer_af
beer
pasta
spirits
herring_salted
chicken
fish_white_fried
fish_prepared_fat
fish_fatty
fish_lean
other_meat_poultry
meat_merge
pork_merge
cooking_oil
egg_baked
liquor
egg_cooked
yogurt_merge
spreads_sweet
wine_fort
butter_b
whipped_cream
chocolate
chocolatemilk
coffeecreamer_merge
buttermilk
crackers
biscuits_s
other_milk_coffee
fruit_juice
rolls
bread
margarine_lfb
milk_coffee
milk_skimmed
milk_semiskimmed
tea
wine_red
wine_white