Research article

Prevotella enterotype associates with diets supporting acidic faecal pH and production of propionic acid by microbiota

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A R T I C L E   I N F O

A B S T R A C T

Metabolism of dietary fibres by colon microbiota plays an important role for human health. Personal data from a nutrition study (57 subjects) were analysed to elucidate quantitative associations between the diet, faecal microbiome, organic acid concentrations and pH. Ratios of the predominant acids acetate, butyrate and propionate ranged from 1:0.67:0.27 to 1:0.17:0.36. Pectin-rich diets resulted in higher faecal acetate concentrations. Negative correlation between faecal pH and BSS was observed. Higher faecal pH and lower acid concentrations were related to the higher abundance of amino acid degrading *Clostridium*, *Odoribacter* and *Eubacterium coprostanoligenes*, which are weak carbohydrate fermenting taxa. Propionic acid correlated especially to high abundance of *Prevotella* and low abundance of proteobacteria. The acetate to propionate ratio of the *Prevotella* enterotype was about half of that of the *Bacteroides* enterotype. Based on the results we suggest the measurement of faecal pH and organic acid composition for research and diagnostic purposes.

1. Introduction

Every organ system of the human body relies on pH balance, however, the renal and the pulmonary systems are the two main pH modulators of the organism. The pulmonary system adjusts pH using carbon dioxide, upon expiration, carbon dioxide is projected into the environment. The renal system affects pH by reabsorbing bicarbonate and excreting fixed acids. Usually the body maintains the pH of blood close to 7.40 [1].

Gastrointestinal tract (GIT) is an organ, separated from blood and lymph circulations by gut epithelial cells and mucus layer, hence, its pH is not tightly controlled by the systems described above. Nevertheless, it is a dynamic environment in which microorganisms interact with the host via their metabolites. Among them, the most important metabolites are fermentation products such as organic acids. These weak acids having pKa 3.9–4.8 are present as organic anions in the gut lumen. The concentration of these anions is negatively correlated with faecal pH [2]. Intestinal pH indicates the acid-alkali balance and metabolic activities of the colon bacteria, which play important role for human health. The main weak acid in the colon is acetic acid, which normally ranges from 40 to 80 mmol/kg faeces followed by propionate (10–20 mmol/kg) and butyrate (5–15 mmol/kg) [2–4]. These bacterial fermentation products are important energy sources for epithelial or other human cells, regulating gut motility, signalling receptors for controlling hormone synthesis, anticancer or anti-inflammatory processes. As small molecules circulate in the blood bacterial products reach tissues and organs outside of the gut having general effect of human physiology, including gut-brain axis [5–7].

Gastrointestinal pH can be measured using a pH sensitive radiotelemetry capsule passing through the GIT [8]. Based on the review of Nugent et al., the luminal pH in the proximal small bowel ranges from 5.5 to 7.0 and gradually rises to 6.5–7.5 in the distal ileum [9]. After a fall from the terminal ileum to the caecum (range 5.7–6.8) pH then rises in the left colon and rectum to 6.1–7.2.

Data on the quantitative relationships of the diet, faecal microbiome, organic acids, pH and human health are scarce. A recent study [4] showed that pH was associated with the abundances of mucin-degraders *Akkermansia* and Ruminococcaceae, while inversely associated with several butyrate-producers such as *Faecalibacterium, Roseburia, Butyriciococcus*. In a study of LaBouyer et al. [10] the individual variations of faecal organic acids, pH (in five studies) and colon microbiota of 163 subjects from ten human studies were followed. In the baseline samples on habitual diet, the amount of faecal butyrate correlated positively with the total amount of short-chain fatty acids, whilst opposite was seen for acetate, iso-butyrate and iso-valerate. It was found that relative abundances of the butyrate-producing species *Roseburia* and *Eubacterium rectale* correlated with the amount of butyrate.

Ammonia is formed in metabolism of proteins, amino acids, and particularly urea by colon bacteria. The influence of ammonia on colonic pH is thought to be smaller than that of bicarbonate and organic acids. Diet has an impact on the luminal pH through its effects on organic acid production.

The production of acidic metabolites and the prevalence of the fermentative microbiota depend on the pH and available substrates, whilst details on bacterial metabolic interactions are largely unknown. *In vitro* experiments of Ilhan et al. [11] showed that pH was the strongest driver of microbial community structure and function. The structure of the microbiota was controlled by the pH that was determined by the balance between bicarbonate alkalinity and formation of organic acids. Walker et al. [12] showed that butyrate production by faecal microbiota was more enhanced at pH 5.5 compared to that at pH 6.5. At pH 6.5 growth enhancement of *Bacteroides* species along with increasing propionate production was observed. Higher abundance of butyric acid producers was observed in batch cultures of faecal microbiota at pH 5.5 on various dietary fibres [13]. Favoured growth of a butyric acid producer *Faecalibacterium* below pH 6 and *Bacteroides* above pH 6.5 have been shown in continuous cultures simulating the gastrointestinal environment [14,15]. These results underscore the impact of pH on microbial function in diverse microbial ecosystems such as human gut. The aim of the current study was to find quantitative relationships between diet, faecal pH, organic acids, Bristol stool score, and faecal microbiota at individual level. Our focus was to find associations between faecal pH, specific bacterial metabolites and specific gut bacteria. These metabolites can be formed only in the presence of fibre degrading and metabolizing taxa and available substrates under supporting environmental conditions including pH.

2. Materials and methods

2.1. Study design

In the current paper, the diet and microbiota data from a previously published human study [16] were re-analysed and correlated with the values of measured faecal pH and organic acids. The nutrition study was carried out with 57 healthy volunteers (36 females and 21 males) aged from 23 to 52 years. The participants did not have food restrictions, any kind of food intolerance, extremes in food consumption practices nor chronic illnesses. They continued their habitual diet and filled detailed food dairies three days before providing blood and faecal samples. The amples were collected and analysed as shown in Adamberg et al. [16]. The study was approved by the Tallinn Medical Research Ethics Committee (TMEK No 1631). All participants signed written informed consent forms before the study.

2.2. Faecal sampling, analyses of pH and organic acids

Faecal samples were self-collected by the participants in two consecutive occasions within each sampling period. One to 2 g of faecal samples were taken immediately after defecation by an integrated scoop into a collection tube (Sarstedt, Germany). The tube was kept at −20 °C until frozen transportation to the lab, maximum two days. In the lab, the samples were stored at −80 °C until further analyses.

For pH and organic acid analyses, the faecal sample was diluted five times with deionized water. The faecal suspension was mixed vigorously and centrifuged at 10 000 g 10 min to remove the undissolved particles. pH of the supernatant was measured at room temperature using a pH-meter (Mettler Toledo, MP125, Switzerland), electrode InLab Pro (Mettler Toledo, Switzerland, calibrated by pH buffers 4.01 and 7).

Concentrations of organic acids were determined from the faecal supernatant filtered through AmiconR Ultra-3K filters (cut-off 3 kDa) according to the manufacturer's instructions (Millipore, USA) at 4 °C. The samples were analysed by high-performance liquid chromatography (HPLC, Alliance 2795 system, Waters, Milford, MA, USA), using BioRad HPX-87H column (Hercules, CA, USA) with isocratic elution of 0.005 M H_2SO_4 at a flow rate of 0.5 mL/min and at 35 °C. The substances were quantified by refractive index (RI) (model 2414; Waters, USA) and UV (210 nm; model 2487; Waters, USA) detectors and analytical grade standards of acetate, butyrate, formate, isobutyrate, isovalerate, lactate, propionate, succinate and valerate. The detection limit for the HPLC method was 0.1 mM.

2.3. Microbiota analyses

Bacterial profiles in faeces were measured using PureLink Microbiome DNA extraction kit (Thermo Fisher Scientific, UK) for DNA extraction, which includes a bead beating step. Universal primers: S-D-Bact-0341-b-S-17 Forward (5′ TCGTCGGCAGCGTCA-GATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and S-D-Bact-0785-a-A-21 Reverse (5′

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) were used for PCR amplification of the V3–V4 hypervariable regions of the 16S rRNA genes. Sequencing libraries were prepared with Nextera XT DNA Library Preparation Kit v2 (Illumina). Prepared libraries were quantified using Qubit™ dsDNA HS Assay Kit (quantitation range 0.2–100 ng; Thermo Fisher Scientific). If the DNA quantity turned out to be too high, the sample was diluted and re-measurement was performed with Qubit™ dsDNA BR Assay Kit (quantitation range 2–1000 ng; Thermo Fisher Scientific, UK). Illumina MiSeq 2 × 250 v2 platform (Illumina) for sequencing of 16S rDNA region V3–V4 as described previously [16].

2.4. Data processing and statistical analyses

This study focuses on personal level relationships between faecal pH and organic acids, and between the diet and faecal microbiota composition. To elucidate the diet-microbiome relationships, the microbiota data of each faecal sample was correlated with the corresponding food consumption data considering the transit time. In general, looser stool is related to a shorter transit and vice versa [17]. Hence, the transit time was evaluated according to the reported Bristol stool score (BSS), and the diet was compared to the faecal microbiota composition. For example, harder stools (BSS <3) were matched with the foods three days earlier, those having BSS between 3 and 5, with the foods two days earlier and the stools with BSS above 5 (loose stools) with those eaten on the previous day.

The food consumption data was recorded by Nutridata program (Version 7, tap.nutridata.ee, National Institute for the Health Development, Estonia) as shown in Adamberg et al. [16]. The foods were quantified as grams per day for every participant. The nutrition data was re-analysed using weight in grams of a food products consumed per day instead of normalized caloric values (kcal of food per 1000 kcal). All foods were categorised into 15 main groups based on the dietary fibre composition and/or the food type (berries, fruits, fats/oils, nuts/seeds, sauces, sweets, vegetables; dairy, egg, fish, meat, refined grain and whole grain cereal products; alcoholic and non-alcoholic drinks). Further, the main food groups were divided into 47 sub-groups as shown in Supplementary Table S1. Very general and heterogeneous groups such as porridge cereals or dried fruits were avoided. Instead, the food groups were reformed based on the content of the microbiota accessible substrates. In cereal products the type of dietary fibres such as beta-glucans in oat and barley or arabinoxylans in rye and wheat were considered. Foods containing polyols or lactose, formed separate groups. The meals or food products containing several components with different fibre types, were divided into primary components. For calculation of consumption pattern amounts of dietary fibre in food groups per day were calculated. For foods not containing dietary fibres (dairy, meat, eggs and drinks), the portion sizes providing above 50 kcal per day (as 1 unit) were taken into calculations.

Four faecal samples were collected from each participant in a period from April to September. To minimize loss of seasonal peculiarities of the food choices, for example the peak time of strawberry consumption in June–July, the samples were analysed in three seasonal periods: taken in April–May, June–July and August–September. Each seasonal period represented a single sampling point per participant.

To find relationships between the diet, faecal pH, organic acids, and bacterial composition, correlation and group-based statistics was applied. For group-based analyses, all samples were divided into three terciles according to their pH and organic acid concentrations (subgroup indicator "low" or "high" represents the values of pH or acid concentrations lower than 33 or higher than 67 percentile). The samples were then compared between seasonal periods as described above. Statistical significance between the groups was calculated using unpaired Wilcoxon test. Results were considered significant at p-value <0.05. The ranges of pH in the pH groups were "low" (pH < 7), medium (pH 7–7.5) and „high" (pH > 7.5). The ranges of "low" and "high" in the acetate (Ace) groups 10–31 and 49–92 mmol/kg, respectively, in the butyrate (But) groups 1.9–8.6 and 14–33 mmol/kg, respectively, and in the propionate (Prop) groups 3.2–8.5 and 14–35 mmol/kg. *Bacteroides* and *Prevotella* prevalent microbiotas were designated by *Bacteroides* to *Prevotella* ratio, ratio 1 ± 0.5 was taken as a mixed enterotype. Samples of the two enterotypes were divided into groups based on the sampling periods (April–May, June–July, August–Sept). Only the bacteria significantly different at least between two groups were considered for further analysis (unpaired Wilcoxon test, p < 0.05 was taken as significant). Each participant is represented by a single sample per sampling period.

For general correlation analysis, Pearson correlations were calculated between foods or faecal bacteria and faecal pH or organic acid concentrations for each seasonal period. To find individual level relationships between food consumption and faecal pH or organic acids and faecal microbiota, Pearson correlations were calculated for data pairs of each participant. As there were four samples from each participant, categorical analysis of correlation coefficients was carried out by counting positive and negative correlations in each quartile of correlation coefficients ($R_1 < -0.5 < R_2 < 0 < R_3 < 0.5 < R_4$). Distribution bias of correlation trends towards positive or negative correlation was estimated by chi-square test.

Data analysis and statistics were done using programs Microsoft 365 Excel and R version 4.1.2. using the packages dplyr, GGally, ggpubr, reshape2, rstatix and tidyverse. Graphics were built using R Studio and package ggplot2.

3. Results

3.1. Variability of faecal pH and acid profiles

High variability between participants were found in faecal pH and organic acid content. Variation of the faecal pH between pH 5.7 and 8.8 was seen (Fig. 1), although the median pH and organic acid concentrations were comparable during all sampling periods. Acetate, butyrate and propionate covered the least 77 % (90th percentile) of the amount of all faecal organic acids detected with acetate as the major acid. The concentration of acetate varied from 11 to 92 mM and that of butyrate from 2 to 33 mM. The ratios of acetate, butyrate and propionate ranged from 1:0.67:0.27 to 1:0.17:0.36, respectively.
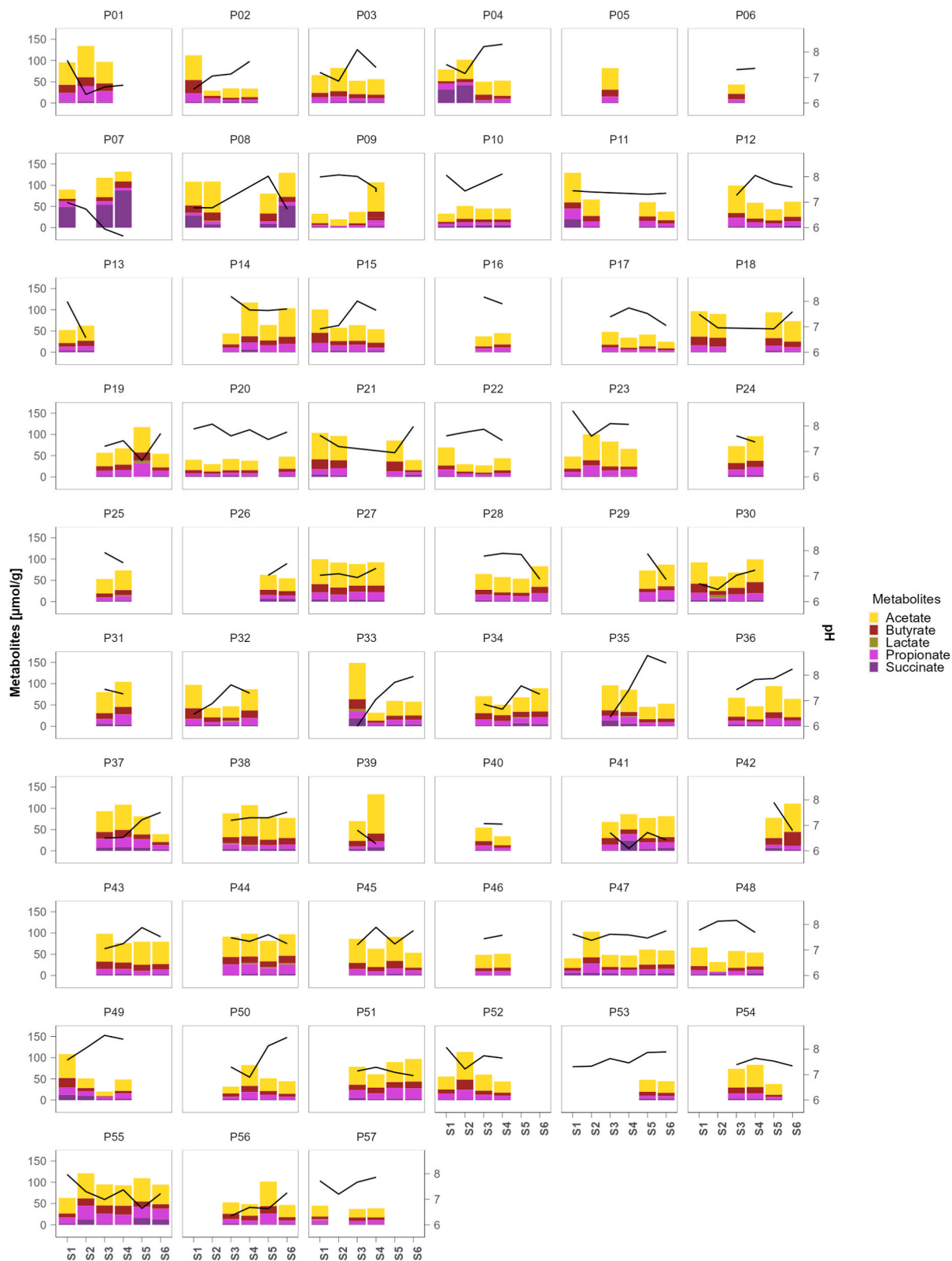
Fig. 1. Distribution of faecal pH and concentrations (mmol/kg) of acetate, lactate, butyrate, propionate and succinate of all participants. Samples S1, S2 were taken in April–May, S3, S4 in June–July and S5, S6 in August–September.

3.2. Seasonal changes in food consumption

In total 855 different food items were recorded, with only 20 common food items consumed by more than half of the subjects. Potatoes, onions, table sugar and rapeseed oil were consumed with the highest frequency (at least once by > 90 % participants, ie 53–55 persons) followed by tomatoes, eggs, carrots, cucumbers and 2.5 % drinking milk, all consumed by more than 80 % of the subjects. 46 common foods were similar for more than 25 % of participants i.e. 15 persons. Accordingly, the food items were categorised to elucidate the relationships between individual diet patterns, faecal microbiota composition, pH and organic acid contents.

Based on the food diaries at least one portion (50 g) of meat and dairy products per day were consumed by more than 70 % of the participants. Other abundantly consumed foods were potatoes, sweets and white wheat products (at least 50 g/day by at least 50 % of the subjects) (Fig. 2). When comparing the diets of spring (April–May) and summer (June–July) periods, seasonal variability could be seen. Consumption of strawberries and cherries was the highest in June–July while that of apples, citrus fruits and cruciferous vegetables (cabbages) was the lowest in this period. Also, consumption of buckwheat and rice decreased in summer period. At individual level, the faecal acid concentrations reflected the food consumption patterns. For example, pectin-rich diets resulted in higher faecal acetate concentrations while abundant consumption of white wheat products was inversely correlated with faecal butyrate concentration (Fig. 3).

3.3. Association of abundance of faecal Prevotella and concentration of propionic acid

The patterns of fermentation products reflected the degradation of dietary fibres by gut bacteria. The metabolites, in turn, affected

Food category	Apr-May	June-July	Aug-Sept
meat	71	71	83
dairy, excl. cheese	74	75	72
sweets	62	62	69
cheese/curd	64	46	55
starchy vegetables	37	50	52
white flour	35	40	52
tomato	42	41	43
rye	40	36	45
pumpkins	23	32	35
banana	32	35	20
apples	39	13	17
oat	21	18	28
fish	25	23	19
eggs	29	17	17
cruciferous vegetables	20	14	26
nuts/seeds	15	23	15
root vegetables	19	14	15
legumes	14	11	22
strawberries	3	32	11
buckwheat	21	14	6
cherries	1	26	9
whole wheat	12	11	12
rice/maize	17	7	11
leaf vegetables	7	7	12
citrus fruits	15	3	3
barley	5	6	0

Fig. 2. Heatmap of the food consumption patterns indicating seasonal variation. The numbers indicate the percentage of the participants consuming the food in the given category at a certain sampling period (April–May, June–July, August–Sept). Only the amounts of foods providing at least 1 g of dietary fibre per day are presented.

the microbial composition. Lower pH associated with higher concentration of total organic acids. In different, branched chain acids derived from amino acid degradation isovalerate and valerate did not have clear associations with faecal pH, possibly because of ammonia released from the amino group.

Higher faecal pH associated with the higher abundance of known amino acid degrading taxa such as *Clostridium* cluster XIII, *Eubacterium coprostanoligenes* and *Odoribacter*, which are weak carbohydrate fermenters (Fig. 4). On the contrary, well-known dietary fibre degrading and acid producing bacteria such as *Roseburia* or *Prevotella* were more abundant in the samples of low pH and high acid concentrations (Supplementary Figs. S2–S5).

The concentration of propionic acid associated with the abundance of genus *Prevotella* (Fig. 4). The relative abundance of *Prevotella* reached up to 42 % with the median values above 26 % in Prop$_{high}$ group while remained below 11 % in Prop$_{low}$ group. Genus *Prevotella* comprises several known producers of succinate [18,19] that can be converted to propionate by other gut bacteria such as *Dialister*. To elucidate the statistical differences of the bacteria and organic acids, the samples were grouped into enterotypes based on the *Prevotella* to *Bacteroides* ratio. Subjects of the Prevotella enterotype had higher abundances of *Holdemanella* but lower abundances of *Alistipes*, *Parasutterella* and butyric acid producing taxa, especially *Eubacterium rectale* throughout the study (Fig. 5). Enhanced propionate production was reflected by 40 % lower median ratios of acetate to propionate in *Prevotella* enterotype compared to that in *Bacteroides* enterotype (2.8 mol/mol vs 3.9 mol/mol, respectively, Fig. 5), while opposite was seen for propionate to butyrate ratios. Moreover, higher amounts of propionate were negatively correlated to the abundances of Proteobacteria (Supplementary Fig. S5), a phylum associated with gut inflammation. In addition, negative correlation between high concentrations of propionate and an opportunistic pathogen *Odoribacter* was observed (Fig. 4).

3.4. Individual level data analysis

The individual level data analyses revealed subject-specific relationships between the diet and faecal parameters - pH, organic acid concentrations and microbiota composition. For example, *Akkermansia* was detected in faecal samples of about half of the participants (29 of 57) and in most of these cases (20) correlation between the abundance of *Akkermansia* and higher faecal pH was found (Fig. 6). Concentrations of total organic acids and pH associated also with the Bristol stool score (BSS), ie more organic acids (μmol/g) were detected in looser stools. More than half of the participants (34) registered different BSS values at different sampling periods while in 23 cases, negative correlation between pH and the BSS was observed (Fig. 7, Supplementary Fig. S7). However, clear associations between BSS and any food items nor gut microbiota were not found. Higher consumption of sweet juicy fruits (melon and watermelon) resulted in more acidic faecal pH in 8 out of 13 subjects reporting these fruits in their food diaries, and higher BSS. Association of oat consumption with higher faecal pH indicate that more proteins and/or less dietary fibres were available for colonic bacteria.

The concentration of faecal succinate remained typically below 1 μmol/g. As an exception, amounts of succinate above 10 μmol/g were detected in all samples from nine participants. Such high amounts of succinic acid triggered the decrease of pH indicating malabsorption or incomplete conversion of succinate to propionate. Seven out of these subjects had also enormous abundance of faecal *Prevotella* (23–42 % of total microbiota).

Significant correlation between the total abundance of butyrogenic bacteria (sum of *Faecalibacterium, Eubacterium* and *Roseburia*) and total acid concentrations was found without common patterns of specific species and butyrate concentration. It might be related to the type of available fibre source as less faecal butyrate was detected in cases of very low fibre consumption (mostly refined wheat products) (Fig. 3). High concentrations of propionate but also acetate and butyrate correlated with higher abundance of *Prevotella* (Fig. 6, Supplementary Figs. S8–S10), a characteristic bacterium of looser stools. Concentration of propionate also correlated with the abundances of *Phascolarctobacterium*, a propionate producing bacterium and *Streptococcus*, a producer of lactate, which is a precursor for propionate (Fig. 6 and Supplementary Fig. S10). Lower abundances of proteobacteria in the presence of higher propionate concentrations cannot be explained by their inhibition by propionic acid only. Individual level analysis also revealed correlation between
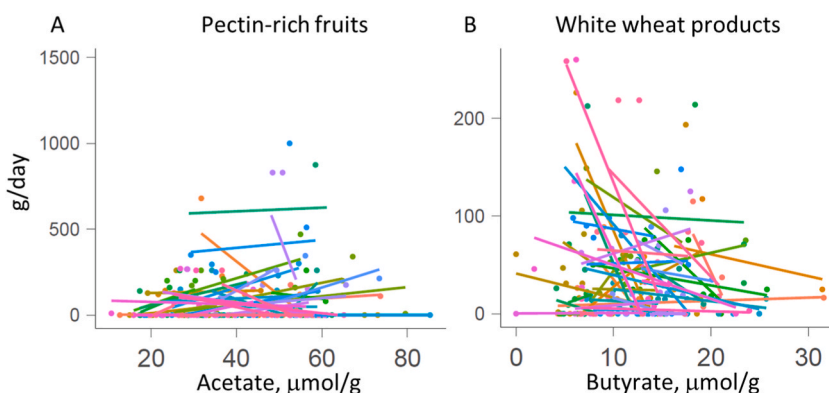
Fig. 3. Pearson correlation between acetate and butyrate concentrations with pectin-rich fruits (A) and white wheat product consumption (B), respectively. The colour of points (individual samples) and regression lines indicate the participant ID. Pectin-rich fruits indicate the sum of apples, plums and black and red currants.
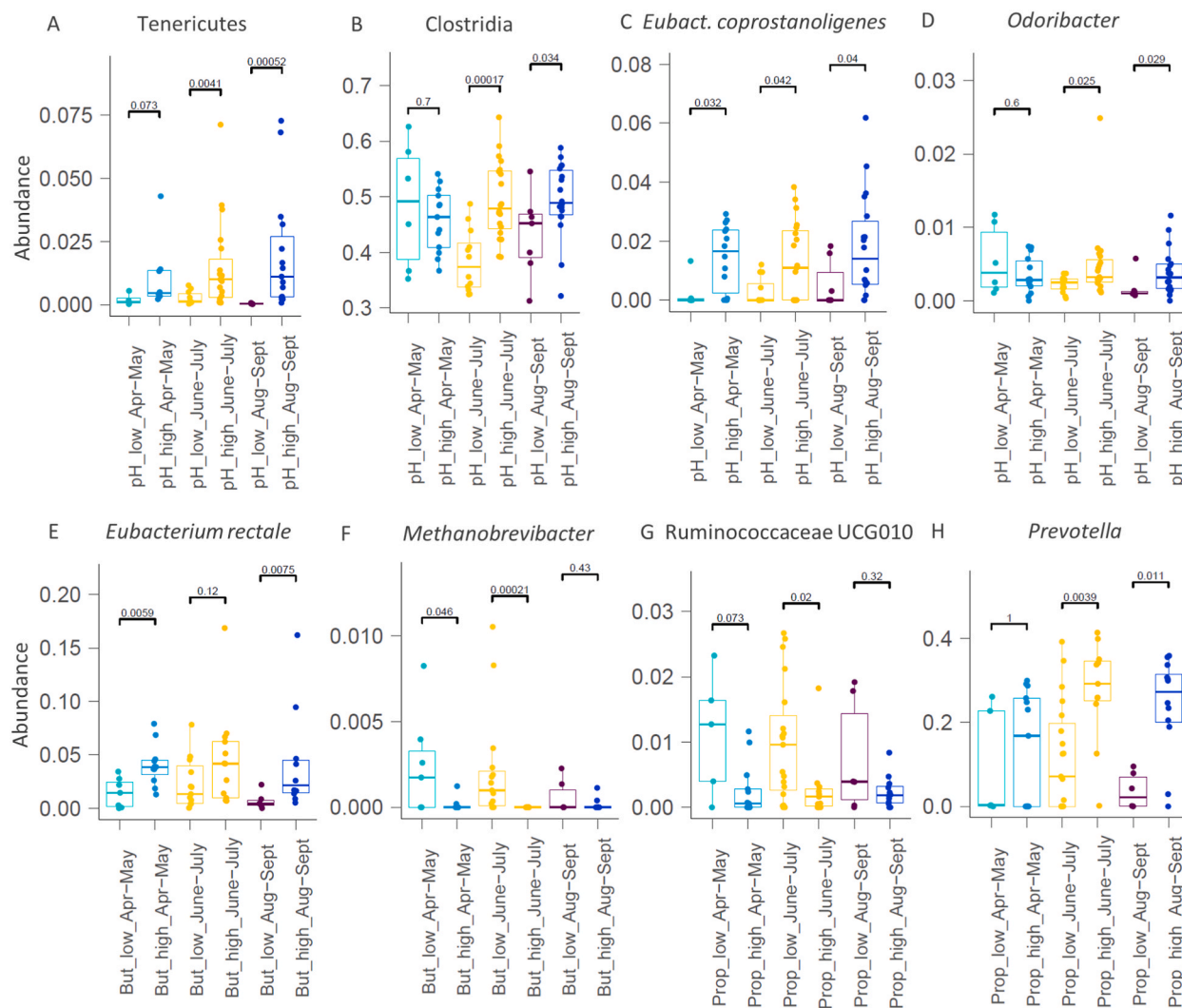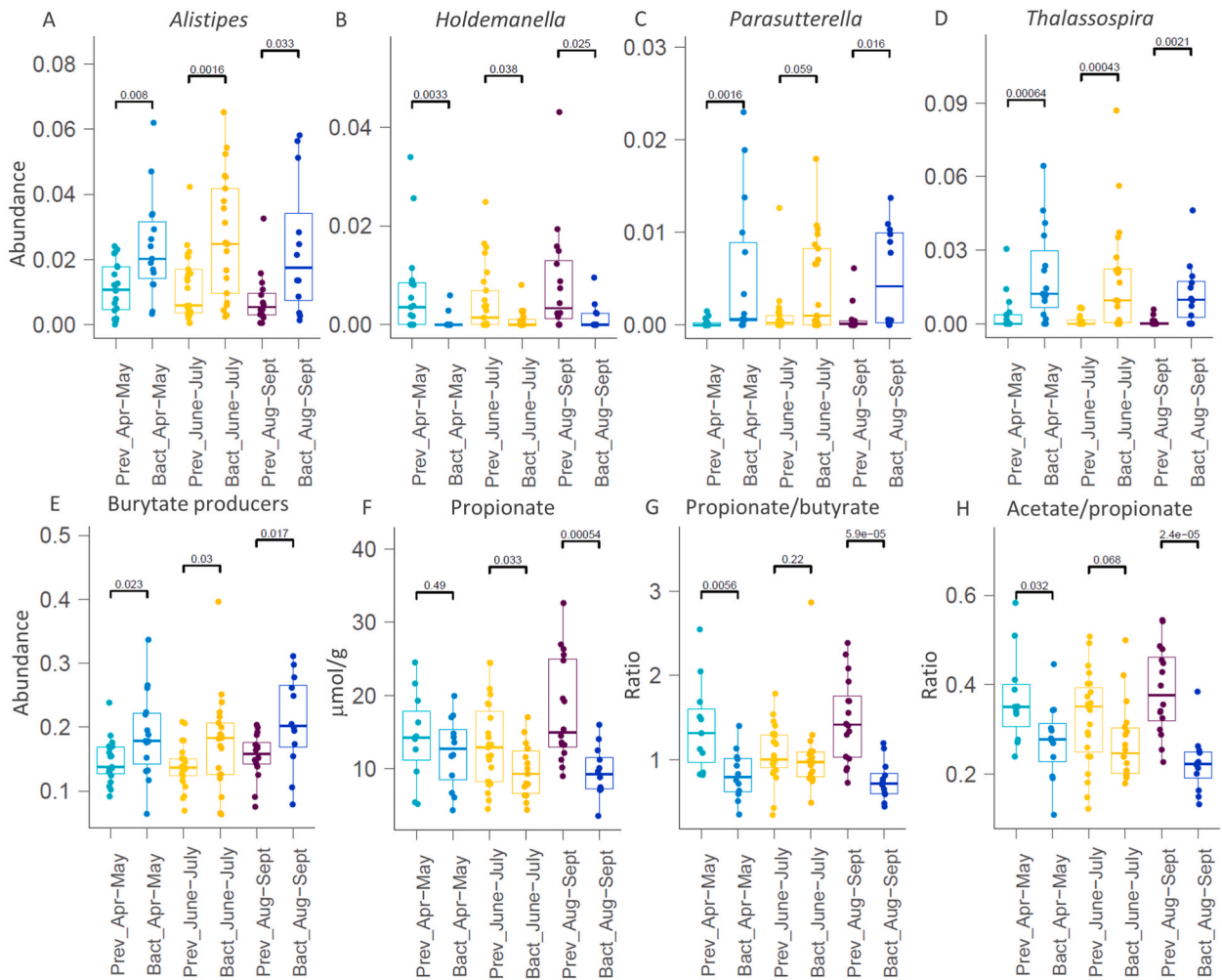
Fig. 4. Bacterial taxa (A–H) significantly different in faecal samples of high and low pH (A–D), as well as high and low concentrations of butyrate (E–F) and propionate (G–H). Samples of each participant were grouped based on the sampling period (Apr–May, June–July, Aug–Sept) and then grouped by pH or acid concentrations. Grouping details are given in materials and methods.

lower abundances of several bacteria such as *Eubacterium coprostanoligenes, Clostridium* cluster XIII and some *Bacteroides* species (*B. cellulosilyticus, B. xylanisolvens*) and higher concentration of faecal butyrate and propionate (Supplementary Figs. S9 and S10).

4. Discussion

In this study, the nutrition data were analysed and correlated with the faecal characteristics: pH, organic acid content, BSS and microbiota composition. The analyses were performed both at group and individual levels. Main advantage of the individual level analysis is the possibility to find important biological relationships that could not be found by group level analysis. Additionally, we analysed food consumptions by dividing foods into groups based on dietary fibre composition, which reflects the nutrient groups available for the gut microbiota and organic acids can only be formed in the presence of specific fibre degrading and metabolizing taxa. We showed that the responses of the gut microbiome and its fermentation products to diet were very clearly individual. As shown by Johnson et al. [20] gut microbiome of individuals was related to food choices, but not to conventional nutrients, that was also observed in our study.

Data on general relationships between food and faecal pH or organic acids are limited. Based on the recent literature data, the colonic pH reflects the balance between carbohydrate and protein fermentation, the effect of food components on faecal pH and concentrations of SCFA (Table 1). In individual level analysis we observed a clear reverse correlation between the consumption of white flour products and faecal butyrate concentration indicating limited supply of wholegrain cereal-derived dietary fibres. In long term perspective, this may affect the colonic pH and gut health. To enhance the growth of butyrogenic taxa and increase the butyric

Fig. 5. Comparison of the abundances of bacterial genera (A–D) and acid profiles (E–H) significantly different in *Bacteroides/Prevotella* ratio.

acid content in the colon, the refined cereals should be replaced by fibre-rich wholegrain products. However, changing the eating habits can be a bigger obstacle than large-scale production of health supporting foods. High-fibre/whole-grain cereals are avoided in low FODMAP diets as rye and wheat contain fructans. Associations between protein-rich and/or low FODMAP diets and higher faecal pH with lower amounts of organic acids have been observed in several human trials (Table 1) [21–26]. This lack of the whole grain cereals in low FODMAP diet can reduce faecal acids more than 10 μmol/g [23].

Table 1 Effect of diets or dietary components on faecal pH and organic acids based on published studies. Values of faecal pH and organic acids (μmol/g) are shown as differences compared to the baseline period or control diet.

Decrease of faecal pH and increase of concentrations of organic acids by inulin, arabinoxylo-oligosaccharides, oat bran, resistant starch, high-amylose barley and wheat bran has been reported (Table 1) [27–30,33–38]. Also, Mediterranean diet or omnivorous diet rich in dietary fibres have positive effect on formation of bacterial fermentation products [39,41]. Our data showed the importance of the colon microbiota composition in control of faecal pH. The formation of propionate in *Prevotella* enterotype was especially affected by pH. We propose a cross-feeding mechanism between succinate producing *Prevotella* and succinate consuming *Dialister* and/or *Phascolarctobacterium* resulting in higher propionate amounts. Alternatively, succinate can be a substrate for butyrate production as we observed positive correlation between the abundance of *Prevotella* and concentration of faecal butyrate. Hence, the diet-derived substrates for *Prevotella* may through cross-feeding promote the production butyrate by *Faecalibacterium*, *Eubacterium* and *Roseburia*. Consistent with a recent studies [4,10], both total SCFA and formation of butyrate increased significantly at lower faecal pH. Acidic colonic pH influences butyrate production through altering the stoichiometry of butyrate formation, resulting in increased acetate uptake, and facilitating increased relative abundance of butyrate-producing taxa (notably *Roseburia* and *Eubacterium rectale*) [10].

Persistent changes of faecal pH are characteristic to substantial diet change or development of a disease. We observed specific associations of faecal pH and bacteria, eg. higher faecal pH of the individuals with detectable amounts of *Akkermansia*. Although *Akkermansia* has been shown to ease chronic gut inflammation processes [42], higher abundance of this bacterium has been observed
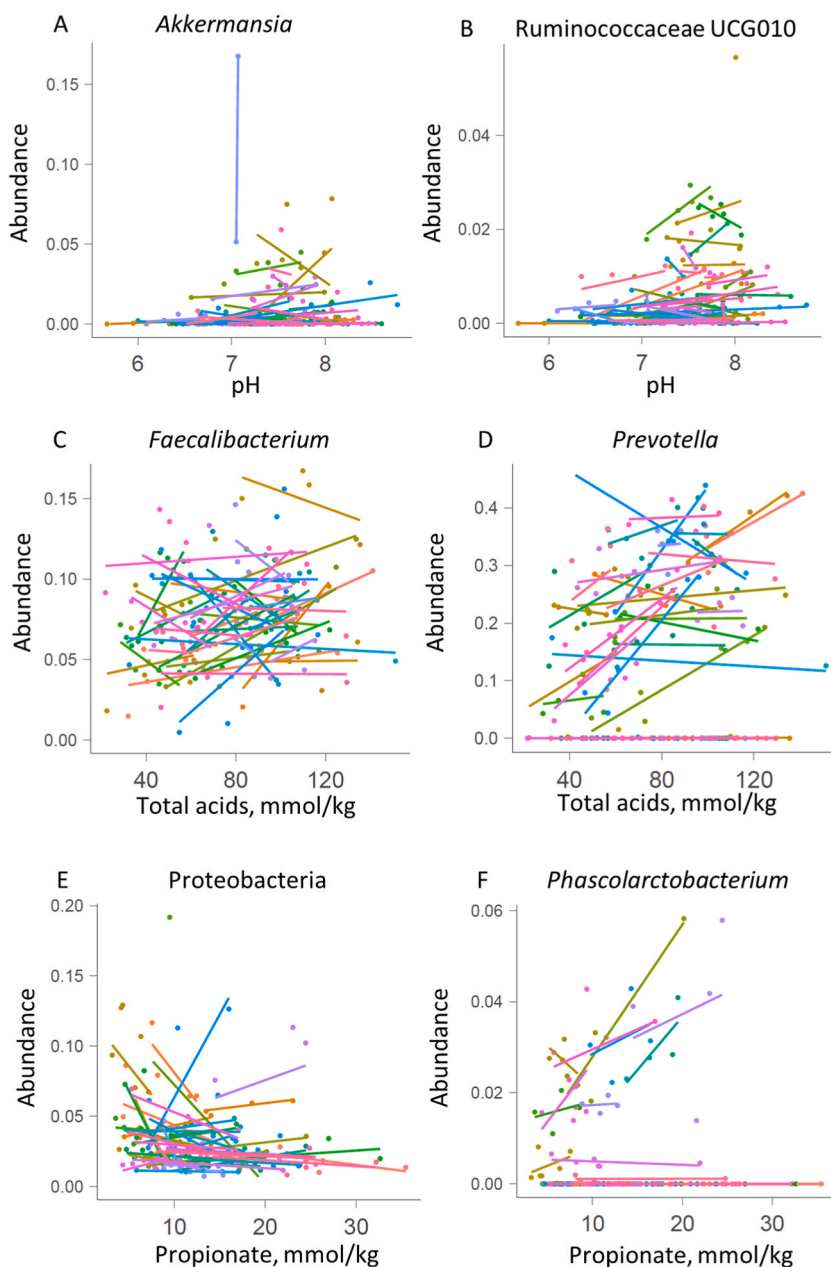
Fig. 6. Correlation between faecal microbiota and faecal pH (A–B), total acid concentrations (C–D) or propionic acid (E–F).

also in Parkinson's disease patients [43,44]. One of the common Parkinson's disease symptoms is constipation, typical to long gut transit time and low BSS (dry stool). Often, low transit rate and low BSS accompany with high abundance of *Akkermansia* [17,45]. Furthermore, constipation and high faecal pH may be related to colorectal cancer (Tabel 1) [3,40]. In accordance with this, enhanced growth of *Akkermansia* at high pH and slow dilution rates was demonstrated in *in vitro* cultivation experiments with faecal cultures [15, 46].

In conclusion, the biochemical characteristics – faecal pH and organic acid content have been underexplored in research and healthcare practices so far. We suggest applying these relatively cheap and simple analyses to elucidate colonic fermentation balance and potential pathological shifts. The increase of faecal butyrate concentration is largely driven by lower colonic pH. Statistically significant correlation between the amount of faecal propionic acid and the abundance of genus *Prevotella* was shown. Cross-feeding between fibre degrading and acid producing taxa resulted in diverse colon microbiota and higher concentrations of total SCFA-s. Thus, fibre-rich diet is a crucial factor for good gut health.
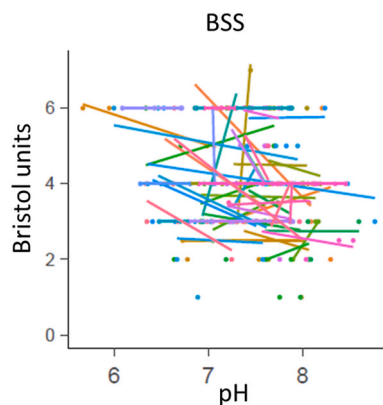
Fig. 7. Pearson correlation between faecal pH and Bristol stool score (BSS). The colour of points (individual samples) and regression lines indicate the participant ID.

Table 1

Effect of diets or dietary components on the change of faecal pH or organic acids (mM) based on the literature data. pH and organic acid values are shown as difference compared to control diet or baseline period.

Diet type or food	Control diet	n*	n_c*	pH	Acetate	Butyrate	Propionate	Reference
Low FODMAP	sham	21	20	0.4	−24.5	−10.2	−8.7	[23]
Low FODMAP	habitual	33		0.2	−2	−2.7	−1	[24]
Yacon (FOS, inulin)	habitual	24		−0.4	−2.9	0.5	−1.6	[27]
Inulin	habitual	32		−1	11	3	−1	[28]
Oat beta-glucan	habitual	25			12.8	2.1	2.8	[29]
High-amylose barley	Refined grain	17		−0.3	10.1	8.8	2.2	[30]
Almond	habitual	35		−0.3	−4.6	0.8	1.1	[31]
Vegan	omnivore	36	36	−0.3	0	3.3	−3.9	[32]
Wholegrain-rich	Refined grain	50		−0.1	no	0.7	no	[4]
Wheat bran (28 g/day)	habitual	13		−0	8.1	10	−2.1	[33]
Wheat bran + RS (12 + 22 g/day	habitual	20		−0.1	8.6	12	−2.4	[34]
Wheat bran fibre (30 g/day)	Low fibre	24			3.5	2.1	0.1	[35]
RS (30 g/day)	Low fibre	24			2.9	3.5	−1.2	[35]
RS rich foods (38 g/day)	Low RS (5 g/day)	11		−0.6	21	7.2	−2.5	[36]
Wheat bran extract (AXOS, 10 g/day)	placebo	57		−0.2	0.3	−0.1	0.1	[37]
AX (15 g/day)	placebo	17		0.2	6.2	2.5	0.5	[38]
Mediterranean	Western	40	31	0.3	3.7	−1.0	−1.9	[39]
Normal protein weight loss	maintenance	18		−0.2	−9	−2.9	−2	[21]
HP, low fat	habitual	11	15	0.7				[25]
HP weight loss	maintenance	18		0.1	−15.9	−9.4	−2.8	[21]
HP MC	maintenance	17		0.1	−6.2	−2.2	−3.7	[22]
HP MC	maintenance	16			−21.6	−8.8	−6.5	[26]
HP LC	maintenance	17		0.5	−22.4	−8.5	−6.4	[22]
HP LC	maintenance	18			−29.6	−13.3	−9.5	[26]
Colorectal cancer	Healthy[a]	21	14	1				[40]
Colorectal cancer	Healthy[a]	93	49	0.5	−10.3	−4.1	−7.1	[3]

n* and n_c* - indicates the number of participants in the study intervention group or control group, respectively. If n_c is missing it indicates to the cross-over study.

[a] Compared with no CRC subjects, RS – resistant starch, AX – arabinoxylan, AXOS – arabinooligosaccharides, HP - high protein, LC - low carbs, MC - medium carbs.

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CRediT authorship contribution statement

Signe Adamberg: Writing – review & editing, Methodology, Conceptualization, Funding acquisition. **Kaarel Adamberg:** Writing – original draft, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31134.

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