

# Minireview

## Analyses of short-chain fatty acids and exhaled breath volatiles in dietary intervention trials for metabolic diseases

Jisun HJ Lee<sup>1,2</sup> and Jiangjiang Zhu<sup>1,2</sup> 

<sup>1</sup>Department of Human Sciences, The Ohio State University, Columbus, OH 43210, USA; <sup>2</sup>James Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210, USA

Corresponding author: Jiangjiang Zhu. Email: zhu.2484@osu.edu

### Impact statement

The prevalence of metabolic diseases such as obesity and diabetes has been rapidly increasing in the 21st century, and it has become a major global health problem. Recently, changes in lifestyle, such as dietary modifications, were found useful in preventing metabolic diseases. Meanwhile, the positive impact of dietary interventions on human health has been linked with the production of short-chain fatty acids (SCFAs) by gut microbiota and exhaled breath volatile metabolites through the complicated metabolic process. Thus, monitoring SCFAs and exhaled breath volatiles as biomarkers can be used to evaluate the effectiveness of dietary interventions on metabolic diseases in clinical studies. The dietary intervention-induced alteration of SCFAs and exhaled volatiles can also be useful for the development of novel prevention approaches and drug-free treatments for metabolic diseases.

### Abstract

As an alternative to pharmacological treatment to diseases, lifestyle interventions, such as dietary changes and physical activities, can help maintain healthy metabolic conditions. Recently, the emerging analyses of volatile organic compounds (VOCs) from breath and short-chain fatty acids (SCFAs) from plasma/feces have been considered as useful tools for the diagnosis and mechanistic understanding of metabolic diseases. Furthermore, diet-induced changes of SCFAs in individuals with diagnosed metabolic abnormalities have been correlated with the composition changes of the gut microbiome. More interestingly, the analysis of exhaled breath (breathomics) has gained attention as a useful technique to measure the human VOC profile altered as a result of dietary interventions. In this mini-review, we examined recent clinical trials that performed promising dietary interventions, SCFAs analysis in plasma/feces, and VOC profile analysis in exhaling breath to understand the relationship between dietary intervention and metabolic health.

**Keywords:** Dietary intervention, short-chain fatty acids, volatiles, breathomics, mass spectrometry

*Experimental Biology and Medicine* 2021; 246: 778–789. DOI: 10.1177/1535370220979952

### Introduction

Normal metabolism is the key to good health. On the other hand, altered metabolic processes generally lead to the development of metabolic diseases such as obesity, diabetes mellitus, dyslipidemia, and osteoporosis.<sup>1</sup> In our modern age, the rapidly elevated prevalence of metabolic diseases is considered as a major health problem.<sup>2</sup> It has been reported that more than a third of adults in the United States are diagnosed with metabolic abnormalities, most likely due to an unhealthy lifestyle.<sup>3</sup> As preferred alternatives to therapeutics or surgical treatments, lifestyle changes such as dietary modifications and adopting a physically more active lifestyle can be beneficial to people with metabolic dysregulations.<sup>4</sup> Considering this, various clinical studies have been undertaken to assess the impact of dietary intervention on different metabolic diseases.<sup>4–6</sup>

The diagnosis of metabolic diseases is generally cumbersome, and causes, risks, or consequences of metabolic diseases have been assessed by measuring altered levels of key physiological metabolite biomarkers as an important diagnostic approach.<sup>7</sup> These biomarkers are used as an indicator of a given biological state or diseased condition.<sup>8</sup> However, very few biomarkers related to the nutritional effects on the development and progression of various metabolic diseases have been identified so far. The complex pathophysiology of metabolic diseases underlines the limitation of known routine markers such as lipid profiles, glucose, and insulin in their diagnostic accuracy, and nutritional interventions add additional layers of complexities on top of these factors. In recent years, metabolomics, a systematic study of metabolites, has been considered as a powerful approach in biomarkers

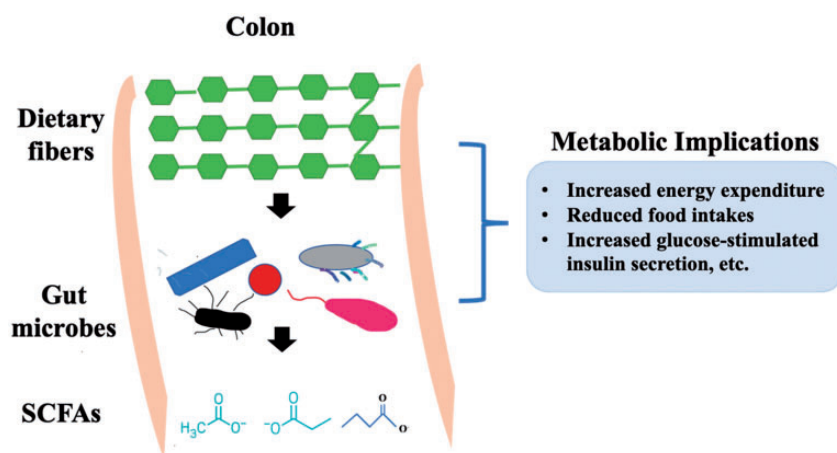
discovery with the promise to be eventually applied to clinical settings.<sup>9</sup>

Thus far, mass spectrometry (MS)-based metabolomics is considered as a useful tool in clinical studies to investigate the effect of dietary interventions in improving metabolic disorders, including discovering potential biomarkers of route-cause pathophysiology of these syndromes.<sup>10</sup> An ultra-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and a gas chromatography equipped to mass spectrometry (GC-MS) are the most popular analytical techniques for metabolomics analyses.<sup>11–14</sup> Nowadays, the use of these analytical tools in dietary interventions associated with human metabolic disorders has been growing, especially when it comes to identifying and quantifying small molecular weight compounds, volatile organic compounds (VOCs) and short-chain fatty acids (SCFAs), in human biofluids such as plasma and feces.<sup>11,15–17</sup> SCFAs are produced as a result of fermentation of indigestible foods by gut microbiota and are normally required for maintaining optimal gut health.<sup>18</sup> These SCFAs act as signaling molecules that can enter the systemic circulation and directly affect metabolic or peripheral tissue functions.<sup>19</sup> They were also found to have a considerable role in the control of body weight and insulin sensitivity.<sup>19</sup> The production of SCFAs is influenced by the diet-mediated changes and physiological activities in the gut.<sup>20</sup> Thus, critical assessment of SCFAs levels can be crucial in understanding healthy and diseased conditions. Recently, the exhaled VOC analysis, called breathomics, has also been considered as a promising tool for investigating metabolic responses in a non-invasive manner. However, the significance of breathomics in clinical trials of dietary interventions, especially in biomarker quantification, remained to be fully explored. In this mini-review, we summarize the research progress of SCFAs production, breathomics, and their potentials as real-time biomarkers in the context of human dietary interventions to human metabolic health.

## Short-chain fatty acids and their connection with diet, gut microbes, and human health

It is well-known that humans cannot digest the bulk of dietary fibers due to the lack of related enzymes, and the non-digestible carbohydrates are fermented in the large intestine by the colonic/gut microbiota.<sup>20,21</sup> SCFAs, saturated aliphatic organic acids, become the major group of metabolites that are generated by the gut microbial community as a consequence of fermentation of dietary fibers (Figure 1).<sup>22,23</sup> SCFAs consist of one to six carbons, and acetate (C<sub>2</sub>), propionate (C<sub>3</sub>), and butyrate (C<sub>4</sub>) have been reported as the most abundant ones by representing 90–95% of the SCFA present in the colon.<sup>20,24</sup> Previous studies have identified that the formation of SCFAs is influenced by various factors, including the pattern of food intake, antibiotics treatment, and microbial populations.<sup>20,25–27</sup> In particular, carbohydrates are the main source of SCFAs production; meanwhile, amino acids, such as valine, leucine, and isoleucine from the protein breakdown are also involved in the production of branched SCFAs such as isobutyrate, isovalerate, and 2-methyl butyrate.<sup>20</sup>

Earlier studies have described the metabolic routes of several SCFAs. For example, two major paths for synthesizing acetate are initiated from formic acid through the Wood–Ljungdahl pathway or from hydrogen and carbon dioxide by acetogenic bacteria.<sup>28,29</sup> In the case of propionate generation, three pathways, involved in succinate, acrylate, and propanediol synthesis, are considered as major routes.<sup>30</sup> Among them, propionate production via succinate pathway is prevailing, and several *Firmicutes* and *Bacteroidetes* were found to be involved in succinate pathways, and their richness was also related to the level of propionate in fecal samples.<sup>31</sup> Another pathway involving the butyryl-CoA:acetate CoA-transferase gene for butyrate formation has been elucidated with the participation of butyrate-producing bacteria,<sup>20</sup> which can convert butyryl-CoA to butyrate with a single step enzymatic reaction in the



**Figure 1.** A diagram demonstrates the relationships of dietary fibers, gut microbes, their SCFA metabolites, and their implications to host metabolic health. (A color version of this figure is available in the online journal.)

presence of butyrate-producing *Firmicutes*, such as *Faecalibacterium*, *Eubacterium*, and *Roseburia*.<sup>32</sup> The connections between these elucidated pathways of SCFA productions and the involvement of multiple members of the gut microbial community highlight the need to study the chemical fingerprints profiles in conjunction with the microbial fingerprints in metabolic studies on the evaluation of dietary intervention effects to patients with metabolic dysregulations.

Besides, epidemiological studies have demonstrated that the gut microbiome may participate in host-signaling mechanisms via microbial metabolites, as well as use SCFAs as energy sources to feed host cells.<sup>20</sup> SCFAs were also found to influence host physiology and potentially modulate the development of metabolic syndrome by incorporating into glucose and lipid metabolism (Figure 1).<sup>33–35</sup> Also, SCFAs play vital roles in the maintenance of the gut barrier function as fuels for the intestinal epithelial cells which may lead to the increase of mucin productions to prevent occurrence and development of gut diseases.<sup>20,36,37</sup> Mainly, butyric acids have been reported to prevent bacterial adhesion and improve the tight-junctions integrity.<sup>38,39</sup> Furthermore, the immunomodulating function of the microbial community that produces SCFAs has been described during the host inflammatory processes.<sup>40</sup> For instance, it has been reported that butyrate and propionate may promote the differentiation of T-regulatory cells into effector and regulatory T cells via suppression of histone deacetylase.<sup>41</sup> Consequently, the reduced incidence of intestinal inflammation may have a beneficial impact on improving host immune tolerance and reducing the risk of inflammatory bowel disease and colorectal cancer.<sup>20</sup>

### Human breath volatile analysis: The new frontier in human health research

Thousands of VOCs are emitted from the human body, and their components can reflect a person's metabolic condition. Therefore, metabolic diseases often result in a change in body smell.<sup>42</sup> These VOCs can be originated from breath, urine, feces, saliva, sweat, and skin. Among them, exhaled breath contains hundreds of reported VOCs that can be attributed to either exogenous or endogenous sources.<sup>43</sup> For example, exogenous VOCs include compounds inhaled or derived from the external environment, such as smoking a cigarette or ingesting food. On the other hand, endogenous VOCs consist of circulating bloodstream compounds that were produced by many metabolic processes at different organs/tissues including ones from colonic microbial metabolism and released through the lungs. The concentrations of VOCs in breath vary from picomolar to nanomolar levels, which make it challenging to distinguish endogenous VOCs from the exogenous compounds.<sup>44</sup> Nonetheless, breath sampling has gained attention for its non-invasive, extremely simple, and painless manners. Therefore, the breath analysis has become the top choice for some clinical studies of which clinicians and researchers have managed to successfully identify disease-specific VOCs. For example, trimethylamine was detected in the breath of patients with trimethylaminuria

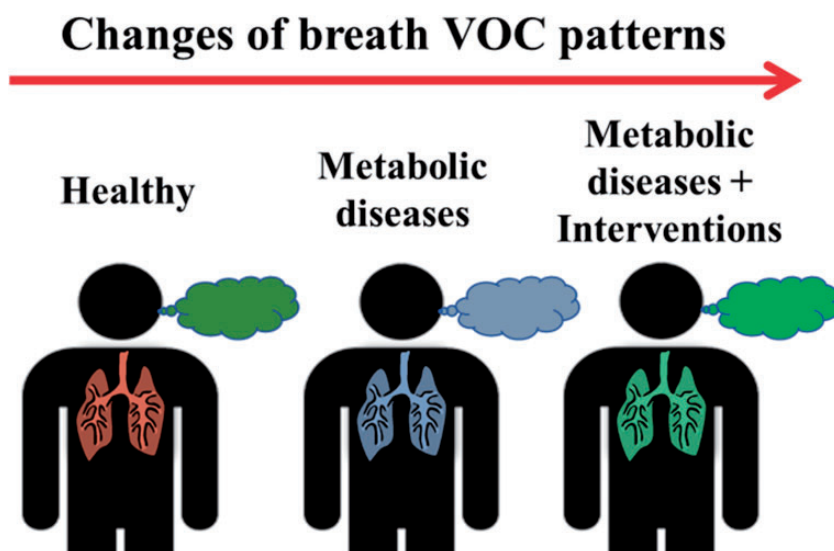
as a potential diagnostic marker; and in the breath of patients with fetor hepaticus, methyl-mercaptan was identified as a useful biomarker for the diagnosis of this disease.<sup>45,46</sup>

Metabolic diseases often result in the production and accumulation of certain metabolites in body fluids due to impairment in normal biochemical pathways. Therefore, the detection of increased concentrations of these metabolites can be used for the diagnosis of these diseases.<sup>47</sup> For example, in the case of diabetes, cells utilize glucose as the main source of energy in the body, and the pancreas produces insulin to signals cells for glucose uptake.<sup>48</sup> Glucose then accumulates in the blood of patients with type 1 diabetes who cannot synthesize insulin, and eventually excreted in the urine. Given these unusual changes, fatty acid catabolism will be promoted as cells tend to use fatty acids rather than glucose as an energy source. As a result, this change creates ketone compounds, such as acetone, acetoacetate, and 3-hydroxybutyrate as by-products, and these ketone bodies cause blood acidity, leading to ketoacidosis of the blood. These excess ketones are secreted in the urine and breath.<sup>48</sup> As a result, the patient's urine and breath could have a fruity odor due to the presence of acetone. Sequentially, it is reasonable to expect that when interventions such as dietary patterns' changes were applied to these patients, their breath patterns could change accordingly (Figure 2).

### Overview of clinical trials of dietary interventions with constant monitoring of SCFAs

It is well known that individuals with prediabetes have increased risks of the development of type 2 diabetes (T2D) and multiple vascular complications.<sup>49</sup> Numerous randomized controlled trials (RCTs) have demonstrated the feasibility of preventing progression from prediabetes to T2D, using lifestyle or pharmacological interventions.<sup>50,51</sup> studies reported in the past decade on the detections of SCFAs associated with dietary interventions were summarized in Table 1. The RCTs reviewed here provide compelling evidence that lifestyle modification can decrease the risk of progression to T2D in people with prediabetes with a maximum effect size of ~60% risk reduction compared with placebo.<sup>52,53</sup> Out of the various lifestyle modifications, precision nutrition has the potential to provide personalized dietary guidance for more effective prevention or management of T2D.<sup>54</sup> A majority of these clinical studies have investigated the effectiveness of dietary interventions for preventing or delaying metabolic abnormality in individuals with MetS or at high risk for MetS (e.g. obese or overweight) by leveraging SCFAs as potential biomarkers.

In Table 1, 2 out of 11 clinical trials used healthy individuals as control groups, in which the same diet was assigned to both healthy and obese/overweight individuals as test groups.<sup>16,55</sup> The sample sizes of these studies ranged from 14 to 88 subjects, and most of these clinical trials were designed in a randomized and blind manner. The duration of the dietary intervention varied



**Figure 2.** A schematic demonstrates the possible breath VOC pattern changes from a healthy individual to a patient with metabolic diseases, and to the patient who went through the dietary interventions of the metabolic diseases. Different colors of the breath indicate different breath VOC patterns. (A color version of this figure is available in the online journal.)

from 7 h to 24 weeks. Mainly, plasma or fecal samples were chosen for metabolic analysis, and particularly, eight cases not only investigated fecal SCFAs, but also reported the diet-induced alteration of fecal microbial composition using the 16S ribosomal RNA analysis.<sup>25,31,55–60</sup> As summarized in Table 1, liquid chromatography<sup>55</sup> and gas chromatography<sup>31,56,57,60–62</sup> were generally combined with derivatization methods and MS, which have been most commonly used as effective analytical techniques. In other cases, gas chromatography was coupled with isotope ratio MS<sup>16</sup> or flame injection detectors<sup>25,58,59</sup> for the determination of small and polar SCFAs.

Overall, several RCTs demonstrated the beneficial effects of dietary components on preventing the risk of developing metabolic derangements according to the diet-induced change in metabolic and anthropometric parameters.<sup>16,25,56,58–60</sup> Thus, the results of these RCTs may suggest a variety of effective dietary intervention strategies that last from days to months, and successful metabolic risk management can be achieved. Meanwhile, small-molecule metabolite biomarkers, especially SCFAs, have been considered as important indicators related to biochemical changes that underlying the physiological changes after dietary intervention. Epidemiologic studies have encouraged dietary fiber intakes in diabetic and metabolic disease patients based on its health benefits such as improving insulin sensitivity and SCFAs producing intestinal microflora.<sup>63,64</sup> Here, we focused on previous RCTs which mostly tested dietary fiber-based ingredients, including barley beta-glucans, inulin, galactooligosaccharides, whole-grain cereal products, healthy-carbohydrate diet containing rich arabinoxylan and resistant starch, inulin-type fructans, rye kernels, concentrated arabinoxylan combined with rye kernels (AXRK), wheat bran non-starch polysaccharides, or non-dietary fiber ingredients such as sodium butyrate and *Lactobacillus*

*salivarius* to examine diet-induced changes of SCFAs (Table 1).

The diet-induced changes of SCFAs in subjects diagnosed with MetS or at a high risk of metabolic diseases varied depending on the types of experimental ingredients. For instance, studies on diets containing rich dietary fibers, such as barley beta-glucans, inulin, whole-grain cereal products, a healthy-carbohydrate diet with rich arabinoxylan and resistant starch resulted in increased amounts of SCFAs (acetate, propionate, and butyrate), compared with control groups.<sup>16,25,57,61</sup> Meanwhile, galactooligosaccharides and inulin-type fructans were not found to lead significant influences by presenting no considerable changes or decrease of SCFAs, respectively.<sup>56,58</sup> In some cases, diverse patterns of SCFAs alteration were observed according to different types of foods. For example, a study by Hartvigsen *et al.* examined the effect of 50 g of semolina porridge as control, or 50 g of control diet supplemented with one of three digestible carbohydrates, namely arabinoxylan, rye kernels, or concentrated AXRK, on individuals with diabetes. As a result, significantly increased acetic acid abundance was found in the group that ingested arabinoxylan diets. Whereas higher levels of acetic acid and butyric acid were detected from the AXRK-intaking group, compared with control groups.<sup>62</sup> Overall, these clinical trials have shown that a dietary fiber-enriched diet can affect the level of plasma or fecal SCFA in a fiber type-specific manner.

### Regulations of SCFA productions and their related gut microbes

Previous studies have shown that the diet is one of the key regulatory factors, in terms of modulation of the symbiosis or dysbiosis of gut microbiota, and ultimately affects human health.<sup>65,66</sup> The food rich in dietary fibers has been reported to prevent and mitigate T2D mellitus,

Table 1. Summary of dietary interventions induced changes in SCFAs productions in populations with metabolic abnormalities.

Study design (duration)	Sample size (control/test)	Age (control/test)	Gender (M/F)	Health condition	Dietary intervention	Analytical tools (sample)	Altered microbiota	Results in test (significance)	References
A randomized, placebo-controlled, double-blind clinical trial (4 weeks)	16/27	54.00 ± 8.07/ 50.93 ± 7.40	M/F	MetS or high risk at MetS	Either 200 g of bread containing 6% of barley beta-glucans or 200 g of bread without added beta-glucans as control	GC-FID (Feces)	<i>Bifidobacterium</i> spp. and <i>Akkermansia muciphila</i> ↑ in test groups	Acetate ↑ (*) and propionate ↑ (*) in the test group	25
Placebo-controlled trials (4 weeks)	9/10	25 ± 2.4/ 42 ± 2.4	M	Healthy or obesity	4 g of sodium butyrate	LC-MS/MS (feces)	<i>Coriobacteriaceae</i> , <i>Clostridiales</i> , <i>Bacteroides</i> , <i>Oscillospira</i> ↓ in MetS groups	Acetate ↑ (*), propionate ↑ (**), and butyrate ↑ (*) in MetS group	55
Double-blind, randomized, placebo-controlled crossover trial (7 h)	14	34 ± 3	M	Healthy, overweight, or obesity	Either high-fat milkshake containing 24 g inulin or 24 g maltodextrin as control	GC-C-IRMS/GC-IRMS (plasma, breath, and feces)	N/A	<sup>13</sup> C-labelled acetate ↑ (**), propionate ↑ (*), and butyrate ↑ (*) in test group (plasma)	16
Double-blinded, placebo-controlled, parallel intervention study (12 weeks)	23/21	58.4 ± 7.3/ 59.2 ± 7.2	M/F	Overweight or obesity	Either 45 g of GOS or 45 g of maltodextrin as control	GC/MS (plasma and feces)	<i>Bifidobacterium</i> ↑ in test groups	No significant changes in acetate, propionate, and butyrate	56
Randomly assigned (12 weeks)	28/26	58.4 ± 1.6/ 57.2 ± 1.9	M/F	Overweight or obesity (MetS)	Either whole-grain cereal products (whole-grain group) or refined cereal goods as control	GC/MS (plasma)	N/A	Propionate ↑ (*) in test group	61
Randomized crossover Study (4 weeks)	19	60 (range 39–75)	M/F	MetS	Either HCD with AX- and RS-enriched cereal foods or WSD based on refined grains as control	GC/MS (feces)	<i>Bifidobacterium</i> ↑ in test groups	Acetate ↑ (**), and butyrate ↑ (***) in HCD diet	57
Randomized, double-blind, parallel, placebo-controlled trial (12 weeks)	15/15	Range 18–65	F	Obesity	Either 16 g of ITF or 16 g of maltodextrin as control	GC-FID (feces)	<i>Bifidobacterium longum</i> , <i>Bifidobacterium pseudocatenulatum</i> and <i>Bifidobacterium adolescentis</i> ↑ in test groups	Acetate ↑ (**), propionate ↓ (*), and caproate ↓ (***) in ITF group	58
Acute, randomized crossover study (6 h)	15	63.5 ± 5.0 (range 52–73)	M/F	Diabetes with MetS	50 g of one of four digestible carbohydrate semolina porridge added AX, RK, concentrated AXRK, and SE as control	GC/MS (plasma)	N/A	Acetate in AX ↑ (**), and AXRK ↑ (***), whereas butyrate in AXRK ↑ (***) than control groups (SE)	62
Randomized crossover design (10 weeks)	14	53 (range 27–73)	M	Obesity	Weight maintenance (M) (1 week) → type III RS or NSP (3 weeks) → WL (3 weeks) diets	GC/MS (feces)	<i>Ruminococcaceae phylotypes</i> ↑ (RS), <i>Lachnospiraceae phylotypes</i> ↑ (NSP), <i>Bifidobacteria</i> ↓ (WL)	Acetate ↑ (**), propionate ↓ (*), butyrate ↓ (*) in NPS than RS groups, whereas isobutyric acid ↓ (**), and isovaleric acid ↓ (***) in RS than NPS groups	31

(continued)

Table 1. Continued.

Study design (duration)	Sample size (control/test)	Age (control/test)	Gender (M/F)	Health condition	Dietary intervention	Analytical tools (sample)	Altered microbiota	Results in test (significance)	References
Randomized, controlled, single blind, parallel design (24 weeks)	11/17/ 22/21/17	54 ± 9.5	M/F	High risk at MetS	One of five diets HS, HM/HGI, HM/LGI, HC/HGI, or HC/LGI	GC-IFD (feces)	<i>Bifidobacterium</i> ↑ (HC), <i>Bacteroides</i> ↑ (HC/HGI), and <i>Faecalibacterium prausnitzii</i> ↑ (HC/LGI) and HS)	Acetate↑(*), propionate↑(**), butyrate↑(**) after HS diet	59
Double-blinded, randomized, placebo-controlled intervention (12 weeks)	23/27	Range 12–15	N/A	Obesity	One capsule of either <i>L. salivarius</i> Ls-33 ATCC SD5208 (1010 CFU per capsule) or maltodextrin as control	GC/MS (feces)	<i>Firmicutes</i> / <i>Bacteroidetes</i> (F/B ratio)↑ in test groups	No significant difference in acetic, propionic, butyric, isobutyric, 2-methylbutyric, valeric, and isovaleric acids	60

M: male; F: female; MetS: metabolic syndrome; GOS: galacto-oligosaccharides; HCD: healthy-carbohydrate diet; WSD: Western-style diet; ITF: inulin-type fructans; AX: arabinoxylan; RK: rye kernels; AXRK: arabinoxylan combined with rye kernels; SE: semolina porridge as control; RS: resistant starch; NSP: non-starch polysaccharides; WL: weight-loss; HS: high saturated fat diet; HM: high monounsaturated fat; HGI: high glycemic index; LGI: low glycemic index; HC: high carbohydrate; GC: gas chromatography; FID: flame ionization detection; LC: liquid chromatography; MS/MS: tandem mass spectrometry; C-IRMS: combustion-isotope ratio mass spectrometer; N/A: not available.

\*, \*\*, and \*\*\* indicate significant difference at  $P < 0.05$ , 0.01, and 0.001, respectively.

cardiovascular disease, and colon cancer.<sup>67,68</sup> Furthermore, SCFAs are produced by the fermentation of fibers and counteract obesity by reducing fat accumulation in adipose tissues and accelerating energy expenditure.<sup>69–71</sup> Commonly, clinical studies perform in-depth gut microbiota analysis while examining metabolic changes in humans in response to diet. Most of the studies reviewed in Table 1 were conducted with the evaluation of the effects of specific dietary ingredients on changes in the gut microbial population along with the SCFA production. Mainly, gut microbes from four bacterial phylum, including *Actinobacteria* (*Bifidobacterium* and *Coriobacteriaceae*), *Verrucomicrobia* (*Akkermansia municipihila*), *Firmicutes* (*Clostridiales*, *Oscillospira*, *Ruminococcaceae*, *Lachnospiraceae*, and *Faecalibacterium*), and *Bacteroidetes* (*Bacteroides*), were identified at genus, species, family, or class levels as described in Table 1. In general, *Actinobacteria* is known as a minority group of commensal bacteria, and it plays a pivotal role in the development and maintenance of gut homeostasis, involved in the modulation of gut permeability and the immune system.<sup>72</sup> Moreover, health beneficial *Actinobacteria* species, such as *Bifidobacterium* and *Coriobacteriaceae*, have been reported to reduce obesity, blood lipids, and cardiovascular risk in patients with MetS and to improve T2D conditions.<sup>73,74</sup> *Akkermansia municipihila* (Phylum *Verrucomicrobia*), a mucin-degrading bacteria, is also described as SCFA-producing (such as propionate and acetate).<sup>75,76</sup> *Firmicutes* and *Bacteroidetes* are the major population in healthy gut microbiota.<sup>77</sup> Consumption of plant-based diets may regulate the growth of *Firmicutes* and *Bacteroidetes*, and the reduced ratio of abundance of *Firmicutes* to *Bacteroidetes* (F/B) has been reported to indicate healthier gut microbiota.<sup>78</sup> In addition, changed F/B ratios have also been found to be associated with various pathologies. For instance, the decreased F/B ratio has been linked to colonic inflammation in patients with inflammatory bowel disease, and the altered F/B also has been observed in obese and T2D.<sup>79–82</sup>

In most cases in Table 1, increased abundances of bacteria were observed along with elevated levels of SCFAs after consuming dietary fibers (barley beta-glucans, resistant starch) or carbohydrate-based diets.<sup>25,31,55–57,59</sup> In a study by Hald *et al.*, a healthy-carbohydrate diet with arabinoxylan- and resistant starch-enriched cereal foods, or a Western diet based on refined grains was given to participants diagnosed with MetS in a randomized and crossover fashion.<sup>57</sup> The substantially enhanced values of acetic acid and butyric acid were observed along with the increased *Bifidobacterium* abundance in healthy-carbohydrate diet groups.<sup>57</sup> In another study, either 200 g of bread containing 6% of barley beta-glucans (beta-glucans bread) as a test diet or 200 g of bread without added beta-glucans as control were provided to a group of individuals either have MetS or have a high risk of developing one. After the dietary intervention, significantly changed levels of SCFAs in feces were observed with elevated propionic acid in the test group (43.2%,  $P = 0.045$ ).<sup>25</sup> Meanwhile, significantly increased *Bifidobacterium* spp. and *Akkermansia municipihila* were detected in a subgroup of participants who have strong cholesterol reduction after the beta-glucans bread

Table 2. Summary of dietary interventions induced changes of exhaled breath VOCs in selected clinical trials.

Study design (duration)	Sample size (control/test)	Age (control/test)	Gender (M/F)	Health condition	Daily dietary intervention (duration)	Sample collection	Analytic tools	Results in test group (P value)	References
A randomized, double-blind, placebo-controlled, parallel study	Young (25/27) and elderly (24/24)	Young (22.8 ± 4.1/23.4 ± 4.5) and elderly (69.8 ± 2.4/69.5 ± 3.2)	M/F	Healthy	Either 15 g of sugar beet pectin or maltodextrin as control (4 weeks)	Exhaled breath, breath was collected into a 3 L Tedlar bag	GC-TOF-MS	No significant difference in acetic, butyric, isobutyric, isovaleric, propionic, and valeric acids <sup>85</sup>	
Randomized	22	12.27 ± 3.17/10.55 ± 3.98	M/F	Epilepsies	One of MAD, cKD, or non-KD (exhaled breath, needle trap)	Exhaled breath, needle trap	GC/MS	2-methylfuran, 2-pentanone, acetaldehyde, acetone, methyl-vinyl-ketone† (**), in KD groups, whereas ethanol† (**) in non-KD groups <sup>84</sup>	
Randomized cross-over (4 weeks)	7	54.3 (38–61 years)	M/F	Healthy with gastrointestinal symptoms	Run-in period (4 weeks): 100% WW daily 6–10 slices (20–25 g/slice) High-fiber test periods (2 weeks): either WGR or WW + BRB) daily 6–10 slices (25–30 g/slice)	Exhaled breath, breath analyses were done using the ChemProVR 100i online analyzer	Electronic nose based on AIMS	Data not shown (NS) <sup>87</sup>	
Randomized cross-over (7 days)	7	32 (25 to 46)	M	Healthy	High fiber diet (44 g/day) and low fiber diet (17 g/day)	Exhaled alveolar breath (150 mL)	SPME-GC-MS	Ethanol, 1-propanol, acetoin, propionic acid, and butyric acid † (NS) and acetone, 1-butanol, diacetyl, and phenol † (NS) <sup>86</sup>	

M: male; F: female; MAD: modified Atkins diet; cKD: classic KD; KD: ketogenic diets; WW: white wheat bread; WGR: whole grain rye bread; BRB: bioprocessed (fermented) rye bran; GC: gas chromatography; TOF: time-of-flight; MS: mass spectrometry; AIMS: aspiration ion mobility technology; SPME: solid-phase microextraction; NS: no significance. \* \*\*, and \*\*\* indicate significant difference at  $P < 0.05$ , 0.01, and 0.001, respectively.

intervention.<sup>25</sup> These results showed that even though a large cohort study may generate a statistically meaningful dataset, a more personalized and precise nutritional intervention that relying on multiple layers of information needs to be tailored to ensure effectiveness.

In other cases, interventions with sodium butyrate and inulin-type fructans that resulted in the decreased SCFAs contents were observed, despite the increased values of some detected bacteria.<sup>55,58</sup> For instance, Salazar *et al.* reported that the significantly decreased levels of acetic acid, propionic acid, and caproic acid were detected in obese people after ingesting 16 g of inulin-type fructans than the observed levels in those who served with 16 g of maltodextrin as controls.<sup>58</sup> Interestingly, however, they did detect a richer abundance of *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, and *Bifidobacterium adolescentis* in the intervention groups.<sup>58</sup>

More complicated, in another study by Fava *et al.*, different combinations among high saturated fat (HS), high monounsaturated fat (HM), high glycemic index (HGI), low glycemic index (LGI), and high carbohydrate (HC) were prepared as HS, HM/HGI, HM/LGI, HC/HGI, or HC/LGI diets, and one of these five was served to those who are in high risk of developing MetS.<sup>59</sup> The fecal SCFA analysis revealed significantly increased acetic acid, propionic acid, and butyric acid levels after the HS diet. Moreover, changes in microbial population were closely connected to the types of diet, for example, considerably richer levels of *Bifidobacterium* and *Bacteroides* were detected in HC and HC/HGI groups, respectively, as well as increased abundance of *Faecalibacterium prausnitzii* was observed in both HC/LGI and HS diets.<sup>59</sup>

Overall, most of these changes in gut bacteria in Table 1 were considered as results of experimental diet intakes, and the production of SCFAs, such as acetate, butyrate, and propionate, were also observed at relatively higher levels compared with control groups. However, exceptions that did not fall into such a pattern also exist. For example, a galactooligosaccharides intervention rendered an increased number of *Bifidobacterium*, but no significant difference of SCFAs was reported among the detected compounds.<sup>56</sup> Similarly, enhanced levels of *B. longum*, *B. pseudocatenulatum*, and *B. adolescentis* were found in a group of participants after the inulin-type fructans-based dietary intervention, with substantially reduced contents of acetate, propionate, and caproate were detected in the same group.<sup>58</sup> Taken together, it is clear that many further studies are needed to deeply understand the interaction between the gut microbiome and the SCFA productions, and their implications in the prevention and treatment of several systemic metabolic abnormalities related to human health.<sup>72</sup>

## The impact of dietary intervention on human volatile

Despite extensive researches have focused on blood metabolite biomarkers, their clinical translations remain challenging, therefore research efforts have been continued by many scientists and physicians in this area. In this context, interests in the metabolic analysis have been shifted to

breathomics studies, which are defined as a special branch of metabolomics to detect VOCs in the breath of individuals as potential biomarkers for screening and detecting diseases.<sup>83</sup> Specific profiles of volatile compounds in exhaled breath, and metabolites in EBC (breathprints) have been proposed to be potentially useful surrogate markers of inflammatory respiratory diseases.<sup>15</sup> In this mini-review, we identified several studies of exhaled breath analysis that focused on whether and how various types of diets served to those who are healthy or with disorders, such as epilepsies and gastrointestinal symptoms, can change the metabolic patterns in these groups of people (Table 2).

A study by Ruzsanyi *et al.* examined the ketogenic diets (KDs), which generally comprise high-fat, low-carbohydrate, and moderate-protein intakes, for their potential beneficial effects on seizures of children who were diagnosed with epilepsies.<sup>84</sup> To analyze VOCs' profiles, the breath samples were collected after the participants were served with a modified Atkins diet (MAD), a classic KD, or a non-KD, and these breath VOCs were analyzed by GC-MS using a needle trap. In total, 18 VOCs were identified and 6 exhaled VOCs were considerably altered, including elevated levels of 2-methylfuran, 2-pentanone, acetaldehyde, acetone, and methyl-vinyl-ketone in KD groups, whereas the increased concentration of ethanol was observed in non-KD groups.<sup>84</sup> This clinical trial also found that KD-induced breath VOC changes, such as acetone, may be involved in  $\beta$ -oxidation. Taken together, this study suggested that the analysis of exhaled VOCs can be a promising technique for exploring a variety of potential biomarkers for dietary interventions. However, as summarized in Table 2, some studies which analyzed breath samples post food ingestions, such as sugar beet pectin and high fiber diets (HFDs), did not result in statistically significant changes in VOC profiles.<sup>84–86</sup> For example, the impact of sugar beet pectin intervention on young and elderly adults in a randomized, double-blind, placebo-controlled parallel design was assessed to understand its role in healthy aging.<sup>85</sup> In total, 100 individuals randomly received either 15 g of sugar beet pectin (young [ $n = 25$ ] and elder [ $n = 24$ ]) or maltodextrin as placebo (young [ $n = 27$ ] and elder [ $n = 24$ ]) twice daily for four weeks. Then, exhaled breath samples were collected into a 3-L Tedlar bag to analyze VOC profiles using a GC-MS.<sup>85</sup> While eventually, no significant difference due to tested diets was reported, this study represented the effort of attempting to reduce aging-related comorbidities via dietary intervention and non-invasive, potentially high-compliance breath analysis. Similarly, seven healthy individuals with gastrointestinal symptoms were provided with 6–10 slices (20–25 g/slice) of 100% white wheat bread daily for a run-in period (4 weeks). Successively, they were served with 6–10 slices (25–30 g/slice) of sourdough whole grain rye bread and wheat bread with bioprocessed, fermented, rye bran daily for two weeks of each diet (a total of four weeks). The alveolar exhaled breath samples (150 mL) were collected and used for detecting signals of aspiration ion mobility spectrometry after whole-grain rye bread diet intervention in comparison with other diets. In contrast to the expected outcome,



there was no significantly different effect of tested diets; however, an individual variation on the breath signals was observed even they were offered with the same diets.<sup>85</sup>

In another study, Raninen *et al.* performed a pilot clinical trial that HFD (44 g/day) and low fiber diet (LFD; 17 g/day) were randomly assigned to seven healthy male subjects for a week.<sup>86</sup> At the end of the diet periods, alveolar exhaled breath samples were taken at fasting state and postprandially 30, 60, and 120 min. In total, 15 VOCs, including acetone, ethanol, 1-propanol, 2-propanol, 1-butanol, acetic acid, propionic acid, butyric acid, valeric acid, isovaleric acid, 2-methylbutyric acid, hexanoic acid, acetoin, diacetyl, and phenol were detected using solid-phase microextraction and GC-MS. Among the screened VOCs, relatively enhanced levels of ethanol, 1-propanol, acetoin, propionic acid, and butyric acid were found in HFD interventional trials as compared to LFD, but without statistical significance.<sup>86</sup> These pilot studies imply that exhaled breath analysis can be a suitable technique for examining diet-induced VOC profiles in fasting and postprandial state simply and efficiently.<sup>86,87</sup> Despite a wide range of VOCs can be screened from exhaled breath samples, a significant difference between tested diets was not always observed, which could be due to various influencing factors, such as sample size, high inter-individual variation, or diet-linked metabolic background. Therefore, standardization of procedures, longitudinal studies, as well as external validation are needed to further develop breathomics into clinical tools with improved sensitivity, accuracy, and specificity.<sup>79,87,88</sup> On the positive note, these exhaled breath analysis studies, while still at their early stage for potential clinic applications, underscored the promising potential of MS-based breathprints analysis as an early screening tool in studying physiological functionality of dietary components to human metabolism.<sup>87</sup>

## Conclusions and future directions

Analyses of plasma/fecal SCFAs and breath VOC profiles have been considered as promising and minimum-invasive/non-invasive tools for evaluating changes in human metabolism in response to a variety of dietary interventions in randomized clinical trials. The biological implications of these small molecular metabolites to the gut microbial metabolism and human metabolic network are a fascinating area of research, which highlighted the need for more comprehensive studies in the future. In this review, we investigated the association between diets and fecal SCFAs in patients with MetS or at high risk of MetS, and diet-induced changes of the gut microbiome and their production of SCFAs are also discussed. Some interesting clinical evidence that proved the usefulness of these metabolite biomarkers in reflecting the effectiveness of investigated dietary interventions was discovered in these microbe-gut-host health nexus studies. In addition, we highlighted the possibility of breath VOCs analysis to examine the dietary intervention effect on human metabolism. However, several factors, including the impact of food components, time, and dose-response of these dietary interventions to these metabolite biomarkers, and the response

stability need to be further investigated between individuals with diverse genetic profiles.

Moreover, the use of many emerging MS-based analytical platforms, such as secondary electrospray ionization (SESI),<sup>26,89,90</sup> proton transfer reaction (PTR),<sup>91</sup> or selected ion flow tube mass spectrometry.<sup>92</sup> have become popular recently. These breath analysis techniques have been reported to have various advantages in facilitating rapid data acquisition based on real-time and online analysis of VOCs.<sup>93</sup> Notably, PTR-MS and SESI-MS have gained attention based on the high sensitivity at trace levels of part per billion (ppb) or a detection limit of 0.2 parts per trillion (ppt), respectively.<sup>89,90,94</sup> This merit enables the determination of potential biomarkers by fast detection and identification of microbial VOCs in the headspace of *in vitro* culture in non-invasive and spontaneously responsive manners.<sup>84,85</sup> Therefore, these recently invented MS-based tools can be well-suited for breathomics analysis, and can be further considered for robust and reproducible analytical measurements in the nutrient intervention trials. Moving forward, we believe plasma/fecal SCFA analysis and human exhaled VOC analysis can be one of the most effective and powerful tools for exploring the impact of dietary intervention on responsive metabolic alteration towards the development of personalized nutrition.

## AUTHORS' CONTRIBUTIONS

Conceptualization, JZ; methodology, JZ and JHJL; formal analysis, JHJL; investigation, JZ and JHJL; resources, JZ; data curation, JHJL; writing—original draft preparation, JHJL; writing—review and editing, JZ; project administration, JZ; funding acquisition, JZ. All authors have read and agreed to the submitted version of the manuscript.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM133510. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## ORCID iD

Jiangjiang Zhu  <https://orcid.org/0000-0002-4548-8949>

## REFERENCES

1. Tabatabaei-Malazy O, Larijani B, Abdollahi M. Targeting metabolic disorders by natural products. *J Diabetes Metab Disord* 2015;14:57
2. Ginter E, Simko V. *Type 2 diabetes mellitus, pandemic in 21st century*. Springer, 2013, pp. 42–50.

3. Senkus KE, Tan L, Crowe-White KM. Lycopene and metabolic syndrome: a systematic review of the literature. *Adv Nutr* 2019;**10**:19–29
4. Pitsavos C, Panagiotakos D, Weinem M, Stefanadis C. Diet, exercise and the metabolic syndrome. *Rev Diabet Stud* 2006;**3**:118–26
5. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, Almeida M, Quinquis B, Levenez F, Galleron N, Gougis S, Rizkalla S, Batto JM, Renault P, Consortium ANRM, Dore J, Zucker JD, Clement K, Ehrlich SD. Dietary intervention impact on gut microbial gene richness. *Nature* 2013;**500**:585–8
6. Cummings DE, Arterburn DE, Westbrook EO, Kuzma JN, Stewart SD, Chan CP, Bock SN, Landers JT, Kratz M, Foster-Schubert KE. Gastric bypass surgery vs intensive lifestyle and medical intervention for type 2 diabetes: the CROSSROADS randomised controlled trial. *Diabetologia* 2016;**59**:945–53
7. Robberecht H, Hermans N. Biomarkers of metabolic syndrome: biochemical background and clinical significance. *Metab Syndr Relat Disord* 2016;**14**:47–93
8. Barazzoni R, Silva V, Singer P. Clinical biomarkers in metabolic syndrome. *Nutr Clin Pract* 2014;**29**:215–21
9. Zhang A, Sun H, Yan G, Wang P, Wang X. Metabolomics for biomarker discovery: moving to the clinic. *Biomed Res Int* 2015;**2015**:354671
10. Perdomo CM, Fruhbeck G, Escalada J. Impact of nutritional changes on nonalcoholic fatty liver disease. *Nutrients* 2019;**11**:677
11. Wan Y, Tong W, Zhou R, Li J, Yuan J, Wang F, Li D. Habitual animal fat consumption in shaping gut microbiota and microbial metabolites. *Food Funct* 2019;**10**:7973–82
12. Liebal UW, Phan ANT, Sudhakar M, Raman K, Blank LM. Machine learning applications for mass spectrometry-based metabolomics. *Metabolites* 2020;**10**:243
13. Lee JHJ, Jayaprakasha GK, Rush CM, Crosby KM, Patil BS. Production system influences volatile biomarkers in tomato. *Metabolomics* 2018;**14**:99
14. Gu H, Carroll PA, Du J, Zhu J, Neto FC, Eisenman RN, Raftery D. Quantitative method to investigate the balance between metabolism and proteome biomass: starting from glycine. *Angew Chem Int Ed* 2016;**55**:15646–50
15. Santini G, Mores N, Penas A, Capuano R, Mondino C, Trove A, Macagno F, Zini G, Cattani P, Martinelli E, Motta A, Macis G, Ciabattini G, Montuschi P. Electronic nose and exhaled breath NMR-based metabolomics applications in airways disease. *Curr Top Med Chem* 2016;**16**:1610–30
16. van der Beek CM, Canfora EE, Kip AM, Gorissen SHM, Olde Damink SWM, van Eijk HM, Holst JJ, Blaak EE, Dejong CHC, Lenaerts K. The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. *Metabolism* 2018;**87**:25–35
17. Jasbi P, Baker O, Shi X, Gonzalez LA, Wang S, Anderson S, Xi B, Gu H, Johnston CS. Daily red wine vinegar ingestion for eight weeks improves glucose homeostasis and affects the metabolome but does not reduce adiposity in adults. *Food Funct* 2019;**10**:7343–55
18. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *mBio* 2019;**10**:e02566–18
19. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015;**11**:577–91
20. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, De Los Reyes-Gavilán CG, Salazar N. Intestinal short chain fatty acids and their link with diet and human health. *Front Microbiol* 2016;**7**:185
21. Den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013;**54**:2325–40
22. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 2012;**336**:1262–7
23. Roy CC, Kien CL, Bouthillier L, Levy E. Short-chain fatty acids: ready for prime time? *Nutr Clin Pract* 2006;**21**:351–66
24. Cook SI, Sellin JH. Review article: short chain fatty acids in health and disease. *Aliment Pharmacol Ther* 1998;**12**:499–507
25. Velikonja A, Lipoglavsek L, Zorec M, Orel R, Avgustin G. Alterations in gut microbiota composition and metabolic parameters after dietary intervention with barley beta glucans in patients with high risk for metabolic syndrome development. *Anaerobe* 2019;**55**:67–77
26. Lee JHJ, Zhu J. Optimizing secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) for the analysis of volatile fatty acids from gut microbiome. *Metabolites* 2020;**10**:351
27. Kasote DM, Jayaprakasha G, Patil BS. Encapsulation of polyphenols: an effective way to enhance their bioavailability for gut health. In: *Advances in plant phenolics: from chemistry to human health*. Washington, DC: ACS Publications, 2018, pp.239–59
28. Miller TL, Wolin MJ. Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. *Appl Environ Microbiol* 1996;**62**:1589–92
29. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014;**12**:661–72
30. Vainio EJ, Muller MM, Korhonen K, Piri T, Hantula J. Viruses accumulate in aging infection centers of a fungal forest pathogen. *ISME J* 2015;**9**:497–507
31. Salonen A, Lahti L, Salojarvi J, Holtrop G, Korpela K, Duncan SH, Date P, Farquharson F, Johnstone AM, Lobley GE, Louis P, Flint HJ, de Vos WM. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J* 2014;**8**:2218–30
32. Louis P, Young P, Holtrop G, Flint HJ. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA: acetate CoA-transferase gene. *Environ Microbiol* 2010;**12**:304–14
33. He J, Zhang P, Shen L, Niu L, Tan Y, Chen L, Zhao Y, Bai L, Hao X, Li X. Short-chain fatty acids and their association with signalling pathways in inflammation, glucose and lipid metabolism. *IJMS* 2020;**21**:6356
34. den Besten G, Gerding A, van Dijk TH, Ciapaitė J, Bleecker A, van Eunen K, Havinga R, Groen AK, Reijngoud DJ, Bakker BM. Protection against the metabolic syndrome by guar gum-derived short-chain fatty acids depends on peroxisome proliferator-activated receptor gamma and glucagon-like peptide-1. *PLoS One* 2015;**10**:e0136364
35. Scoville DK, Li CY, Wang D, Dempsey JL, Raftery D, Mani S, Gu H, Cui JY. Polybrominated diphenyl ethers and gut microbiome modulate metabolic syndrome-related aqueous metabolites in mice. *Drug Metab Dispos* 2019;**47**:928–40
36. Diehl GE, Longman RS, Zhang JX, Breart B, Galan C, Cuesta A, Schwab SR, Littman DR. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. *Nature* 2013;**494**:116–20
37. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunker MK, Bultman SJ. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* 2011;**13**:517–26
38. Jung TH, Park JH, Jeon WM, Han KS. Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway. *Nutr Res Pract* 2015;**9**:343–9
39. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in caco-2 cell monolayers. *J Nutr* 2009;**139**:1619–25
40. Macia L, Thorburn AN, Binge LC, Marino E, Rogers KE, Maslowski KM, Vieira AT, Kranich J, Mackay CR. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. *Immunol Rev* 2012;**245**:164–76
41. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, Kim CH. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* 2015;**8**:80–93
42. Anton A, Ben de Lacy C, Wolfram M, Jochen S, Bogusaw B, Joachim P, Norman R, Terence R. The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva. *J Breath Res* 2014;**8**:034001
43. Shirasu M, Touhara K. The scent of disease: volatile organic compounds of the human body related to disease and disorder. *J Biochem* 2011;**150**:257–66

44. Phillips M, Greenberg J. Ion-trap detection of volatile organic compounds in alveolar breath. *Clin Chem* 1992;**38**:60–5
45. Chen S, Zieve L, Mahadevan V. Mercaptans and dimethyl sulfide in the breath of patients with cirrhosis of the liver. Effect of feeding methionine. *J Lab Clin Med* 1970;**75**:628–35
46. Kaji H, Hisamura M, Saito N, Murao M. Gas chromatographic determination of volatile sulfur compounds in the expired alveolar air in hepatopathic subjects. *J Chromatogr* 1978;**145**:464–8
47. Agnes WB, Joep JBNvB, Jan WD, Agnieszka S, Emile FW, Frederik JvS. The versatile use of exhaled volatile organic compounds in human health and disease. *J Breath Res* 2012;**6**:027108
48. Zhennan W, Chuji W. Is breath acetone a biomarker of diabetes? A historical review on breath acetone measurements. *J Breath Res* 2013;**7**:037109
49. Bell K, Shaw JE, Maple-Brown L, Ferris W, Gray S, Murfet G, Flavel R, Maynard B, Ryrie H, Pritchard B, Freeman R, Gordon BA. A position statement on screening and management of prediabetes in adults in primary care in Australia. *Diabetes Res Clin Pract* 2020;**164**:108188
50. Lindstrom J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson JG, Hemio K, Hamalainen H, Harkonen P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Mannelin M, Paturi M, Sundvall J, Valle TT, Uusitupa M, Tuomilehto J. Finnish Diabetes Prevention Study G. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *Lancet* 2006;**368**:1673–9
51. Norris SL, Carson S, Roberts C. Comparative effectiveness of pioglitazone and rosiglitazone in type 2 diabetes, prediabetes, and the metabolic syndrome: a meta-analysis. *Curr Diabetes Rev* 2007;**3**:127–40
52. Group DPPR. Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: the Diabetes Prevention Program Outcomes Study. *Lancet Diabetes Endocrinol* 2015;**3**:866–75
53. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, Shuai Y, Zhang B, Zhang J, Thompson TJ, Gerzoff RB, Roglic G, Hu Y, Bennett PH. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. *Lancet* 2008;**371**:1783–9
54. Wang DD, Hu FB. Precision nutrition for prevention and management of type 2 diabetes. *Lancet Diabetes Endocrinol* 2018;**6**:416–26
55. Bouter K, Bakker GJ, Levin E, Hartstra AV, Kootte RS, Udayappan SD, Katiraei S, Bahler L, Gilijsma PW, Tremaroli V, Stahlman M, Holleman F, van Riel NAW, Verberne HJ, Romijn JA, Dallinga-Thie GM, Serlie MJ, Ackermans MT, Kemper EM, van Dijk K, Backhed F, Groen AK, Nieuwdorp M. Differential metabolic effects of oral butyrate treatment in lean versus metabolic syndrome subjects. *Clin Transl Gastroenterol* 2018;**9**:155
56. Canfora EE, van der Beek CM, Hermes GD, Goossens GH, Jocken JW, Holst JJ, van Eijk HM, Venema K, Smidt H, Zoetendal EG. Supplementation of diet with galacto-oligosaccharides increases bifidobacteria, but not insulin sensitivity, in obese prediabetic individuals. *Gastroenterology* 2017;**153**:87–97 e3
57. Hald S, Schioldan AG, Moore ME, Dige A, Laerke HN, Agnholt J, Bach Knudsen KE, Hermansen K, Marco ML, Gregersen S, Dahlerup JF. Effects of arabinoxylan and resistant starch on intestinal microbiota and short-chain fatty acids in subjects with metabolic syndrome: a randomised crossover study. *PLoS One* 2016;**11**:e0159223
58. Salazar N, Dewulf EM, Neyrinck AM, Bindels LB, Cani PD, Mahillon J, de Vos WM, Thissen JP, Gueimonde M, de Los Reyes-Gavilan CG, Delzenne NM. Inulin-type fructans modulate intestinal bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clin Nutr* 2015;**34**:501–7
59. Fava F, Gitau R, Griffin B, Gibson G, Tuohy K, Lovegrove J. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome ‘at-risk’ population. *Int J Obes* 2013;**37**:216–23
60. Larsen N, Vogensen FK, Gobel RJ, Michaelsen KF, Forssten SD, Lahtinen SJ, Jakobsen M. Effect of *Lactobacillus salivarius* Ls-33 on fecal microbiota in obese adolescents. *Clin Nutr* 2013;**32**:935–40
61. Vetrani C, Costabile G, Luongo D, Naviglio D, Rivellesse AA, Riccardi G, Giacco R. Effects of whole-grain cereal foods on plasma short chain fatty acid concentrations in individuals with the metabolic syndrome. *Nutrition* 2016;**32**:217–21
62. Hartvigsen ML, Laerke HN, Overgaard A, Holst JJ, Bach Knudsen KE, Hermansen K. Postprandial effects of test meals including concentrated arabinoxylan and whole grain rye in subjects with the metabolic syndrome: a randomised study. *Eur J Clin Nutr* 2014;**68**:567–74
63. Weickert MO, Pfeiffer AF. Metabolic effects of dietary fiber consumption and prevention of diabetes. *J Nutr* 2008;**138**:439–42
64. Post RE, Mainous AG 3rd, King DE, Simpson KN. Dietary fiber for the treatment of type 2 diabetes mellitus: a meta-analysis. *J Am Board Fam Med* 2012;**25**:16–23
65. Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T, Liao W. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017;**15**:73
66. Peng M, Luo H, Kumar V, Kajbaf K, Hu Y, Yang G. Dysbiosis of intestinal microbiota induced by dietary oxidized fish oil and recovery of diet-induced dysbiosis via taurine supplementation in rice field eel (*Monopterus albus*). *Aquaculture* 2019;**512**:734288
67. Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and Colon cancer. *Metabolism* 2012;**61**:1058–66
68. Jenkins DJ, Kendall CW, Axelsen M, Augustin LS, Vuksan V. Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. *Curr Opin Lipidol* 2000;**11**:49–56
69. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009;**58**:1509–17
70. Keenan MJ, Zhou J, McCutcheon KL, Raggio AM, Bateman HG, Todd E, Jones CK, Tulley RT, Melton S, Martin RJ, Hegsted M. Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity* 2006;**14**:1523–34
71. Barczynska R, Slizewska K, Litwin M, Szalecki M, Kapusniak J. Effects of dietary fiber preparations made from maize starch on the growth and activity of selected bacteria from the firmicutes, bacteroidetes, and actinobacteria phyla in fecal samples from obese children. *Acta Biochim Pol* 2016;**63**:261–6
72. Binda C, Lopetuso LR, Rizzatti G, Gibiino G, Cennamo V, Gasbarrini A. Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Dig Liver Dis* 2018;**50**:421–8
73. Bernini LJ, Simao AN, Alfieri DF, Lozovoy MA, Mari NL, de Souza CH, Dich I, Costa GN. Beneficial effects of *Bifidobacterium lactis* on lipid profile and cytokines in patients with metabolic syndrome: a randomized trial. Effects of probiotics on metabolic syndrome. *Nutrition* 2016;**32**:716–9
74. Liu H, Zhang H, Wang X, Yu X, Hu C, Zhang X. The family coriobacteriaceae is a potential contributor to the beneficial effects of roux-en-Y gastric bypass on type 2 diabetes. *Surg Obes Relat Dis* 2018;**14**:584–93
75. Parada Venegas D, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, Harmsen HJ, Faber KN, Hermoso MA. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol* 2019;**10**:277
76. De La Cuesta-Zuluaga J, Mueller NT, Corrales-Agudelo V, Velásquez-Mejía EP, Carmona JA, Abad JM, Escobar JS. Metformin is associated with higher relative abundance of mucin-degrading *Akkermansia muciniphila* and several short-chain fatty acid-producing microbiota in the gut. *Diabetes Care* 2017;**40**:54–62
77. Duncan SH, Louis P, Flint HJ. Cultivable bacterial diversity from the human colon. *Lett Appl Microbiol* 2007;**44**:343–50
78. Han Y, Xiao H. Whole food-based approaches to modulating gut microbiota and associated diseases. *Annu Rev Food Sci Technol* 2020;**11**:119–43
79. Yang Y, Jobin C. Microbial imbalance and intestinal pathologies: connections and contributions. *Dis Model Mech* 2014;**7**:1131–42

80. Mariat D, Firmesse O, Levenez F, Guimaraes V, Sokol H, Dore J, Corthier G, Furet JP. The firmicutes/bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol* 2009;**9**:123
81. Luoto R, Kalliomaki M, Laitinen K, Isolauri E. The impact of perinatal probiotic intervention on the development of overweight and obesity: follow-up study from birth to 10 years. *Int J Obes* 2010;**34**:1531-7
82. Larsen N, Vogensen FK, Van Den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010;**5**:e9085
83. Brusselmans L, Arnouts L, Millevert C, Vandernickt J, van Meerbeeck JP, Lamote K. Breath analysis as a diagnostic and screening tool for malignant pleural mesothelioma: a systematic review. *Transl Lung Cancer Res* 2018;**7**:520
84. Ruzsanyi V, Peter Kalapos M, Schmidl C, Karall D, Scholl-Burgi S, Baumann M. Breath profiles of children on ketogenic therapy. *J Breath Res* 2018;**12**:036021
85. An R, Wilms E, Smolinska A, Hermes GD, Masclee AA, de Vos P, Schols HA, van Schooten FJ, Smidt H, Jonkers DM. Sugar beet pectin supplementation did not alter profiles of fecal microbiota and exhaled breath in healthy young adults and healthy elderly. *Nutrients* 2019;**11**:2193
86. 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**:612-9
87. Raninen K, Lappi J, Kolehmainen M, Kolehmainen M, Mykkanen H, Poutanen K, Raatikainen O. Diet-derived changes by sourdough-fermented rye bread in exhaled breath aspiration ion mobility spectrometry profiles in individuals with mild gastrointestinal symptoms. *Int J Food Sci Nutr* 2017;**68**:987-96
88. Fischer S, Bergmann A, Steffens M, Trefz P, Ziller M, Miekisch W, Schubert JS, Kohler H, Reinhold P. Impact of food intake on in vivo VOC concentrations in exhaled breath assessed in a caprine animal model. *J Breath Res* 2015;**9**:047113
89. Li H, Zhu J. Differentiating antibiotic-resistant *Staphylococcus aureus* using secondary electrospray ionization tandem mass spectrometry. *Anal Chem* 2018;**90**:12108-15
90. Li H, Xu M, Zhu J. Headspace gas monitoring of gut microbiota using targeted and globally optimized targeted secondary electrospray ionization mass spectrometry. *Anal Chem* 2018;**91**:854-63
91. Lindinger W, Jordan A. Proton-transfer-reaction mass spectrometry (PTR-MS): on-line monitoring of volatile organic compounds at pptv levels. *Chem Soc Rev* 1998;**27**:347-75
92. Smith D, Spanel P. Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. *Mass Spectrom Rev* 2005;**24**:661-700
93. Chan DK, Leggett CL, Wang KK. Diagnosing gastrointestinal illnesses using fecal headspace volatile organic compounds. *World J Gastroenterol* 2016;**22**:1639
94. Crespo E, Cristescu SM, de Ronde H, Kuijper S, Kolk AH, Anthony RM, Harren FJ. Proton transfer reaction mass spectrometry detects rapid changes in volatile metabolite emission by *Mycobacterium smegmatis* after the addition of specific antimicrobial agents. *J Microbiol Methods* 2011;**86**:8-15