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Noninvasive monitoring of fibre fermentation in healthy volunteers by analyzing breath volatile metabolites: lessons from the FiberTAG intervention study

Audrey M. Neyrinck[a*], Julie Rodriguez[a*], Zhengxiao Zhang[b], Benjamin Seethaler[c], Florence Mailleux[a], Joeri Vercammen[d,e], Laure B. Bindels[a], Patrice D. Cani[a,f], Julie-Anne Nazare[g], Véronique Maquet[h], Martine Laville[g], Stephan C. Bischoff[c], Jens Walter[i,j], and Nathalie M. Delzenne[a]

[a]Metabolism and Nutrition Research Group, Louvain Drug Research Institute, UCLouvain, Université catholique de Louvain, Brussels, Belgium; [b]Department of Medicine, University of Alberta, Edmonton, Canada; [c]Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany; [d]Interscience, Louvain-la-Neuve, Belgium; [e]Engineering, Industrial Catalysis and Adsorption Technology (INCAT), Ghent University, Ghent, Belgium; [f]WELBIO-Walloon Excellence in Life Sciences and BIOtechnology, UCLouvain, Université catholique de Louvain, Brussels, Belgium; [g]Université Claude Bernard Lyon1, Hospices Civils de Lyon, CENS, FCRIN/FORCE Network, Lyon, France; [h]KitoZyme, Herstal, Belgium; [i]Department of Agricultural, Food & Nutritional Science and Department of Biological Sciences, University of Alberta, Edmonton, Canada; [j]APC Microbiome Ireland, School of Microbiology, and Department of Medicine, University College Cork – National University of Ireland, Cork, Ireland

ABSTRACT

The fermentation of dietary fibre (DF) leads to the production of bioactive metabolites, the most volatile ones being excreted in the breath. The aim of this study was to analyze the profile of exhaled breath volatile metabolites (BVM) and gastrointestinal symptoms in healthy volunteers after a single ingestion of maltodextrin (placebo) versus chitin-glucan (CG), an insoluble DF previously shown to be fermented into short-chain fatty acids (SCFA) by the human microbiota in vitro. Maltodextrin (4.5 g at day 0) or CG (4.5 g at day 2) were added to a standardized breakfast in fasting healthy volunteers (n = 15). BVM were measured using selected ion flow tube mass spectrometry (SIFT-MS) throughout the day. A single ingestion of 4.5 g CG did not induce significant gastrointestinal discomfort. Untargeted metabolomics analysis of breath highlighted that 13 MS-fragments (among 408 obtained from ionizations of breath) discriminated CG versus maltodextrin acute intake in the posprandial state. The targeted analysis revealed that CG increased exhaled butyrate and 5 other BVM – including the microbial metabolites 2,3-butanedione and 3-hydroxybutanone – with a peak observed 6 h after CG intake. Correlation analyses with fecal microbiota (Illumina 16S rRNA sequencing) spotlighted *Mitsuokella* as a potential genus responsible for the presence of butyric acid, triethylamine and 3-hydroxybutanone in the breath. In conclusion, measuring BMV in the breath reveals the microbial signature of the fermentation of DF after a single ingestion. This protocol allows to analyze the time-course of released bioactive metabolites that could be proposed as new biomarkers of DF fermentation, potentially linked to their biological properties.

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Introduction

Dietary fibre (DF) are non-digestible polysaccharides considered as beneficial for human health, namely through their effect on gut function such as the modulation of stool production.[1] They are classified according to their chemical structure, their food source, their water solubility and viscosity, or their fermentability.[1] The categorization of DF as viscous soluble (e.g. pectin), nonviscous soluble (e.g. inulin) or as insoluble (e.g. cellulose) is an attempt to link their physico-chemical properties to physiological effects. However, this may link to erroneous conclusions regarding their fermentability by the gut microbiota. Indeed, several insoluble DF can be fermented by gut microbes, and could thus be considered as potential prebiotics.[1,2] A prebiotic refers to a substrate that is selectively utilized by host microorganisms conferring a health benefit.[3] Chitin-glucan (CG) is an insoluble DF considered as a safe food ingredient by the European Food

CONTACT Nathalie Delzenne nathalie.delzenne@uclouvain.be Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Brussels, UCLouvain, Université Catholique de Louvain, Brussels, Belgium.

*These authors contributed equally to this work.

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Safety Authority.[4] It is extracted from the cell walls of the mycelium of *Aspergillu niger* fungi and is composed of branched β-1,3/1,6 glucan linked to chitin via a β-1,4 linkage. Marzorati *et al.* demonstrated that CG was fermented with enhanced short-chain fatty acids (SCFA) production (mainly propionate and butyrate) using an *in vitro* approach (Simulator of the Human Intestinal Microbial Ecosystem).[5] In previous studies in rodents, we and others showed that CG significantly improved metabolic parameters, an effect linked to an increase of butyrate-producing *Roseburia* spp. and reduced cardiovascular risk factors.[6,7] In humans, CG administration for 6 weeks reduced oxidized low-density lipoproteins (LDL) in the serum, the mechanism behind this observation being unexplored.[8]

The identification of bioactive metabolites produced upon fermentation of DF in the gut is important to afford the biological events involved in health improvement, and to propose biomarkers of nutrient intake. Volatile organic compounds (VOCs) in the exhaled breath have been proposed as potential surrogate markers of gut dysbiosis in gastrointestinal diseases and diabetes mellitus.[9,10] Bacteria being a major source of volatile metabolites, it was suggested that breath volatile metabolites (BVM) such as H_2, SCFA or alkanes may be indicators of host health.[11,12]

Usually, the analysis of exhaled breath biomarkers is carried out by means of gaz chromatography-mass spectrometry (GC-MS), preferably in combination with a suitable enrichment technique prior to analysis. Although very powerful, GC-MS is not particularly compatible with routine clinical practice. It is a relatively slow technique, requiring analysis times that take on average 30–45 min for comprehensive analysis without post-run data processing.[13] Alternatively, direct MS techniques such as selected ion flow tube (SIFT)-MS, proton transfer reaction (PTR)-MS and more recently secondary electrospray ionization (SESI) MS have gained more attention because they hold the promise of speed, selectivity, and sensitivity in a single instrument.[14] SIFT-MS is particularly well suited for the purpose of exhaled breath analysis.[15] It combines very soft chemical ionization under controlled conditions with three precursor ions (H_3O+, NO+, and O_2+), preserving molecular ion integrity for maximum sensitivity and selectivity. Precursor ions are generated *in situ* from humidified air that is gently fed into a high energy microwave discharge source to create an ion plasma. Selected precursor ions are extracted from this plasma by means of a short upstream quadrupole and introduced in the flow tube, where they are thermalized by means of a high flow of helium and allowed to react with the sample molecules for a defined amount of time. During the reaction, formed product ions are diverted toward a downstream quadrupole by means of the excess helium flow where they are separated from each other and detected. Since reaction conditions are accurately controlled, SIFT-MS allows direct quantification of target components in real-time from raw ion count rates and associated reaction kinetics. Typical concentration levels are situated at low parts per billion by volume levels. Of note, the applied precursor ions are very effective in ionizing a broad range of organic and inorganic components but they do not react with nitrogen, oxygen and carbon dioxide. Usually, SIFT-MS is applied in target mode, which means that only a limited set of components are measured simultaneously.[15] If target components are not known, SIFT-MS is used in untargeted scan mode, where it provides pattern-based classification capacity, the identification of probable biomarkers, and retrospective quantification of target components.[16]

In the context of the project FiberTAG (Joint Programming Initiative "A Healthy Diet for a Healthy Life" 2017–2020 https://www.fibertag.eu/), we aimed to identify bioactive bacterial metabolites linking DF intake and gut microbiota-related health effect.[17] In this present study, exhaled BVM have been analyzed by SIFT-MS,[10,18]) in healthy volunteers to monitor the potential interaction of a single exposure to CG as insoluble DF with the gut microbiota.

Results

Characterization of the subjects (baseline fecal gut microbiota, SCFA composition and hydrogen production)

The interventions were conducted in March 2018 following the study design shown in Figure 1. Fifteen subjects respecting inclusion and exclusion criteria (Sup Data 1) were selected as H_2-producers based on

the lactulose test (Sup Data 2) and completed the study. Baseline characteristics of participants are shown in Sup Data 3. Before the intervention, the mean energy intake was 2046 ± 130 kcal/day and their nutrient intakes were 229 ± 23, 81 ± 6, 70 ± 5, and 20 ± 4 g per day for carbohydrates, lipids, proteins, and fibre, respectively.

The overall composition of gut microbiota was assessed by the measure of both α-diversity and β-diversity indexes. Subjects seemed to display a different gut microbiota composition as shown by the principal coordinate analysis of the Bray-curtis index; subject I being the most distant from others (Figure 2a). However, this difference was reduced when the distance measure considers the fraction of branch length in a phylogenic tree, as assessed by the unweighted uniFrac distance; subject G being the most distant from others on this principal coordinate analysis (PCoA). α-Diversity related to bacterial richness (Observed OTU), evenness (Pielou), or both (Shannon) was systematically lower for subject I (Figure 2b). Gut microbiota analysis presented at the phylum level confirmed the lower α-diversity found for this subject for which Verrucomicrobia and Tenericutes were absent from its bacterial profile in feces (Figure 2c). Those phyla were also absent in the fecal matter of subject N who presented again a low α-diversity indexes. Firmicutes was the most abundant bacterial phylum for all subjects (> 60%) followed by either Bacteroidetes or Actinobacteria depending on the subject. The total amount of SCFA in the fecal matter is presented in Figure 2d. Subjects E and G exhibited the highest levels of fecal SCFA whereas subject L presented the lowest total amount of fecal SCFA. We noted lower proportions of valeric and iso-valeric acids in subject I as compared to other subjects. Those SCFAs were not detectable for subject C (Figure 2d). The total amount of SCFA in all subjects varied between 1 mg/g to 4 mg/g of fecal dry matter, acetate being the most abundant one.

Gastrointestinal tolerance after CG versus maltodextrin ingestion in healthy subjects

In order to evaluate the tolerance of ingesting CG after a unique intake during the breakfast, visual analogue scale (VAS) scores were recorded. The baseline scores of gastrointestinal parameters between the 2 test days were not significantly different (Sup Data 4). The evolution of the different scores throughout the day was not significantly affected by CG intake, suggesting a good gastrointestinal tolerance (Figure 3). Only flatulence was more important as soon

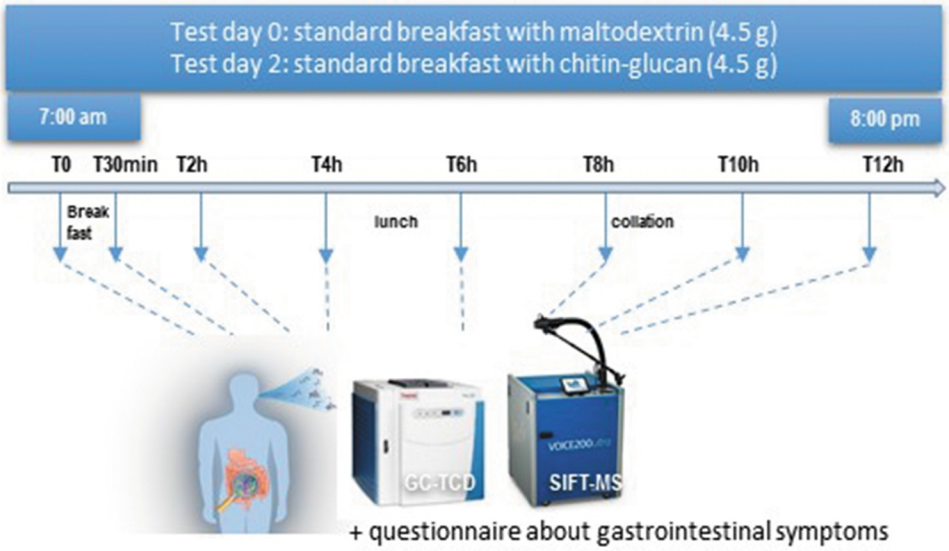

Figure 1. Overview of the study design.

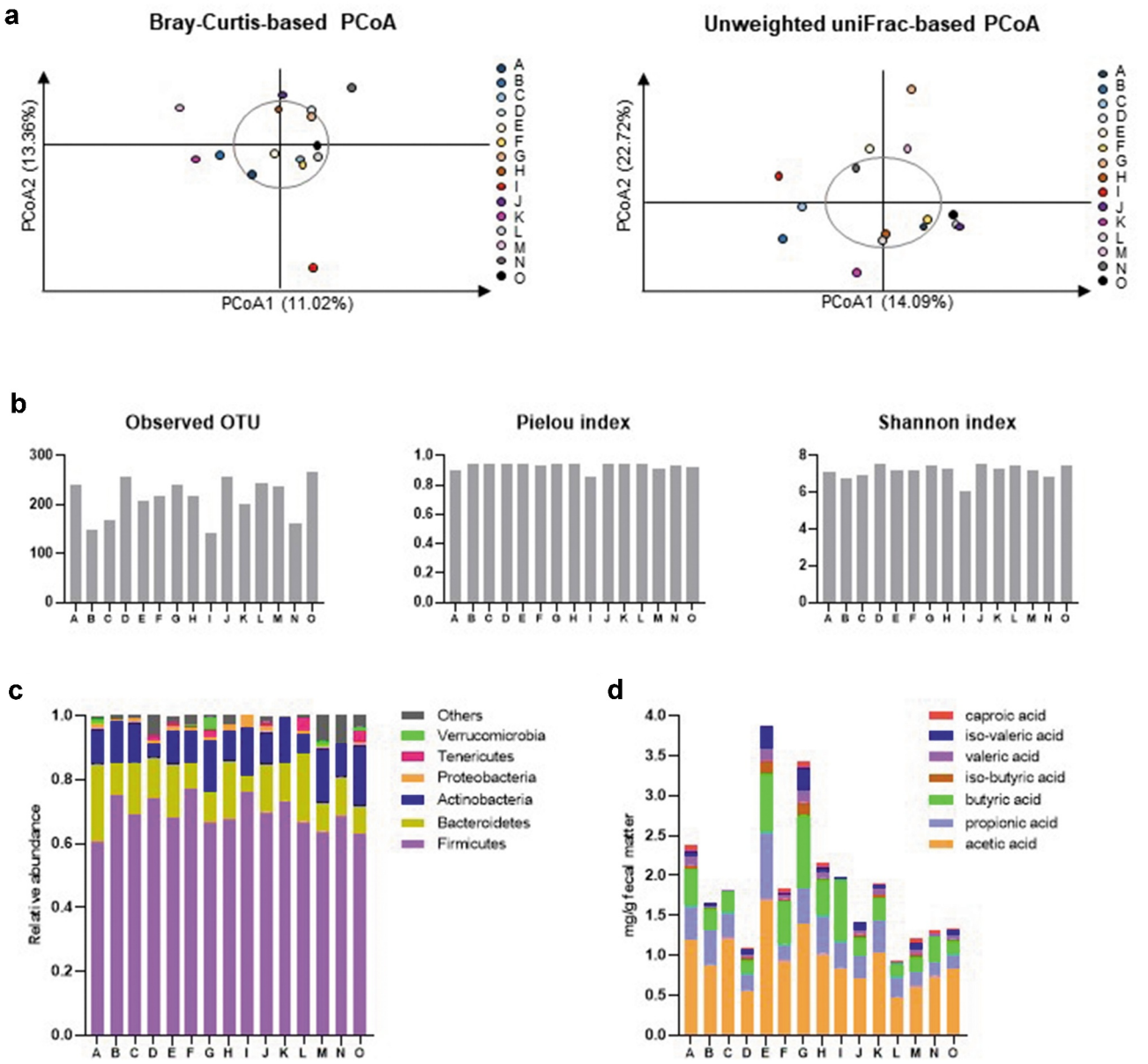

Figure 2. Gut microbiota and short-chain fatty acids (SCFA) composition in fecal matter of 15 healthy subjects before intervention days. (a) Principal coordinate analysis (PCoA) score plot of β-diversity estimated using the using the Bray-curtis and unweighted uniFrac indexes. (b) α-diversity indexes related to bacterial richness (Observed OTU), evenness (Pielou) or both (Shannon) for each subject. (c) Bar plots of relative abundance of phylum levels accounting for more than 0.5% for each subject. (d) Bar plots of SCFA concentration for each subject.

as 6 h after CG intake, as compared to maltodextrin intake (p = .054).

BVM profile after single ingestion of CG or maltodextrin in healthy subjects

The SIFT-MS analysis was carried out using three precursor ions (H_3O+, NO+, and O_2+) to record the resulting fingerprints of the breath (untargeted analysis) (Figure 4a). Principal component analysis (PCA) analysis of MS-fragments in overnight-fasted state (time 0 h), in preprandial state meaning before lunch (time 4 h) and in the postprandial-post absorptive state (time 6 h) showed that the 15 subjects formed separate clusters depending on the time of measurement, and therefore on the nutritional status (fasted, preprandial, postprandial). The clustering at time 6 h was more pronounced

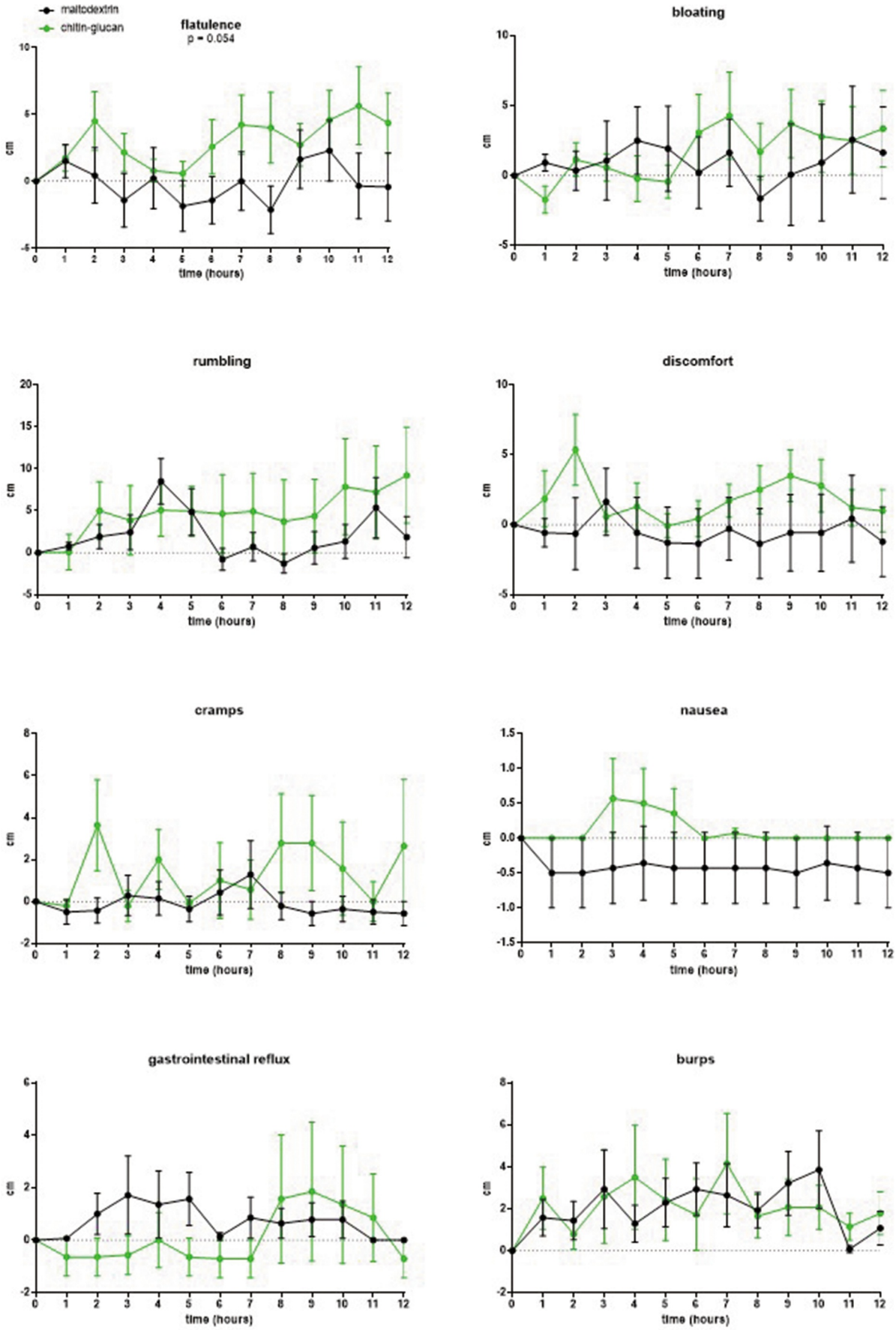

Figure 3. Gastrointestinal tolerance assessed by visual analog scale of 15 healthy subjects about 8 symptoms after chitin-glucan intake of maltodextrin intake. Data are means ± SEM ($p > .05$; matched-pairs Wilcoxon signed-rank test on net AUC).

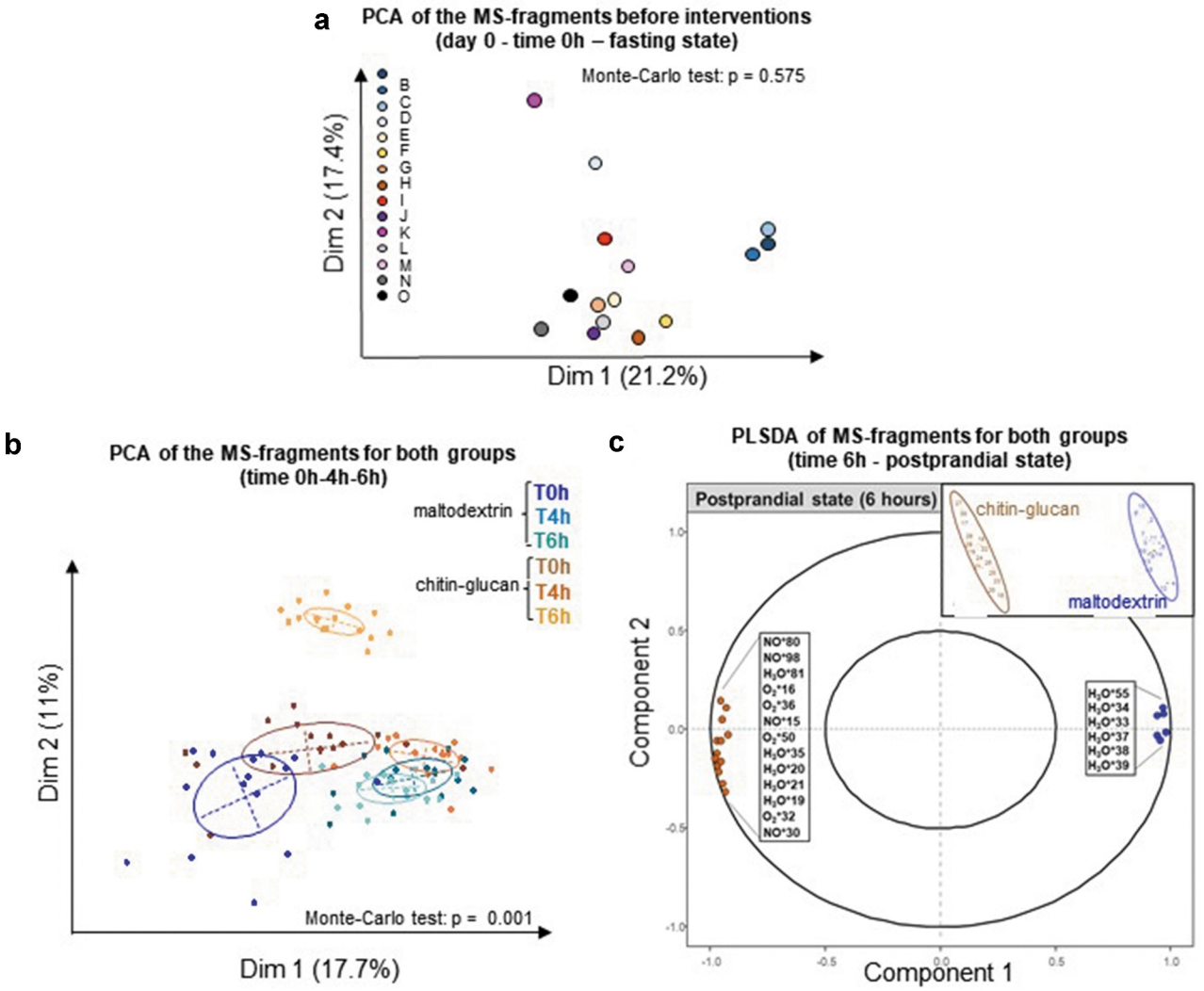

Figure 4. Untargeted analysis of breath from 15 healthy subjects after chitin-glucan and maltodextrin intake. Principal component analysis (PCA) score plot of the MS-fragments from the SIFT-MS spectra (from H_3O+, NO+ and O_2+ ionizations) obtained in fasted state (time 0 h) the first test day (a) or in fasted state (time 0 h), in pre-prandial state (time 4 h) and in postprandial state (time 6 h) (b). PLS-DA (with cutoff 0.9) of 408 MS-fragments after chitin-glucan intake and maltodextrin intake at time 6 h (c). Statistical analysis was assessed by a Monte Carlo rank test.

in CG than in the maltodextrin group, indicating a difference in BVM profile between both treatments (Figure 4b). Before the lunch (time 4 h), the trends were distinct from the overnight fasting state (time 0 h) and we observed a slight overlap between both groups (Figure 4b). Interestingly, after the lunch at time 6 h, a clear separation (from partial least squares discriminant analysis (PLS-DA) analysis) appeared for participants that received CG compared to subjects receiving maltodextrin and was linked to 13 MS-fragments (Figure 4c). The targeted analysis was conducted to identify 26 BVM known to be involved in energy metabolism in particular those coming from bacterial metabolism.[11,12] Consistently with the untargeted analysis, we observed that only one BVM exhaled in the breath (pentane) was more important on the test day with maltodextrin (Sup Data 5). All other BMV had similar value in fasted state upon both days (day 0 and day 2). The concentrations (in ppm) of all BVM exhaled in the breath over time for both test days are presented in Supplemental Data 6. Repeated measures ANOVA revealed a significant effect for time and for subject inter-variation for all targeted BVM ($p < .05$). A significant effect of the treatment was obtained

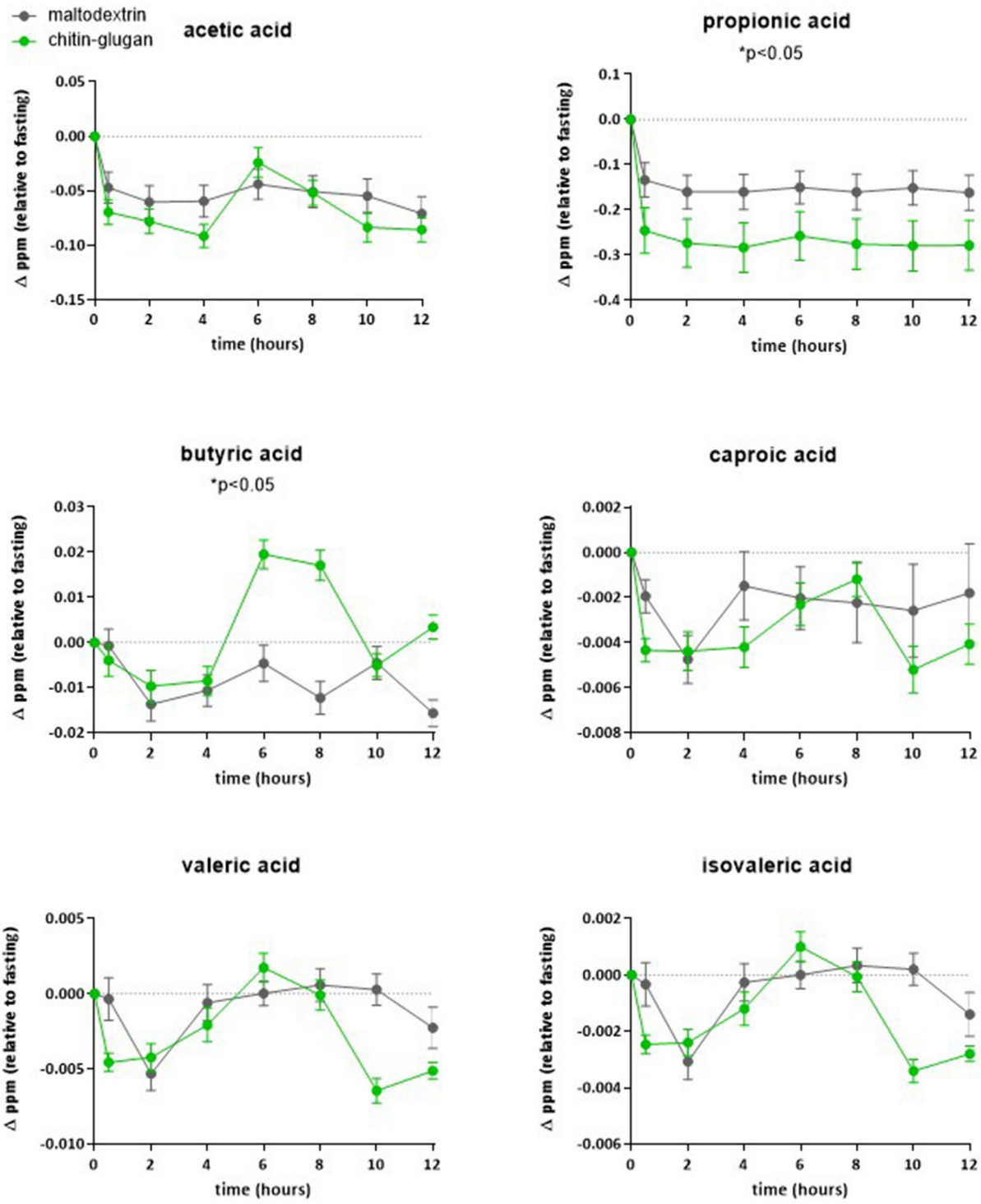

Figure 5. Targeted short-chain fatty acids (SCFA) concentrations (changes from baseline) exhaled in breath of healthy subjects in response to chitin-glucan and maltodextrin intake. Data are means ± SEM (*p < .05; matched-pairs Wilcoxon signed-rank test on net area under the curve).

only with butyric acid, triethylamine, 2,3-butanedione, 3-hydroxybutanone, ethane, methanol, and pentane (repeated measures ANOVA $p < .05$). Figure 5 presents the evolution of exhaled SCFA over time corrected by the baseline value at a fasted state. Among all SCFA identified, the evolution of propionic acid and butyric acid in the breath was significantly affected after a single ingestion of CG.

Of note, the level of exhaled propionic acid was lower after CG intake than maltodextrin intake whatever the time considered. More importantly, exhaled butyric acid increased after CG intake after the lunch and reached the highest level at time 6 h in the postprandial state compared to participants ingesting maltodextrin. The evolution of other exhaled SCFAs was not significantly different between the 2 test days. Figure 6 revealed six other BVM with an AUC that was significantly affected after CG versus maltodextrin intake. Among them, triethylamine, 2,3-butanedione, 3-hydroxybutanone (p = .054) and pentane followed the same trends than the butyric acid with a peak observed at time 6 h for triethylamine, 3-hydroxybutanone and 2,3-butanedione, and at time 8 h for pentane. Methanol increased whereas ethane decreased just after CG-breakfast intake and a peak was observed at 8 h for methanol. Although some of them were increased after CG ingestion, the evolution of well-known markers of gut fermentation such as exhaled H_2 or CH_4 as well as other targeted BVM were not significantly affected after CG compared to maltodextrin intake (Sup Data 7). We observed that the highest levels of acetone, acetonitrile, acetic acid, propionic acid, caproic acid, 1- and 2-propanol, benzene, and benzaldehyde were found at time 0 (meaning in the fasting state) since all changes from baseline were negative after treatment and throughout the test day for those metabolites (Figure 5, Sup Data 7). In accordance with the untargeted analysis, several identified BVM such as acetic acid, (iso) valeric acids, acetone, acetonitrile, ethanol, butanol, phenol, isoprene, methane, and H_2S were different between the pre- and postprandial states (time 4 h *versus* time 6 h) without any significant effect of CG treatment. Isoprene was the sole BVM which decreased after lunch in both groups (Figure 5, Sup Data 7). We performed correlation analyses between different indexes of α-diversity and exhaled BVM (net AUC) in both groups (Spearman r and p values are given in Sup Data 6). Correlations obtained with observed OTU and Shannon indexes were not significant whatever targeted BVM exhaled in the breath when CG is consumed. In contrast, the Shannon diversity index was negatively and significantly correlated with butyric acid, methanol, triethylamine, and 3-hydroxybutanone exhaled in the breath throughout the day when no DF was consumed (placebo group). Relevant correlations were also seen with the Pielou index with C5 acids (valeric, isovaleric and 2-methylbutyric acids) that were exhaled in the breath (mostly after the lunch) but exclusively when CG is consumed. It is worth to note that the level of exhaled butyrate (at time 6 h and the net AUC) after CG intake was not correlated with the initial concentration of fecal butyrate (r = 0.40 and r = −0.07, respectively; p > .05). More importantly, baseline *Mitsuokella* relative abundance was the sole genus positively correlated with net AUC of exhaled butyrate (Sup Data 6). In addition, net AUC of triethylamine was positively correlated with *Mitsuokella* and *Catenibacterium*, 3-hydroxybutanone was positively correlated to *Mitsuokella, Alloprevotella, Lachnospiraceae FCS020* group and *Ruminococcaceae NK4A214* group whereas exhaled pentane was positively correlated to *Faecalibacterium* and 2,3-butanedione was positively correlated with *Muribaculaceae metagenome, Clostridium sensu stricto*, and *Flavonifractor*.

Discussion

Effects of DF are usually investigated using urine, blood, and fecal analysis, but exhaled breath is of increasing interest because it allows noninvasive sampling. There are almost 900 VOCs in the exhaled breath of healthy humans[19] which could be considered as potential biomarkers of metabolic disorders, specific diets, or both. Consistently, breath analysis (mainly hydrogen and methane) is already used in clinics for monitoring gastrointestinal disorders.[12,20–22] Recently, Rondanelli *et al.* reviewed biomarkers present in exhaled breath of gastrointestinal diseases and nutritional status.[23] For example, exhaled acetone has been reported to be an important biomarker of diabetes.[9] A few studies have also monitored the effects of diet (gluten-free diet, high *versus* low-fat dairy drink, high *versus* low fibre diets) on the composition of exhaled breath.[12,24–27] In the present study, we compared for the first time the effect of unique ingestion of an insoluble DF (CG prone to be fermented by the gut microbiota) *versus* maltodextrin (a fully digestible carbohydrate) as placebo on BVM

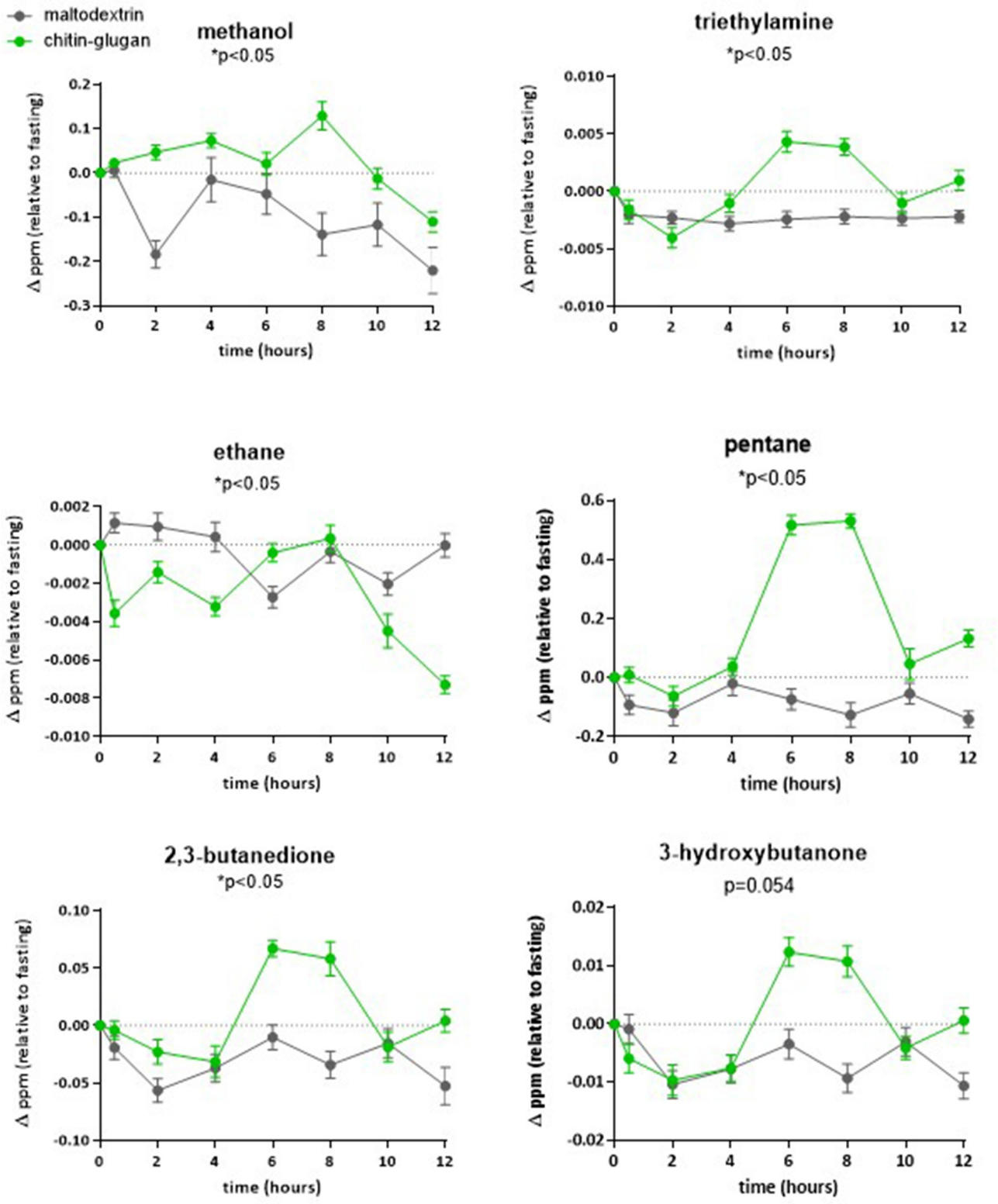

Figure 6. Targeted breath volatile metabolites (BVM) concentrations (changes from baseline) exhaled in breath of healthy subjects significantly changed after chitin-glucan intake compared to maltodextrin intake. Data are means ± SEM (*p < .05; matched-pairs Wilcoxon signed-rank test on net area under the curve).

profile in young healthy adults. The interpretation of our data considered characterization prior to intervention in terms of fecal microbiota composition and fecal SCFA. We hypothesized that some BVM may be the reflect of DF gut fermentation with respect to inter-subject variation.

It has already been described that the presence of some metabolites such as acetone, ethanol, isoprene, and methanol in the breath is conditioned by the fasting state.[11] In our study, the highest levels of acetone but also acetonitrile, acetic acid, propionic acid, caproic acid, 1- and 2-propanol, benzene, and benzaldehyde were found at fasting state in the morning. The potential origin of such BVM was already discussed elsewhere focusing on diet, environmental exposure (exposure to air pollutants), and biological pathways.[11,28] Some of them were decreased after the breakfast without any further variation due to the lunch, the collation, or the CG intake whatever the time considered (1- and 2-propanol, benzene, and benzaldehyde). We hypothesized that the presence in a breath at time 0 h of this kind of BVM with high affinity with fat tissue may be the result of the high rate of lipolysis at a fasted state. Other BVMs were different between the pre- and postprandial states without any significant influence of CG intake. It is the case of acetic acid, (iso)valeric acids, acetone, acetonitrile, ethanol, and methane for which levels increased after the lunch in both groups. Acetone, produced via fatty acid oxidation, has been one of the earliest identified gaseous disease biomarkers, being distinctively present in the breath of patients with diabetic ketoacidosis, hypoinsulinemic states, and starvation.[29] Subtle acetone fluctuations also systematically occur in response to modest, physiological changes in insulin/glucose levels.[29] In contrast, ethanol is not produced by human cells, but small concentrations in blood and breath derive from the alcoholic fermentation of glucose by gut bacteria expressing pyruvate decarboxylase. Following carbohydrate ingestion, blood and breath ethanol increase.[29] Furthermore, the levels of exhaled breath ethanol increased after intravenously administered glucose load, supporting the hypothesis that glucose enrichment of gastrointestinal capillaries per se may stimulate gut bacterial fermentation to some degree.[30] The source and physiological effect of isoprene in humans are matters of debate. In animals and humans, this intermediary metabolite of cholesterol synthesis is formed in the liver from isopentenyl pyrophosphate (IPP) and its isomer (DMAPP).[31] However, as this reaction is slow and may be insignificant at physiological pH values, it is unlikely to completely explain the endogenous isoprene production by animals. So far, two major metabolic pathways leading to DMAPP have been identified: the mevalonic acid (MVA) pathway and the 1-deoxy-D-xylulose-4-phosphate/2-C-methylerythriol 5-phosphate (DOXP/MEP) pathway. The DOXP/MEP pathway was demonstrated to prevail in plants and most bacteria, whereas the MVA pathway is mainly present in higher eukaryotes. In bacteria, DMAPP is converted into isoprene enzymatically by isoprene synthase. Surprisingly, isoprene increased with the time of fasting and fell down in a postprandial state independently of the DF intake. Another study showed that isoprene concentrations did not change significantly following feeding with a liquid protein-energy meal.[32] It would be interesti ng to assess the role of isoprene as a marker of cholesterol synthesis, which can be affected differently following meal composition. A pilot study of seven healthy subjects suggested that increased metabolic activity in the gastrointestinal tract after ingestion of a meal could explain, at least partly, the increase of BVM such as ethanol, 1-propanol, 3-hydroxybutanone, propionic acid, and butyric acid.[12] Here, we did not observe any change with a meal of propionic acid and 1-propanol. Anyway, our data led us to conclude that a lot of BVM from our targeted analysis, principally produced by host metabolism, reflected metabolic state rather than a response to a DF ingestion. In addition, our study revealed that bacterial metabolites coming from the gut microbiota and exhaled in the breath such as H_2S, methane and SCFA (acetic acid, butyric acid, (iso)valeric acids, propionic acid, caproic acid) may be influenced by the ingestion of a meal or by the fasting state.

Hexose and pentose sugars are fermented by isolated human colonic bacteria via pathways leading to the formation of SCFA, ethanol, and hydrogen depending on the strain and species.[33] In particular, butyrate formation occurs in Firmicutes bacteria, either via butyrate kinase (in many *Clostridium* and *Coprococcus* species) or via

butyryl CoA:acetate CoA transferase.[34] Marzorati *et al* have shown that fermentation of CG led to an increased production of both propionate and butyrate *in vitro*.[5] In the present study, a single intake of 4.5 g CG led to an increase of exhaled butyrate suggesting that this SCFA is not restricted to enterocyte metabolism, but may be absorbed in the circulation and then released in the breath as a signature of gut fermentation of CG, since the increase did not appear when volunteers received placebo. We were unable to relate exhaled butyrate to fecal butyrate. It should be kept in mind that SCFA production mainly occurs in the proximal part of the colon where the availability of substrates is most abundant. Accordingly, up to 95% SCFA are rapidly absorbed by the colonocytes resulting in decreasing concentrations from the proximal to distal colon and only about 5% is excreted in feces.[35] In addition, intestinal microbial fermentation is a dynamic process influenced by a wide range of factors. Therefore, the levels of each metabolite are a result of metabolic fluxes of highly variable rates, which are not adequately represented in steady-state metabolite profiles in feces. Our kinetic analysis offered the possibility to assess the dynamic processes of gut fermentation. Interestingly, exhaled butyrate after CG intake was positively correlated with *Mitsuokella* from the Firmicutes phylum. It has been demonstrated that concentration of exhaled methanol increased from a physiological level of ~0.4 ppm up to ~2 ppm a few hours after eating around 500 g of fruits, the effect being principally due to pectin fermentation.[11,36] Here, the basal levels of breath methanol were in the same range than the physiological level but did not reach a concentration higher than 0.5 ppm. In addition, bacterial fermentation in the mouth or throat can partly explain increased postprandial levels of exhaled breath BVM.[12] Breath methanol increased already just after taking the CG with the breakfast, suggesting that this metabolite was not a reflect of CG fermentation in the lower part of the gut. The kinetics of triethylamine release was different, since it increased 4 h after CG intake with a peak obtained at time 6 h as observed for butyric acid but the range of changes was very weak (maximum 0.005 ppm). Although no literature exists about metabolic pathway explaining a potential origin from gut bacteria, the kinetic study performed in the present study supported the hypothesis that this BVM appeared following gut fermentation of CG as observed for butyric acid. We found that production in the breath was positively correlated with the presence of *Mitsuokella* and *Catenibacterium* in the fecal matter. Breath ethane decreased after CG intake whereas pentane followed the same kinetics than exhaled butyric acid. The presence of pentane in exhaled breath is considered as a result of lipid peroxidation of polyunsaturated fatty acids in cellular membranes, a process mediated by free radicals and oxidative stress.[10] One study reported that exhaled pentane was dependent on dietary linoleate intake, and was blunted upon an antibiotic treatment, an observation that leads them to conclude that intestinal bacteria were a major source of breath pentane in addition to endogenous membrane lipid peroxidation.[11,37] Since CG can slightly modify lipid absorption in the upper part of the gut,[6] it is conceivable that the higher pentane exhalation could be linked to the higher production of this metabolite from undigested fatty acids by specific gut microbes. Data show that lactobacilli can produce 3-hydroxybutanone (acetoin) and 2,3-butanedione (diacetyl).[38] The production of 3-hydroxybutanone and 2,3-butanedione has already been studied on different *Lactobacillus* strains growing on Jerusalem artichoke juice rich in inulin that served as substrate.[38] Their production is related to citrate metabolism through the citrate–oxaloacetate–pyruvate–acetolactate–acetoin/diacetyl pathway, where pyruvate is considered as the precursor. In our study, although 3-hydroxybutanone and 2,3-butanedione were exhaled after CG ingestion, no correlation was found with lactobacilli. Regarding the correlations obtained with observed α-diversity indexes, we can conclude that the richness or both abundance and evenness of the species present in the gut microbiota were not crucial to determine the response to CG in terms of exhaled microbial metabolites mostly produced after the lunch (butyric acid, triethylamine, pentane, 2,3-butanedione, and 3-hydroxybutanone). However, the bacterial richness was determinant for some of those bacterial metabolites (butyric acid, triethylamine, and 3-hydroxybutanone) exhaled throughout the day when no DF was consumed (placebo group). Interestingly,

3-hydroxybutanone production in the breath was positively correlated with *Mitsuokella*, the same bacteria revealed for butyric acid production. The genus *Mitsuokella*, comprising only two named species, are Gram-negative obligate anaerobes that utilize fermentable carbohydrates to form acetate, lactate, and succinate.[39] Of these two species, *M. multacida* is regularly found in the intestinal tract of humans and appears to be responsive to diet changes. The acids produced were potentially available to other acid-utilizing bacteria for the formation of butyrate, such as *Roseburia*.[40]

In conclusion, this study showed that CG intake changed the exhaled BVM profile and that meal intake influenced the BVM profile. Even if the limitation of the study is to focus on a small cohort and refer to hydrogen producers only, we have validated a protocol allowing to analyze the effect of DF intake in the morning on the profiling of breath metabolites throughout the day, showing that postprandial – postabsorptive period is probably the most relevant timing for the evaluation of BVM. Exhaled butyric acid, triethylamine, pentane, 3-hydroxybutanone, and 2,3-butanedione are reflecting the bacterial metabolism after CG intake. This implies that analysis of BVM in exhaled air can be used as a dynamic approach to noninvasively assess the responses to DF intake related to gut fermentation.

Materials and methods

Participants

The FiberTAG study is an interventional monocentric study including two test days aiming at characterizing the fermentation of CG (Kiotransine® from KitoZyme, Belgium) versus maltodextrin (Cargill, Belgium), a nonfermentable digestible carbohydrate given as placebo, by assessing the volatile compounds released in the breath. For this purpose, blinded subjects received a single administration of maltodextrin (4.5 g) and two days later, a single administration of CG (4.5 g). After the single administration of CG fibre or maltodextrin given together with the breakfast, the kinetic of BVM production was measured repeatably during 12 h. Healthy subjects were recruited by the Center of Investigation in Clinical Nutrition (CICN-UCLouvain) by displaying posters on the university site and by mails, by social networks, local newspapers and local flyers disposed in shops and doctor's offices in nearby cities. Subjects were pre-screened by phone or mail using a question naire to test if they met the inclusion criteria listed in Supplemental Data 1. The participants were invited to perform a screening test using lactulose to select H_2-producers. Forty subjects passed the screening lactulose test at least 4 weeks before the intervention. Briefly, fasted subjects (at least 10 h) received an oral load of 10 g of lactulose; then, exhaled H_2 was measured every 30 min for 4 h using Lactotest 202 (Medical Electronic Construction, MEC). A minimal increase of 10 ppm of H_2 during three successive measurements was considered as the criteria of subject selection. No increase should be observed in the first 30 min to avoid subjects with small intestinal bacteria overgrowth. After the screening test, 15 subjects were selected respecting inclusion and exclusion criteria (Sup Data 1) and were subjected to a medical examination with the physician investigator to verify that they were healthy. At least 12 days before the first test day, the volunteers were asked by a dietician to follow dietary recommendations to avoid eating fibre-rich food products containing high amounts of prebiotic DF more than once a week. These foods comprised, among others whole grains, artichokes, Jerusalem artichokes, salsifies, leeks, onions. Prebiotics and probiotics supplementation as well as the consumption of fibre-enriched-food were forbidden. The subjects were asked to complete a food diary for 3 days the week before the intervention. The Nubel Pro program and the table of composition from Nubel 2010 were used to assess macronutrient and total fibre intake. Up to 4 days before the intervention period, stool samples were collected and stored directly at −20°C for further transport within 4 days to the CICN for storage at −80°C until analyses. This study was approved by the local ethical committee (Comité d'Ethique Hospitalo-Facultaire UCLouvain/Cliniques Universitaires Saint-Luc) and written informed consent was obtained from all subjects. The trial was carried out in accordance with the Good Clinical Practice as required by the following regulations: the Belgian law of 7 May 2004 regarding experiments on human persons and the EU Directive 2001/20/EC on Clinical Trials (registration at clinicaltrials.gov as NCT03494491).

Intervention days

Both test days were organized in the same way. An overview of the study design is shown in Figure 1. Subjects were blinded regarding the compound they received. The evening before the two intervention days, subjects were asked to eat rice and meat, without vegetables and to avoid alcohol. Fasted subjects (from 9 pm the day before) arrived at 7 am at the CICN. The study coordinator reviewed the inclusion and exclusion criteria and verified if instructions were adequately followed. After mouth wash (with Perio Aid Intensive Care), breath samples were taken to analyze BVM at baseline. In the meantime, subjects were asked to complete a VAS questionnaire about the eight gastrointestinal symptoms usually described after the consumption of fibre. Then the subject received a standardized breakfast composed of white bread and butter, with 240 ml of water and 4.5 g of CG or the placebo. The breakfast should be eaten within 15 min. Breath samples were collected after 30 min and then every 2 h during 12 h after the CG or placebo ingestion to measure BVM. In the meantime, every hour, subjects were asked to complete the VAS questionnaire. Of note, 2 h after the CG or placebo ingestion, subjects were free to drink water. Five hours after the start, the subjects received a standardized lunch composed of white bread, ham, and cheese. At 4 pm (8 h after the start) subjects received a collation (sweet waffle). The test day ended at 8 pm.

Gastrointestinal symptoms

The 100-mm VAS about the eight gastrointestinal (cramps, bloating, rumbling, discomfort, flatulence, burps, gastrointestinal reflux, and nausea) was filled out at baseline and every hour for 12 h after the ingestion of CG or maltodextrin load. The scales were scored by measuring the distance (in mm) from 0 with a ruler. Scores were expressed as changes from baseline in cm.

Breath volatile metabolites (BVM) analysis

Breath samples were collected into bags (FMONP.SBAG1.01 from MEC R&D SPRL, Belgium) before and 30 min after the ingestion of CG or the placebo and then every 2 h for 12 h. The bags were directly sent to Interscience (Louvain-La-Neuve, Belgium) for BVM measurement (within 2 h after the harvest of the exhaled air) by GC using a TCD detector (for H_2 analysis every 4 h; Trace 1310 GC, Thermo Scientific, USA) and using SIFT-MS (for other BVM; Voice 200ultra, Syft Technologies, New Zealand). For this last methodology, the reagent ions (H_3O+, NO+, and O_2+) were generated by a microwave air discharge at 0.5 Torr, selected by using a quadrupole mass filter and injected into a stream of helium carrier gas in the flow tube. All the ion products of the chemical ionization reaction and un-reacted reagent ions were monitored by a quadrupole mass spectrometer in the full scan mode in the mass-to-charge ratio (m/z) range of 10–200 amu (unit mass resolution) over 60 s. Bags filled with helium were used as blank. The full scan data (ion counts per second) averaged over the sampling time for each m/z value was used for the statistical analyses. LabSyft software package (version 1.4.4, Syft Technologies) was used for the mass spectrum acquisition and data exportation as comma-separated values (CSV) files. In total, three data sets were obtained from H_3O+, NO+, and O_2+ ionizations. We targeted volatile metabolites known to study energy metabolism and to study the gut-related metabolic effects of DF, in particular those known to be a reflect of gut fermentation (coming from bacterial metabolism).[11,12] Identified BVM were expressed as changes from baseline in ppm. Concentrations in ppm (presented in Sup Data 5–6) were determined by using pseudo-first-order reaction kinetics according to the equation [A] = [P+] ICFP/k [R+] ICFR (LabSyft software, Syft Technologies, New Zealand); [A] is the concentration of the identified compound in ppm; [P+] and [R+] are the signal in counts per second of product ion and precursor ion, respectively; tr is the reaction time (s); k is the rate constant of the reaction between R+ and A ($cm^3\ s^{-1}$ $molecule^{-1}$); ICFP and ICFR, used to normalize raw ion counts for ion transmission inefficiencies, are mass-dependent transmission functions that are determined by analysis of a calibration gas mixture with known composition and concentrations.[41]

Gut microbiota analysis

Bacterial DNA was extracted from fecal samples from baseline (before interventions) using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany), as previously described.[42] All samples of this study were sequenced in the same run. α-diversity indexes and β diversity indexes were calculated using QIIME2. PCoA plot of the β-diversity indexes was visualized using R software. The raw sequencing data are deposited into the Sequence Read Archive (SRA) of NCBI (http://www.ncbi.nlm.nih.gov/sra) under BioProject PRJNA636138.

Fecal SCFA analysis

SCFAs were analyzed in the native fecal samples as previously described.[42]

Data integration and statistical analysis

Data are expressed as means ± SEM. Baseline values were compared by matched-pairs Wilcoxon signed-rank test; to adjust for multiple testing, the *p*-value of the Wilcoxon test was adjusted with a false discovery rate (FDR) of 5% according to the Benjamini-Hochberg procedure (q-value, significant if $q < 0.05$). Net area under the curve (AUC) was calculated for each symptom recorded every hour and for each identified BVM recorded every 2 h after maltodextrin or CG intake. Wilcoxon matched-pairs signed rank test was used to compare net AUC. In addition, repeated measure ANOVA was performed to compare the evolution of BVM over time. For the gut microbiota analysis, relative abundances performed in QIIME2 are expressed as means and SEM and were calculated on R for each taxon. PCA, PCoA and PLS-DA models were built based on selected variables in R (v3.5.1, package "ade4" and "mixOmics"). For PLS-DA, a loading > 0.9 was chosen. For PCA, statistical differences among the group were determined by Monte Carlo permutations to handle nonparametric data distributions (n = 999, implemented in R). Correlation analysis was performed between BVM and α-diversity or all genera identified (genera with a mean of relative abundance <0.01% were removed) by Spearman's correlation test using the "corrplot" package in R. A significance level of $p < .05$ (q-value for adjusted *p* value, using FDR correction) was adopted for all analyses.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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ORCID

Audrey M. Neyrinck http://orcid.org/0000-0002-9435-3338
Julie Rodriguez http://orcid.org/0000-0002-8271-3893
Zhengxiao Zhang http://orcid.org/0000-0002-6977-4309
Benjamin Seethaler http://orcid.org/0000-0003-1889-8085
Laure B. Bindels http://orcid.org/0000-0003-3747-3234
Patrice D. Cani http://orcid.org/0000-0003-2040-2448
Julie-Anne Nazare http://orcid.org/0000-0003-3545-3264
Martine Laville http://orcid.org/0000-0002-6252-9853
Stephan C. Bischoff http://orcid.org/0000-0003-2614-5948
Jens Walter http://orcid.org/0000-0003-1754-172X
Nathalie M. Delzenne http://orcid.org/0000-0003-2115-6082

References

1. Stephen AM, Champ MM, Cloran SJ, Fleith M, van Lieshout L, Mejborn H, Burley VJ. Dietary fibre in

Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev. 2017;30:149–190. doi:10.1017/S095442241700004X.

2. EFSA. Scientific opinion on the substantiation of health claims related to dietary fibre (ID 744, 745, 746, 748, 749, 753, 803, 810, 855, 1415, 1416, 4308, 4330) pursuant to article 13(1) of regulation (EC)No 1924/20061. EFSA Journal. 2010;8:1735. doi:10.2903/j.efsa.2010.1735.
3. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, et al. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14:491–502. doi:10.1038/nrgastro.2017.75.
4. EFSA Panel on Dietetic Products NaAN. Scientific opinion on the safety of 'Chitin-glucan' as a novel food ingredient. EFSA Journal. 2010;8:1687. doi:10.2903/j.efsa.2010.1687.
5. Marzorati MMV, Possemiers S. Fate of chitin-glucan in the human gastrointestinal tract as studied in a dynamic gut simulator (SHIME). J Funct Foods. 2017;30:313–320. doi:10.1016/j.jff.2017.01.030.
6. Neyrinck AM, Possemiers S, Verstraete W, De Backer F, Cani PD, Delzenne NM. Dietary modulation of clostridial cluster XIVa gut bacteria (Roseburia spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. J Nutr Biochem. 2012;23:51–59. doi:10.1016/j.jnutbio.2010.10.008.
7. Berecochea-Lopez A, Decorde K, Ventura E, Godard M, Bornet A, Teissedre PL, Cristol JP, Rouanet JM. Fungal chitin-glucan from Aspergillus niger efficiently reduces aortic fatty streak accumulation in the high-fat fed hamster, an animal model of nutritionally induced atherosclerosis. J Agric Food Chem. 2009;57:1093–1098. doi:10.1021/jf803063v.
8. Bays HE, Evans JL, Maki KC, Evans M, Maquet V, Cooper R, Anderson JW. Chitin-glucan fiber effects on oxidized low-density lipoprotein: a randomized controlled trial. Eur J Clin Nutr. 2013;67:2–7. doi:10.1038/ejcn.2012.121.
9. Das S, Pal S, Mitra M. Significance of exhaled breath test in clinical diagnosis: a special focus on the detection of diabetes mellitus. J Med Biol Eng. 2016;36:605–624.
10. Rieder F, Kurada S, Grove D, Cikach F, Lopez R, Patel N, Singh A, Alkhouri N, Shen B, Brzezinski A, et al. A distinct colon-derived breath metabolome is associated with inflammatory bowel disease, but not its complications. Clin Transl Gastroenterol. 2016;7:e201. doi:10.1038/ctg.2016.57.
11. Ajibola OA, Smith D, Spanel P, Ferns GA. Effects of dietary nutrients on volatile breath metabolites. J Nutr Sci. 2013;2:e34. doi:10.1017/jns.2013.26.
12. Raninen KJ, Lappi JE, Mukkala ML, Tuomainen TP, Mykkanen HM, Poutanen KS, Raatikainen OJ. Fiber content of diet affects exhaled breath volatiles in fasting and postprandial state in a pilot crossover study. Nutr Res. 2016;36:612–619. doi:10.1016/j.nutres.2016.02.008.
13. Lawal O, Ahmed WM, Nijsen TME, Goodacre R, Fowler SJ. Exhaled breath analysis: a review of 'breath-taking' methods for off-line analysis. Metabolomics. 2017;13:110. doi:10.1007/s11306-017-1241-8.
14. Bruderer T, Gaisl T, Gaugg MT, Nowak N, Streckenbach B, Muller S, Moeller A, Kohler M, Zenobi R. On-line analysis of exhaled breath focus review. Chem Rev. 2019;119:10803–10828. doi:10.1021/acs.chemrev.9b00005.
15. Spanel P, Smith D. Quantification of volatile metabolites in exhaled breath by selected ion flow tube mass spectrometry, SIFT-MS. Clini Mass Spectrom. 2020;16:18–24. doi:10.1016/j.clinms.2020.02.001.
16. Stefanuto PH, Zanella D, Vercammen J, Henket M, Schleich F, Louis R, Focant JF. Multimodal combination of GC x GC-HRTOFMS and SIFT-MS for asthma phenotyping using exhaled breath. Sci Rep. 2020;10:16159. doi:10.1038/s41598-020-73408-2.
17. Neyrinck AM, Rodriguez J, Vinoy S, Maquet V, Walter J, Bischoff SC, Laville M, Delzenne NM. The FiberTAG project: tagging dietary fibre intake by measuring biomarkers related to the gut microbiota and their interest for health. Nutrition Bulletin. 2020;45:59–65. doi:10.1111/nbu.12416.
18. Boshier PR, Marczin N, Hanna GB. Repeatability of the measurement of exhaled volatile metabolites using selected ion flow tube mass spectrometry. J Am Soc Mass Spectrom. 2010;21:1070–1074. doi:10.1016/j.jasms.2010.02.008.
19. de Lacy Costello B, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, Osborne D, Ratcliffe NM. A review of the volatiles from the healthy human body. J Breath Res. 2014;8:014001. doi:10.1088/1752-7155/8/1/014001.
20. Rezaie A, Buresi M, Lembo A, Lin H, McCallum R, Rao S, Schmulson M, Valdovinos M, Zakko S, Hydrogen PM. Methane-based breath testing in gastrointestinal disorders: the North American consensus. Am J Gastroenterol. 2017;112:775–784. doi:10.1038/ajg.2017.46.
21. Smolinska A, Tedjo DI, Blanchet L, Bodelier A, Pierik MJ, Masclee AAM, Dallinga J, Savelkoul PHM, Jonkers D, Penders J, et al. Volatile metabolites in breath strongly correlate with gut microbiome in CD patients. Anal Chim Acta. 2018;1025:1–11. doi:10.1016/j.aca.2018.03.046.
22. Raman M, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, Greenwood R, Sikaroodi M, Lam V, Crotty P, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2013;11(868–75):e1–3. doi:10.1016/j.cgh.2013.02.015.
23. Rondanelli M, Perdoni F, Infantino V, Faliva MA, Peroni G, Iannello G, Nichetti M, Alalwan TA,

Perna S, Cocuzza C. Volatile organic compounds as biomarkers of gastrointestinal diseases and nutritional status. J Anal Methods Chem. 2019;2019:7247802. doi:10.1155/2019/7247802.
24. Kistler M, Szymczak W, Fedrigo M, Fiamoncini J, Hollriegl V, Hoeschen C, Klingenspor M, Hrabe de Angelis M, Rozman J. Effects of diet-matrix on volatile organic compounds in breath in diet-induced obese mice. J Breath Res. 2014;8:016004.
25. Baranska A, Tigchelaar E, Smolinska A, Dallinga JW, Moonen EJ, Dekens JA, Wijmenga C, Zhernakova A, van Schooten FJ. Profile of volatile organic compounds in exhaled breath changes as a result of gluten-free diet. J Breath Res. 2013;7:037104. doi:10.1088/1752-7155/7/3/037104.
26. Barros R, Moreira A, Fonseca J, Delgado L, Castel-Branco MG, Haahtela T, Lopes C, Moreira P. Dietary intake of alpha-linolenic acid and low ratio of n-6:n-3 PUFA are associated with decreased exhaled NO and improved asthma control. Br J Nutr. 2011;106:441–450. doi:10.1017/S0007114511000328.
27. Hageman JHJ, Nieuwenhuizen AG, van Ruth SM, Hageman JA, Keijer J. Application of volatile organic compound analysis in a nutritional intervention study: differential responses during five hours following consumption of a high- and a low-fat dairy drink. Mol Nutr Food Res. 2019;63:e1900189. doi:10.1002/mnfr.201900189.
28. Filipiak W, Ruzsanyi V, Mochalski P, Filipiak A, Bajtarevic A, Ager C, Denz H, Hilbe W, Jamnig H, Hackl M, et al. Dependence of exhaled breath composition on exogenous factors, smoking habits and exposure to air pollutants. J Breath Res. 2012;6:036008. doi:10.1088/1752-7155/6/3/036008.
29. Lee J, Ngo J, Blake D, Meinardi S, Pontello AM, Newcomb R, Galassetti PR. Improved predictive models for plasma glucose estimation from multi-linear regression analysis of exhaled volatile organic compounds. J Appl Physiol. 1985;2009:155–160.
30. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–857. doi:10.1038/s41587-019-0209-9.
31. King J, Mochalski P, Unterkofler K, Teschl G, Klieber M, Stein M, Amann A, Baumann M. Breath isoprene: muscle dystrophy patients support the concept of a pool of isoprene in the periphery of the human body. Biochem Biophys Res Commun. 2012;423:526–530. doi:10.1016/j.bbrc.2012.05.159.
32. Smith D, Spanel P, Davies S. Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study. J Appl Physiol. 1985;1999:1584–1588.
33. Russell WR, Hoyles L, Flint HJ, Dumas ME. Colonic bacterial metabolites and human health. Curr Opin Microbiol. 2013;16:246–254. doi:10.1016/j.mib.2013.07.002.
34. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009;294:1–8. doi:10.1111/j.1574-6968.2009.01514.x.
35. Verbeke KA, Boobis AR, Chiodini A, Edwards CA, Franck A, Kleerebezem M, Nauta A, Raes J, van Tol EA, Tuohy KM. Towards microbial fermentation metabolites as markers for health benefits of prebiotics. Nutr Res Rev. 2015;28:42–66. doi:10.1017/S0954422415000037.
36. Taucher J, Lagg A, Hansel A, Vogel W, Lindinger W. Methanol in human breath. Alcohol Clin Exp Res. 1995;19:1147–1150. doi:10.1111/j.1530-0277.1995.tb01593.x.
37. Gelmont D, Stein RA, Mead JF. The bacterial origin of rat breath pentane. Biochem Biophys Res Commun. 1981;102:932–936. doi:10.1016/0006-291X(81)91627-2.
38. Zalan Z, Hudacek J, Toth-Markus M, Husova E, Solichova K, Hegyi F, Plockova M, Chumchalova J, Halasz A. Sensorically and antimicrobially active metabolite production of Lactobacillus strains on Jerusalem artichoke juice. J Sci Food Agric. 2011;91:672–679. doi:10.1002/jsfa.4232.
39. Gorham JB, Kang S, Williams BA, Grant LJ, McSweeney CS, Gidley MJ, Mikkelsen D. Addition of arabinoxylan and mixed linkage glucans in porcine diets affects the large intestinal bacterial populations. Eur J Nutr. 2017;56:2193–2206. doi:10.1007/s00394-016-1263-4.
40. Duncan SH, Hold GL, Barcenilla A, Stewart CS, Flint HJ. Roseburia intestinalis sp. nov., a novel saccharolytic, butyrate-producing bacterium from human faeces. Int J Syst Evol Microbiol. 2002;52:1615–1620.
41. Kharbach M, Kamal R, Mansouri MA, Marmouzi I, Viaene J, Cherrah Y, Alaoui K, Vercammen J, Bouklouze A, Vander Heyden Y. Selected-ion flow-tube mass-spectrometry (SIFT-MS) fingerprinting versus chemical profiling for geographic traceability of Moroccan Argan oils. Food Chem. 2018;263:8–17. doi:10.1016/j.foodchem.2018.04.059.
42. Rodriguez J, Neyrinck AM, Zhang Z, Seethaler B, Nazare JA, Robles Sanchez C, Roumain M, Muccioli GG, Bindels LB, Cani PD, et al. Metabolite profiling reveals the interaction of chitin-glucan with the gut microbiota. Gut Microbes. 2020;12:1810530. doi:10.1080/19490976.2020.1810530.