Check for updates

Molecular Nutrition & Food Research
www.mnf-journal.com

Gut Microbiome Analysis for Personalized Nutrition: The State of Science

Marie-Christine Simon, Christian Sina, Paola G. Ferrario, Hannelore Daniel,* the Working Group "Personalized Nutrition" of the German Nutrition Society*

Whereas most concepts of personalized nutrition (PN) in the past, included genotyping, recent years have brought new approaches that include microbiome analysis to optimize recommendations for diet and lifestyle changes. The new approach, offered by companies, that microbiome analysis provides a real benefit to either more concise recommendations or for increased compliance to PN, is largely lacking scientific validation. Although the microbiome field shows enormous proliferation, it has some major flaws that make its use in the public health domain currently critical. Starting with the quality and representative character of the stool samples, its processing and analysis as well as assembly of metagenome data and the interpretation. Moreover, there is still no consensus of what constitutes a "normal/healthy" microbiome, nor what features characterize a dysbiotic microbiome. And, based on hundreds of individual parameters and environmental factors, the intestinal microbiome shows a huge variability and consequently changing one factor—such as food intake—is likely to have a limited impact in achieving optimized health. The present review intends to summarize the state of consolidated knowledge on human gut microbiome in the context of diet and disease, its key features, and its influencing factors as well as its "add-on" quality for PN offers.

M.-C. Simon,
Department of Nutrition and Food Science
Nutrition and Microbiome
University of Bonn
53115 Bonn, Germany
E-mail: msimon2@uni-bonn.de

C. Sina
Institute of Nutritional Medicine
University Hospital Schleswig-Holstein
23538 Campus Lübeck, Germany

P. G. Ferrario
Department of Physiology and Biochemistry of Nutrition
Max Rubner-Institut
76131 Karlsruhe, Germany

H. Daniel
Technical University of Munich
85354 Freising, Germany
E-mail: contact@hdaniel.de

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202200476

© 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

DOI: 10.1002/mnfr.202200476

1. Prolog

The ecosystem in the intestine was called intestinal flora till the beginning of the new millennium with the appearance of the term *microbiome* in recognition of its metagenomic dimension. The oldest publication found in PubMed with reference to *intestinal flora* dates to 1914[1] and first publications describing the use of probiotics in humans appeared in 1920.[2] A very productive period of flora-research started in the mid-1950s with studies linking cancers to altered bacterial spectra and by assessing the role of dietary components, bile acids, and other factors in the genesis particularly of colorectal cancer. First studies determining the concentrations of short chain fatty acids as the prime fermentation products in colon appeared also some 50 years ago[1], while studies that for the first time estimated the density of bacteria excreted with the stool in humans were published in the mid-1970s.[3,4] This early research also led to the identification of more than 100 different bacterial species by classical anaerobic culturing techniques.[3] By use of agents that alter the intestinal motility it was also shown that the intestinal transit time affects the density of bacteria in stool samples.[5] This observation was only recently confirmed with the rediscovery of the intestinal transit time as one of the most important determinants of stool microbiome composition.[6,7] This is just one example of the many groundbreaking findings from the heydays of research on the *intestinal flora* that are often neglected. For almost two decades—despite enormous scientific progress—the *intestinal flora* was almost forgotten; only seldomly a reference was given to it as an important biosphere in the health-disease trajectory. That has changed drastically and these days it is hard to keep up with new microbiome science delivered every day. We here review the scientific basis of the diet-microbiome interdependence and the concepts of personalized nutrition that build on or integrate microbiome profiling.

2. Host-Specific Parameters Affecting the Microbiome

Studies on the role of host genetics in defining the diversity of the fecal microbiome have been conducted at the population level

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and in twin cohorts. With both approaches, inheritance of gut microbiome signatures was found to be low—ranging between 2% and 9%—of the variance in genera.[8,9] In a study with >18,300 participants from 24 cohorts, microbiome analysis of fecal samples revealed an impressive variability across the cohorts with only nine genera out of more than 400 consistently detected in all samples.[10] Some 30 genetic host loci were identified as associated with microbiome diversity but only one locus, encoding the intestinal lactase (*LCT*), reached study-wide significance and associated particularly with the abundance of *Bifidobacteria* in stool.[10] Taken together, the genetic make-up of the host seems to have a rather low impact on microbiome composition, but other phenotypic parameters show stronger and more consistent effects and those are sex, age, and BMI that all rank highest among the identified variables of gut microbiome diversity in population studies.[10,11] Besides these easy to obtain anthropometric data, many other phenotypic differences appear to relate to microbiome diversity as well. That includes simple measures of gut function with the frequency and timing of stool passing[12] or the consistency and color of the stool[10] as described by the Bristol Stool Scale (BSS), but those are not often determined in cohort studies.

Other host-specific variables identified in large cohorts as significantly contributing to microbiome diversity are diet, medication, occupational status, ethnicity, birth mode, socio-economic status, or geographical location.[13] For assessment of dietary factors as determinants of stool microbiome diversity, 24 h recall or food frequency questionnaires are used. Although these methods are prone to under-/misreporting and may not deliver the information at a level that one would like to have, they are the basis of almost all population studies that assess diet effects. In microbiome studies with reported food intake dozens of individual food items among many other variables associated with microbiome diversity have been identified. However, each of these individual dietary parameters has only minimal influence. In a cohort of 1135 participants (Netherlands/Belgium) a total of 126 factors significantly associated with microbiome diversity and thereof around 60 food/diet items were found, but those 126 factors altogether explained only 18.7% of the variance.[10] In a population study with 3400 participants 72 determinants significantly associated with microbiome diversity were found and among them 10 were food/dietary factors, each one individually explaining 0.2 to max. 2% of the observed diversity (Shannon diversity).[11] When taken together, all studies conducted on the human gut microbiome and its determinants currently explain around only 15–20% of the inherent variance.[14] As a yet undefined but seemingly accepted measure of a "healthy microbiome" serves currently a large diversity of species in a stool sample. This diversity varies considerably between samples from "industrialized" and "non-industrialized" societies, including pre-historic samples.[15] That leaves space for speculation and interpretation but even here, very basic aspects seldomly get addressed. This includes the fact that for example stool volumes and frequencies are quite different[12] between low income and high income populations[16] and samples from "non-industrialized" or rural domains almost all contain numerous parasites as demonstrated in various studies and parasitic infections have been proven to increase species diversity.[17,18] That may put in question of whether a higher diversity is indeed the best parameter or the prime target.[19] The lack of an accepted definition of a "healthy microbiome"[14] addresses of course what guidance can be provided to humans or patients to affect the microbiome for improving or maintaining health and well-being and that applies to recommendations at population level or in the context of personalized nutrition strategies.

3. Microbiome Analysis from Stool Samples—Approaches and Drawbacks

Over the years, methods and algorithms for the analysis of gut microbiota have been developed and are constantly improved, yet they are still prone to errors. A major problem is that there is no standardized procedure for analysis,[20] and there are often major differences in profiles when the same sample is analyzed in different laboratories.[21] Thus, results from studies are hardly comparable. Furthermore, analytics often lack proper controls to detect false positive or false negative read-outs.[22] When analyzing stool samples for microbiota composition, many individual steps are required and each one is prone to errors. At the experimental level, a proper study design, careful sample collection, and handling and preparation for sequencing are most critical for outcome. During subsequent computer analysis, difficulties can arise in quality control, in assembly of sequences, and their taxonomic or functional assignment. Any error at any of these individual steps affects subsequent analysis and thus the overall result.[20,21] **Figure 1** summarizes the critical steps in the workflow at which data obtained may be affected by the corresponding procedure or, technique/method. In summary, a lack of a time- and cost-efficient standardized method contributes to limited comparability of results from different studies. Controls should be used in all steps of the analysis, if possible, to reduce potential biases. However, there are still no simple solutions for this at all stages of the analysis.

Usually, the dataset generated delivers only relative abundance of the bacteria, i.e., the ratio of the detected bacterial species/genera to each other. This approach however ignores the absolute number of bacteria and thus interpretation of these data could potentially be misleading.[23] For example, an intervention that doubles bacterial species A and leaves bacterial species B unchanged may have the same effect on the relative ratio of species to each other as an intervention that reduces bacterial species B by half and leaves bacterial species A unchanged. In absolute terms, however, the outcome would be different.[23,24] Relative abundance therefore makes it difficult to identify the bacteria that are truly affected by an intervention or disease state[25] but it also limits interpretation, for example in association studies with metabolite concentrations.[26] Analysis of relative abundance does also not take into account the different bacterial load in stool samples between subjects that can vary up to 10-fold.[26] Furthermore, insufficient sequencing depth can lead to zero/non-detectable relative abundance.[23] In bioinformatics analysis, this depth is also a major confounder when calculating distance or dissimilarity indices.[24]

There are already some methods that can be used to detect absolute bacterial load. These include fluorescence spectroscopy, flow cytometry, spike-in with internal reference material, 16S-qPCR (quantitative polymerase chain reaction), 16S-qRT-PCR (reverse transcription qPCR), and ddPCR (droplet digital PCR). However, all methods have advantages and disadvantages.[23] The

 © 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

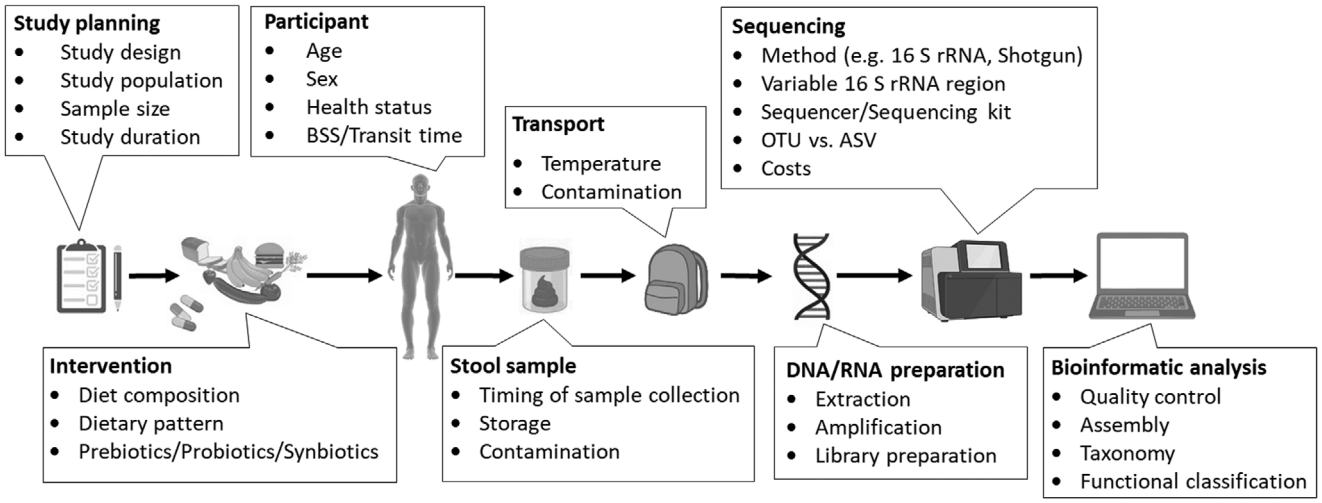

Figure 1. Workflow of human microbiome research. Many individual steps are required for the analysis of stool samples for microbiota composition. Each step is prone to errors, however a proper study design, careful sample collection, handling, and preparation for sequencing are most critical for a valid outcome. During subsequent computer analysis, difficulties can arise in quality control, in assembly of sequences and their taxonomic or functional assignment. Any error at any of these individual steps affects the overall outcome. ASV, amplicon sequence variant; OTU, operational taxonomic units.

selection of the appropriate method depends on the question to be answered, such as of whether a differentiation between living and dead cells is required or whether there is a need for absolute quantification of specific taxa for diagnostic purposes. Absolute quantification of low biomass bacterial samples as well quantification when analyzing large number of samples are hurdles.[23] It should also be considered that the quantification method applied may lead to different quantitative bacterial profiles and thus bias the results.[25] Taken together, absolute quantification appears necessary, when microbiome analysis is used as a diagnostic tool, but currently available methods are all time-consuming and laborious[23] and may—dependent on method—lead to different results.[25]

Specific difficulties for data interpretation arise when assigning biological pathways to the detected bacteria. This is generally done based on the homology to reference genes with previously characterized functions.[27] The reconstruction is often used to further classify the data in a biological context for a meaningful interpretation.[28] Yet, this is still a challenge since it is difficult to define all metabolic pathways encoded by a genome bec different pathways can have the same biological function, overlap, or interfere with each other. In addition, a single protein can perform different functions, or a protein could be assigned to homologous proteins with different functions.[27] However, new approaches are being constantly developed in this area as well.[27,28]

4. What is a Healthy Microbiome?

Already in the early days of gut flora research it was suspected that gut microorganisms are involved in host health.[29] Meanwhile it has been shown for a large number of diseases that there are compositional differences in the microbiota compared to healthy individuals and these may translate as well into functional differences.[30] Observed changes in microbiome composition are frequently described as "dysbiosis", suggesting that there is either an expansion in the density of harmful microorganisms, a reduction of microbes considered as beneficial and/or an overall decrease of microbial diversity.[31] Moreover, it is postulated that novel probiotics or prebiotics provide new treatment options to correct such disturbed ecosystems.[32] Although widely used in the scientific and public domain, there is no consensus on what constitutes a "healthy" microbiome and which deviations define a dysbiosis.[14] The human microbiome project which started in 2007 was not able to define a "healthy core microbiome" by collecting data from average healthy individuals with microbial species, their genes and their derived metabolites. And even 15 years and thousands of publications later a "healthy microbiome" cannot be defined.[31] The Microbiome is are shaped by a combination of genetic, environmental, and lifestyle factors starting with the mode of birth and constantly changing throughout life.[33] This dynamic complicates the identification of essential microbial components relevant for host health and health-dependent alterations of microbiome composition. There is in general not sufficient knowledge about the relationship between gut microorganisms and host health beyond the observed associations. Moreover, in humans it is still difficult to assess whether a possible microbiome shift is the cause, or the consequence of a disease and it has been stated that the concept of a "healthy" microbiome is a prescientific assumption at the current state of research.[31,34]

5. Personalized Nutrition Concepts Based on or Including Microbiome Information

In parallel to the concept of "precision medicine" that includes microbiome analysis,[35] there is increasing interest in microbiome signatures for predicting individual responses to food. The individual microbiome can metabolize identical food components differently and thereby induce different metabolic responses in the host.[36] For example, it has been shown that obese individuals with low microbial gene richness (low microbiota diversity) in their initial fecal microbiota showed a greater

© 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

microbiota response in terms of gene richness to an energy restricted high protein diet compared to obese individuals represented by high microbial gene richness in the pre-treatment microbiota.[37]

Although the concept of enterotypes, i.e., the hypothesis that all humans can be divided into only a few distinct groups based on their core microbiome, is open to discussion,[38,39] there are interesting data in this context that show a correlation between enterotypes and diet. In specific, interindividual differences in food responses related to the microbiome have also been described at the enterotype level.

Among others, it was shown that the *Prevotella* enterotype associated with the intake of complex carbohydrates and had lower glucose levels under a fiber-rich dietary intervention than individuals with a Western diet and a *Bacteroides* enterotype.[40] Low fasting glucose levels are generally considered as a good indicator of metabolic health and it is thus not surprising that a higher *Prevotella*:*Bacteroides* (P:B) ratio was associated with significantly higher weight loss under a high-fiber diet (mean difference: 5.1 kg (95% CI, 1.7;8.6, $p = 0.003$).[41] A validation study confirmed the observation that overweight study participants with an elevated P:B ratio lost more weight on average than subjects with a low P:B ratio during a 24-week low-calorie, high-fiber diet. In addition, this study also revealed evidence of the predictive quality of microbiome diagnostics for the outcome of a prebiotic diet.[41] Subsequently, through deeper sequencing approaches, the *Prevotella* enterotype was shown to consist of at least four distinct clades with substantial functional diversity and differences including the ability to degrade whole-grain fiber.[42] Also, the bacterial species *Eubacterium ruminantium* and *Clostridium felsineum* appear to have a role in the response of a prebiotic nutritional intervention in the context of a weight loss diet,[43] although meaningful prospective studies on the diagnostic value of a species-specific approach are still largely lacking.

In the context of individual responses to food and different microbiome signatures, the study by Zeevi et al. received much attention as the group predicted individual postprandial glucose responses to different food items by machine learning based on multidimensional data.[44] What is remarkable about this study is on the one hand the high number of subjects (800 subjects in the main cohort and 100 subjects in the validation cohort) and the large amount and variety of data collected. Continuous glucose monitoring (CGM) was used to record the postprandial glucose response of over 45,000 meals, including standardized test meals. A comprehensive food database with nutritional values was also integrated into the used study app. The glycemic response of each meal was calculated by combining reported mealtime with CGM data and computing the incremental area under the glucose curve for a 2 h time period after the meal. In addition to medical history and anthropometry, 16sRNA and metagenomics analysis of stool and other blood tests (including HbA1c% and HDL cholesterol) were performed. The results showed not only a high variability of the interindividual postprandial glucose responses to identical test meals among the participants, but also a relatively low accuracy of the prediction of the postprandial response by sole consideration of the number of calories or the carbohydrate portion of a meal with Pearson correlation coefficients of $r = 0.33$ and $r = 0.38$, respectively.

The prediction improved considerably after applying a machine learning glycemic response algorithm based on the data collected, including 16sRNA microbiome data. This increased the goodness of prediction of postprandial glucose response by a factor 2 for the correlation coefficient r ($r = 0.68$ main cohort and $r = 0.7$ validation cohort) and by a factor 4 for R squared, which was of similar quality as the prediction result of dietary counseling by experienced dieticians in a direct comparison.[44] Analogously, the glycemic response prediction algorithm was applied in a study investigating the effect of a prediction-based diet versus a Mediterranean diet. For this purpose, 225 subjects with prediabetes received either personalized dietary recommendations based on a glycemic response prediction algorithm or standardized dietary recommendations in the sense of a Mediterranean diet over a period of 6 months. The results showed not only a reduction in the spike frequency of continuously monitored glucose levels above 140 mg dL^{-1}, which was considered metabolically unfavorable, but also a small but statistically significant reduction in the HbA1c% value.[45] This approach proved to be less successful in a recently published study in which 200 volunteers with obesity and abnormal glucose metabolism underwent a 6-month weight-loss program. Participants were randomized into an arm that followed a classical low-fat diet whereas the other personalized diet group used CGM in combination with an App based on the predictive algorithm developed by Popp et al.[46] Volunteers in both groups lost weight but there was no significant difference in body weight reduction between groups.

How much the microbiome contributes to an individual's postprandial glucose response was also studied by Berry et al.[47] in a similarly complex study design including CGM and app-based food diary by employing 1000 individuals including >400 monozygotic and dizygotic twin pairs. In addition to postprandial glucose response also individual postprandial triglyceride (TG) concentration in response to test meals was recorded. The results of this study also showed large interindividual differences for both postprandial glucose response and serum TG levels after identical test meals. The contribution of various influencing variables (given in % of all) such as of meal composition, nature of the previous meal, genetics, and microbiome was analyzed as well. It was finally shown that the microbiome contributed significant to the different responses in postprandial lipemia, but not in postprandial glycemia. Nevertheless, a machine learning algorithm based on 16sRNA data was able to predict the glycemic responses to food intake relatively accurately.[47] A developed prediction algorithm for postprandial triglycerides appeared not as accurate as the glycemic response-prediction algorithm ($r = 0.47$ vs $r = 0.77$). The fact that the microbiome analysis in this study did not reveal a significant advantage in the glycemic response prediction should not lead to the conclusion that the microbiome has no weight in the machine learning approach. It should be emphasized that the prediction algorithms published by Zeevi et al.[44] and Berry et al.[47] are all based on the integration 16sRNA data and those are often limited in their overall quality (see above).

While Pearson's r, or R squared (squaring the value obtained for Pearson's r) are widely used in PN context as an indicator of model fit,[48] the predictive capability of a statistical model should be further explored in test datasets by cross-validation techniques for possible overfitting and validity. Finally, Zeevi

© 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

et al.[44] and Berry et al.[47] also showed repeatability of the postprandial glucose response after consumptions of the same foods. This means that although having other variables in the model, it should also be possible to relate the foods consumed to their effect on glycemia simply by combining information on the food (meal content and meal timing) with the postprandial glucose response. Whether the determination of microbiome signatures is indeed superior for proper prediction of the individual´s glycemic response cannot be answered, but it has been questioned as a real improvement over conventional methods in detecting hyperglycaemia and for personalized nutrition advice as superior to standard dietary advice to manage high postprandial glucose response.[49]

6. Discussion

With the almost explosive developments in microbiome research, the intestine, and the role of the diet in intestinal and overall health has gained attention in the academic world and the public domain not seen in decades. In addition, in many diseases changes in the bacterial composition based on 16sRNA profiling have been found in stool samples and the microbiota is becoming a target for intervention or even treatment of diseases.[50–52] Despite the fact that individual bacterial strains or phyla have been identified as either detrimental or protective for intestinal diseases or overall health, due to the analytical constraints the vast majority of studies on microbiome composition lack quantitative information on the species identified. Quantitative information however is a dogma in clinical laboratory measurements and with defined threshold levels also for medical intervention. The current state of microbiome analysis, however, provides almost exclusively relative abundance data (see above) and those cannot hold up to the standards of clinical analysis required otherwise.

Despite all current limitations in microbiome analysis and quality assurance there is a growing number of commercial offers in the direct to customer service environment. Offers include either dietary/lifestyle recommendations or even products with some level of individualization as for example a muesli or a micronutrient supplement. In particular the studies by Zeevi et al.[44] and Berry et al.[47] brought a number of new commercial services to the markets in which microbiome analysis is combined with glucose sensors as a new health-management approach. What makes such offers attractive is that interstitial glucose can be monitored continuously via Bluetooth-coupled devices and any change in carbohydrate intake towards products with low glycemic load or low glycemic index provides an immediate positive feed-back to the individual and that is likely a key factor for high compliance.

Most traditional PN offers provide advice or products based on statistical or epidemiological evidence and communicate relative disease risks and those are usually all long-term. Volunteers usually seek short-term measures of success for any dietary/lifestyle intervention and to have a reduced disease-risk in the future is not the most motivating parameter. That is one of the reasons that weight loss—easily measured with proper response in decent time—is the most valued parameter in PN offers for customers.[52] Recording continuously interstitial glucose levels is particularly attractive by the ease to measure with the modern minimal invasive devices and upcoming new non-invasive sensors will make it likely a commodity build into smart electronic companions.[53] While the importance of balanced glucose metabolism in the context of metabolic diseases such as obesity and type 2 diabetes mellitus is widely accepted, the relevance of a personalized glucocentric nutrition for other disease entities with complex pathophysiology, such as neurodegenerative diseases or cancers, needs to be shown. Similarly, scientific data have yet to demonstrate whether a glucose centered PN leads to better health maintenance in primarily healthy individuals and whether the continuous glucose monitoring is useful beyond high-risk populations or pre-diseased individuals.

One important question however is, of whether there is an underlying biological logic in the association of microbiome signatures in stool samples and blood/interstitial postprandial glucose levels. A recent landmark study by the group of S. Berry[7] sheds light on a likely causal interrelationship. In more than 800 participants gut transit time was measured using a blue dye and data obtained predicted microbiome composition in volunteers with a high correlation coefficient ($r = 0.98$) and associated as well with relative abundance of distinct bacterial species. But transit time also correlated with visceral fat mass and postprandial glucose and triglyceride responses ($r = 0.67$). That transit time and microbial diversity but also bacterial mass in feces is interconnected, is also known[5,54] and stool water content and transit are also closely related and associate thus with microbiome composition.[55] Gastrointestinal transit time is controlled by the enteric nervous system of the gut and the gut intrinsic hormone system receives numerous input signals from G-protein-coupled receptors and other sensors to adjust transit time to digestive and absorptive capacity. This is schematically displayed in **Figure 2** in the context of a carbohydrate-rich meal with the physiological responses of the gut and peripheral organs connected mainly via the incretins GIP (gastric insulinotrophic peptide), GLP-1 (glucagon-like peptide 1) and PYY (Peptide YY). These hormones critically control gastrointestinal motility and glucose-dependent insulin secretion and thus glycemic responses to carbohydrate intake. But they simultaneously also affect microbiome composition via the different substrate load that passes from ileum into colon for utilization by bacteria. The highest density of GLP-1 and PYY producing cells is found in ileum and colon and the amount of non-absorbed food/calories reaching the terminal ileum elicits the so-called *ileal break*[56] that causes mainly via GLP-1 and PYY a slow-down of gastric emptying and transit through the small intestine and thus a reduction of substrate load for fermentation and growth of bacteria in colon.

That gastrointestinal transit time is a determinant in postprandial glucose responses has been demonstrated by use to prokinetic agents and compounds that reduce motility[57], which simultaneously alter the bacterial mass in feces[5] and change the substrate load for fermentation as shown in ileostomy patients.[58] What also needs to be considered is that high blood glucose changes gastric emptying of liquid or solid food and reduces for example bile acid output by altered bladder contraction.[59] Changes in the motility of the gastrointestinal tract leading to changes transit time are well known to occur in obesity as well as a variety of diseases including diabetes and others.[60,61] Observed changes in microbiome composition in patients suffering from such diseases could thus have impaired gut motility as a common denominator with the consequence of

 © 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

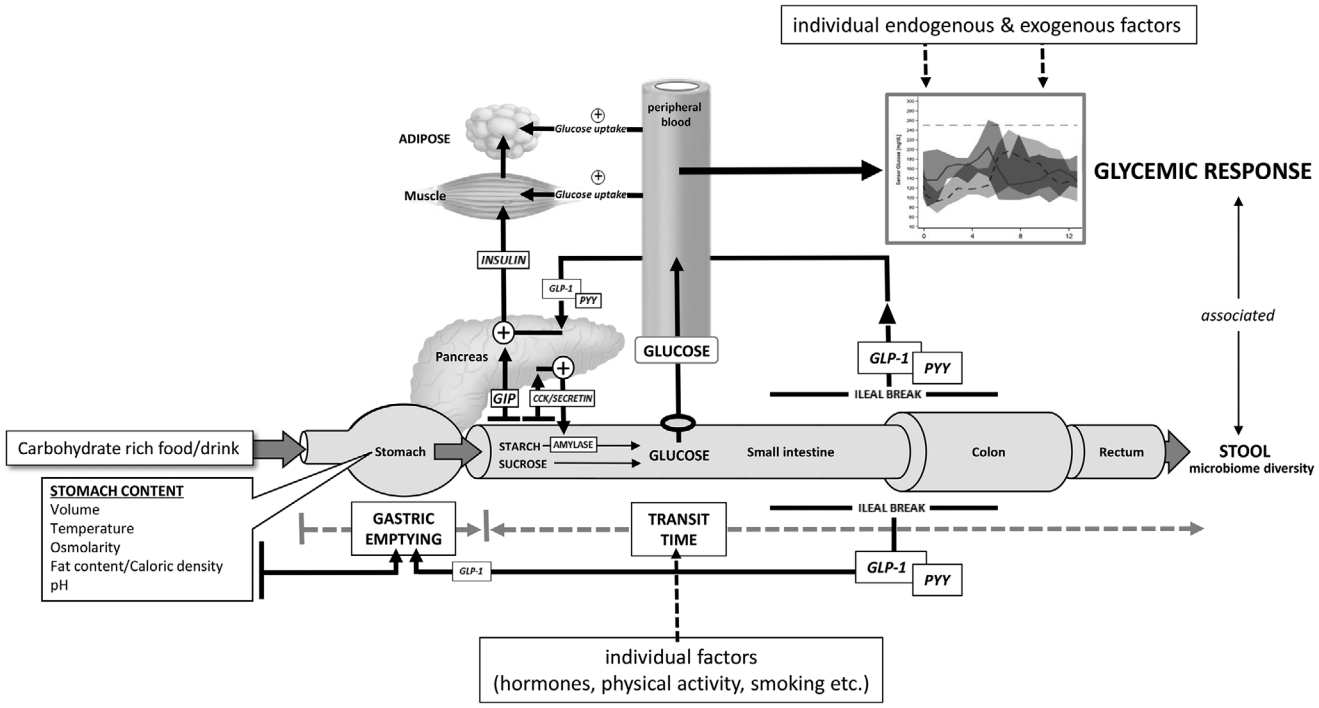

Figure 2. Schematic representation of the physiological responses to a carbohydrate-rich meal in the gut and peripheral tissues and how gastric emptying and small intestinal transit time are controlled via the ileal break with secretion of gastrointestinal peptide hormones that affect simultaneously both, postprandial glycemic responses and stool microbiome quantity and quality. This interplay provides a plausible biological link for the observed association of microbiome and individual glycemic responses that form the basis for some novel approaches in PN. PN, personalized nutrition.

changes in microbiome composition. At this point it becomes obvious that assessing normal gastrointestinal physiology and the differences between individuals is central to understanding the effects of diets and other environmental factors but also of diseases on microbiota quantity and quality. Unfortunately, not many studies collect data on gut functions such as transit time,[6] amount and appearance of feces, or number of defecations per day or week.[12,62] Each of those variables has been associated independently with distinct microbiome signatures.

Given the huge variance in fecal microbiome patterns and an even great variance in the same individual with day-by-day variation,[63] the question of the validity of an analysis—usually based on a single sample—arises. Moreover, based on lack of a definition of what constitutes a "healthy microbiome," interpretation of data obtained from a stool sample requires caution and communication should emphasize that the findings may not be representative for the individual. In the framework of PN, microbiome analysis from a single undefined stool sample is thus scientifically not valid enough to base on the relative abundance data concise recommendation for alterations towards a "healthier" microbiome. Moreover, there are currently no dietary interventions that have proven to cause major changes in the microbiota—and even extreme diets[64] or proper doses of fermentable fibers were shown to have only marginal effects.[26,65] Generated changes are usually limited to very few species/phyla of bacteria[66] and whether those translate into a health-promoting and lasting beneficial effect needs to be demonstrated.

Taken together, the approaches that combine microbiome analysis with continuous glucose monitoring via sensors have likely as underlying causal factor in the association of glycemic responses and microbiome diversity an individual gastrointestinal motility signature. The microbiome in its composition may thus serve as a reporter of an individual´s gastrointestinal phenotype that also may determine or contribute to the phenotypic responses to a meal and in postprandial glycemia. Recording glycemic alterations over extended periods with the new devies is easy and can provide immediate success when dietary changes translate into diminished glucose levels. Although recognizing the important role of flattened glucose profiles and lower fasting levels for metabolic risk groups or pre-diseased individuals these parameters are not the surrogate of overall health. Whether PN offers that include or are solely based on microbiome data can improve consumer compliance to change in long-term the individual´s eating behavior and health trajectory, awaits scientific proof.

Acknowledgements

The work of M.C.S. is supported by funding of the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF, grant number: 01EA1707). The authors would like to thank the other members of the Working Group "Personalized Nutrition" of the German Nutrition Society: Janine Bröder, Anette Buyken, Kurt Gedrich, Christina Holzapfel, Michael Leitzmann, Jakob Linseisen, Stefan Lorkowski, Britta Renner, Melanie Waldenberger, Bernhard Watzl, Jan Wirsam.

Conflict of Interest

The authors declare no conflict of interest.

© 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Author Contributions

H.D. prepared the first draft of the manuscript, which was subsequently finalized in close collaboration with M.C.S.; C.S. and P.G.F. All authors provided substantial content contributions and edited the manuscript; H. D. and M.C.S. created and edited the figures. All authors have read and approved the final manuscript.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Keywords

evidence, glucose monitoring, gut, microbiome, personalized nutrition

Received: July 18, 2022
Revised: October 17, 2022
Published online: November 24, 2022

[1] J. Leidy, *Science* **1914**, *40*, 302.
[2] H A. Cheplin, L F. Rettger, *Proc. Natl. Acad. Sci. USA* **1920**, *6*, 704.
[3] W. E. C. Moore, L V. Holdeman, *Appl. Microbiol.* **1974**, *27*, 961.
[4] L. V. Holdeman, I. J. Good, W. E. Moore, *Appl. Environ. Microbiol.* **1976**, *31*, 359.
[5] A. M. Stephen, H. S. Wiggins, J. H. Cummings, *Gut* **1987**, *28*, 601.
[6] H. M. Roager, L. B. S. Hansen, M. I. Bahl, H. L. Frandsen, V. Carvalho, R. J. GÃ¸Bel, M. D. Dalgaard, D. R. Plichta, M. H. Sparholt, H. Vestergaard, T. Hansen, T. Sicheritz-Pontén, H. B. Nielsen, O. Pedersen, L. Lauritzen, M. Kristensen, R. Gupta, T. R. Licht, *Nat. Microbiol.* **2016**, *1*, 1.
[7] F. Asnicar, E. R. Leeming, E. Dimidi, M. Mazidi, P. W. Franks, H. Al Khatib, A. M. Valdes, R. Davies, E. Bakker, L. Francis, A. Chan, R. Gibson, G. Hadjigeorgiou, J. Wolf, T. D. Spector, N. Segata, S. E. Berry, *Gut* **2021**, *70*, 1665.
[8] J. K. Goodrich, J. L. Waters, A. C. Poole, J. L. Sutter, O. Koren, R. Blekhman, M. Beaumont, W. Van-Treuren, R. Knight, J. T. Bell, T. D. Spector, A. G. Clark, R. E. Ley, *Cell* **2014**, *159*, 789.
[9] D. Rothschild, O. Weissbrod, E. Barkan, A. Kurilshikov, T. Korem, D. Zeevi, P. I. Costea, A. Godneva, I. N. Kalka, N. Bar, S. Shilo, D. Lador, A. V. Vila, N. Zmora, M. Pevsner-Fischer, D. Israeli, N. Kosower, G. Malka, B. C. Wolf, T. Avnit-Sagi, M. Lotan-Pompan, A. Weinberger, Z. Halpern, S. Carmi, J. Fu, C. Wijmenga, A. Zhernakova, E. Elinav, E. Segal, *Nature* **2018**, *555*, 210.
[10] A. Zhernakova, A. Kurilshikov, M. J. Bonder, E F. Tigchelaar, M. Schirmer, T. Vatanen, Z. Mujagic, A. V. Vila, G. Falony, S. Vieira-Silva, J. Wang, F. Imhann, E. Brandsma, S A. Jankipersadsing, M. Joossens, M. C. Cenit, P. Deelen, M. A. Swertz, R. K. Weersma, et al., *Science* **2016**, *352*, 565.
[11] O. Manor, C. L. Dai, S. A. Kornilov, B. Smith, N. D. Price, J. C. Lovejoy, S. M. Gibbons, A. T. Magis, *Nat. Commun.* **2020**, *11*, 5206.
[12] F. Hadizadeh, S. Walter, M. Belheouane, F. Bonfiglio, F. A. Heinsen, A. Andreasson, L. Agreus, L. Engstrand, J. F. Baines, J. Rafter, A. Franke, M. D'amato, *Gut* **2017**, *66*, 559.
[13] V. K. Gupta, S. Paul, C. Dutta, *Front. Microbiol.* **2017**, *8*, 1162.
[14] F. Shanahan, T. S. Ghosh, P. W. O'Toole, *Gastroenterology* **2021**, *160*, 483.
[15] M. C. Wibowo, Z. Yang, M. Borry, A. Hubner, K. D. Huang, B. T. Tierney, S. Zimmerman, F. Barajas-Olmos, C. Contreras-Cubas, H. Garcia-Ortiz, A. L. Martínez-Hernández, J. M. Luber, P. Kirstahler, T. Blohm, F. E. Smiley, R. Arnold, S. A. Ballal, S. J. Pamp, J. Russ, F. Maixner, O. Rota-Stabelli, N. Segata, K. Reinhard, L. Orozco, C. Warinner, M. Snow, S. Leblanc, A. D. Kostic, *Nature* **2021**, *594*, 234.
[16] C. Rose, A. Parker, B. Jefferson, E. Cartmell, *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 1827.
[17] M. A. Toro-Londono, K. Bedoya-Urrego, G. M. Garcia-Montoya, A. L. Galvan-Diaz, J. F. Alzate, *PeerJ* **2019**, *7*, e6200.
[18] M. A. Rubel, A. Abbas, L. J. Taylor, A. Connell, C. Tanes, K. Bittinger, V. N. Ndze, J. Y. Fonsah, E. Ngwang, A. Essiane, C. Fokunang, A. K. Njamnshi, F. D. Bushman, S. A. Tishkoff, *Genome Biol.* **2020**, *21*, 122.
[19] F. Shanahan, C. Hill, *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 387.
[20] R. Bharti, D. G. Grimm, *Brief Bioinform.* **2021**, *22*, 178.
[21] D. Vandeputte, R. Y. Tito, R. Vanleeuwen, G. Falony, J. Raes, *FEMS Microbiol. Rev.* **2017**, *41*, S154.
[22] B. V. H. Hornung, R. D. Zwittink, E. J. Kuijper, *FEMS Microbiol. Ecol.* **2019**, *95*, fiz045.
[23] X. Wang, S. Howe, F. Deng, J. Zhao, *Microorganisms* **2021**, *9*, 1797.
[24] G. B. Gloor, J. M. Macklaim, V. Pawlowsky-Glahn, J. J. Egozcue, *Front. Microbiol.* **2017**, *8*, 2224.
[25] G. Galazzo, N. Van Best, B. J. Benedikter, K. Janssen, L. Bervoets, C. Driessen, M. Oomen, M. Lucchesi, P. H. Van Eijck, H. E. F. Becker, M. W. Hornef, P. H. Savelkoul, F. R. M. Stassen, P. F. Wolffs, J. Penders, *Front. Cell Infect. Microbiol.* **2020**, *10*, 403.
[26] D. Vandeputte, G. Kathagen, K. D'Hoe, S. Vieira-Silva, M. Valles-Colomer, J. O. Sabino, J. Wang, R. Y. Tito, L. De Commer, Y. Darzi, S. V. Vermeire, G. Falony, J. Raes, *Nature* **2017**, *551*, 507.
[27] Y. Ye, T. G. Doak, *PLoS Comput. Biol.* **2009**, *5*, e1000465.
[28] C. S. Magnano, A. Gitter, *NPJ Syst. Biol. Appl.* **2021**, *7*, 12.
[29] F. Guarner, J. R. Malagelada, *Lancet* **2003**, *361*, 512.
[30] A. Vijay, A M. Valdes, *Eur. J. Clin. Nutr.* **2022**, *76*, 489.
[31] C. R. Tiffany, A. J. Bäumler, *Am. J. Physiol. - Gastrointest. Liver Physiol.* **2019**, *317*, G602.
[32] K. Dixit, D. Chaudhari, D. Dhotre, Y. Shouche, S. Saroj, *Life Sci.* **2021**, *278*, 119622.
[33] J. A. Gilbert, M. J. Blaser, J. G. Caporaso, J. K. Jansson, S. V. Lynch, R. Knight, *Nat. Med.* **2018**, *24*, 392.
[34] S. W. Olesen, E. J. Alm, *Nat. Microbiol.* **2016**, *1*, 1.
[35] Xu Zhang, L. Li, J. Butcher, A. Stintzi, D. Figeys, *Microbiome* **2019**, *7*, 154.
[36] N. B. Danneskiold-Samsã¸E, H. Dias De Freitas Queiroz Barros, R. Santos, J. L. Bicas, C. B. B. Cazarin, L. Madsen, K. Kristiansen, G. M. Pastore, S. Brix, Maróstica Júnior MR, *Food Res. Int.* **2019**, *115*, 23.
[37] A. L. Cotillard, S. P. Kennedy, L. C. Kong, E. Prifti, N. Pons, E. Le Chatelier, M. Almeida, B. Quinquis, F. Levenez, N. Galleron, S. Gougis, S. Rizkalla, J. M. Batto, P. Renault, J. Dore, J. D. Zucker, K. Clement, S. D. Ehrlich, H. Blottiã¨Re, M. Leclerc, C. Juste, T. De Wouters, P. Lepage, C. Fouqueray, A. Basdevant, C. Henegar, C. Godard, M. Fondacci, A. Rohia, F. Hajduch, et al., *Nature* **2013**, *500*, 585.
[38] M. Arumugam, J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, G R. Fernandes, J. Tap, T. Bruls, J.-M. Batto, M. Bertalan, N. Borruel, F. Casellas, L. Fernandez, L. Gautier, T. Hansen, M. Hattori, T. Hayashi, M. Kleerebezem, K. Kurokawa, M. Leclerc, F. Levenez, C. Manichanh, H. B. R. Nielsen, T. Nielsen, N. Pons, J. Poulain, J. Qin, T. Sicheritz-Ponten, S. Tims, et al., *Nature* **2011**, *473*, 174.
[39] M. Cheng, K. Ning, *Genomics Proteomics Bioinformatics* **2019**, *17*, 4.
[40] P. Kovatcheva-Datchary, A. Nilsson, R. Akrami, Y. S. Lee, F. De' Vadder, T. Arora, A. Hallen, E. Martens, I. Björck, F. Bäckhed, *Cell Metab.* **2015**, *22*, 971.
[41] M. F. Hjorth, T. L. Q. Bendtsen, J. K. Lorenzen, J. B. Holm, P. Kiilerich, H M. Roager, K. Kristiansen, L. H. Larsen, A. Astrup, *Int. J. Obes.* **2005**, *43*, 149.
[42] A. Tett, K. D. Huang, F. Asnicar, H. Fehlner-Peach, E. Pasolli, N. Karcher, F. Armanini, P. Manghi, K. Bonham, M. Zolfo, F. De Filippis,

© 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

C. Magnabosco, R. Bonneau, J. Lusingu, J. Amuasi, K. Reinhard, T. Rattei, F. Boulund, L. Engstrand, A. Zink, M. C. Collado, D. R. Littman, D. Eibach, D. Ercolini, O. Rota-Stabelli, C. Huttenhower, F. Maixner, N. Segata, *Cell Host Microbe* **2019**, *26*, 666.e7.
[43] K. Korpela, H. J. Flint, A. M. Johnstone, J. Lappi, K. Poutanen, E. Dewulf, N. Delzenne, W. M. De Vos, A. Salonen, *PLoS One* **2014**, *9*, e90702.
[44] D. Zeevi, T. Korem, N. Zmora, D. Israeli, D. Rothschild, A. Weinberger, O. Ben-Yacov, D. Lador, T. Avnit-Sagi, M. Lotan-Pompan, J. Suez, J. A. Mahdi, E. Matot, G. Malka, N. Kosower, M. Rein, G. Zilberman-Schapira, L. Dohnalovãį, M. Pevsner-Fischer, R. Bikovsky, Z. Halpern, E. Elinav, E. Segal, *Cell* **2015**, *163*, 1079.
[45] O. Ben-Yacov, A. Godneva, M. Rein, S. Shilo, D. Kolobkov, N. Koren, N. Cohen Dolev, T. Travinsky Shmul, B. C. Wolf, N. Kosower, K. Sagiv, M. Lotan-Pompan, N. Zmora, A. Weinberger, E. Elinav, E. Segal, *Diabetes Care* **2021**, *44*, 1980.
[46] C. J. Popp, Lu Hu, A. Y. Kharmats, M. Curran, L. Berube, C. Wang, M. L. Pompeii, P. Illiano, D. E. St-Jules, M. Mottern, H. Li, N. Williams, A. Schoenthaler, E. Segal, A. Godneva, D. Thomas, M. Bergman, A. M. Schmidt, M. A. Sevick, *JAMA Netw Open* **2022**, 1e2233760.
[47] S. E. Berry, A. M. Valdes, D. A. Drew, F. Asnicar, M. Mazidi, J. Wolf, J. Capdevila, G. Hadjigeorgiou, R. Davies, H. Al Khatib, C. Bonnett, S. Ganesh, E. Bakker, D. Hart, M. Mangino, J. Merino, I. Linenberg, P. Wyatt, J. M. Ordovas, C. D. Gardner, L. M. Delahanty, A. T. Chan, N. Segata, P. W. Franks, T. D. Spector, *Nat. Med.* **2020**, *26*, 964.
[48] D. Kirk, C. Catal, B. Tekinerdogan, *Comput. Biol. Med.* **2021**, *133*, 104365.
[49] T. M. S. Wolever, *Eur. J. Clin. Nutr.* **2016**, *70*, 411.
[50] R. Trivedi, K. Barve, *Biochem. J.* **2020**, *477*, 2679.
[51] B A. Helmink, M. A. W Khan, A. Hermann, V. Gopalakrishnan, J A. Wargo, *Nat. Med.* **2019**, *25*, 377.
[52] C. Holzapfel, *MMW Fortschr. Med.* **2019**, *161*, 52.
[53] L. Tang, S. J. Chang, C.-J. Chen, J.-T. Liu, *Sensors* **2020**, *20*, 6925.
[54] D. Vandeputte, G. Falony, S. Vieira-Silva, R. Y. Tito, M. Joossens, J. Raes, *Gut* **2016**, *65*, 57.
[55] G. Falony, S. Vieira-Silva, J. Raes, *Nat. Microbiol.* **2018**, *3*, 526.
[56] P. W. J. Maljaars, H. P. F. Peters, D. J. Mela, A. A. M. Masclee, *Physiol. Behav.* **2008**, *95*, 271.
[57] S. Gonlachanvit, C.-W. Hsu, G. H. Boden, L. C. Knight, A. H. Maurer, R. S. Fisher, H. P. Parkman, *Dig. Dis. Sci.* **2003**, *48*, 488.
[58] R. W. Chapman, J. K. Sillery, M. M. Graham, D. R. Saunders, *Am. J. Clin. Nutr.* **1985**, *41*, 1244.
[59] C K. Rayner, M. Samsom, K L. Jones, M. Horowitz, *Diabetes Care* **2001**, *24*, 371.
[60] M. Zhao, D. Liao, J. Zhao, *World J. Diabetes* **2017**, *8*, 249.
[61] J Kw. Mok, J M. Makaronidis, R L. Batterham, *Curr. Opin. Endocr. Metab. Res.* **2019**, *4*, 4.
[62] H. J. Kwon, J. H. Lim, D. Kang, S. Lim, S Ja Park, J. H. Kim, *Intest. Res.* **2019**, *17*, 419.
[63] A. J. Johnson, P. Vangay, G. A. Al-Ghalith, B. M. Hillmann, T. L. Ward, R. R. Shields-Cutler, A. D. Kim, A. K. Shmagel, A. N. Syed, J. Walter, R. Menon, K. Koecher, D. Knights, *Cell Host Microbe* **2019**, *25*, 789.e5.
[64] L. A. David, C. F. Maurice, R. N. Carmody, D. B. Gootenberg, J. E. Button, B. E. Wolfe, A. V. Ling, A. S. Devlin, Y. Varma, M. A. Fischbach, S. B. Biddinger, R. J. Dutton, P. J. Turnbaugh, *Nature* **2014**, *505*, 559.
[65] E. E. Canfora, C. M. Van Der Beek, G. D. A. Hermes, G. H. Goossens, J. W. E. Jocken, J. J. Holst, H. M. Van Eijk, K. Venema, H. Smidt, E. G. Zoetendal, C. H. C. Dejong, K. Lenaerts, E. E. Blaak, *Gastroenterology* **2017**, *153*, 87.e3.
[66] K. J. Portune, A. Benitez-Paez, E. M. G. Del Pulgar, V. Cerrudo, Y. Sanz, *Mol. Nutr. Food Res.* **2017**, *61*, 1600252.
[67] R. Berni Canani, L. Paparo, R. Nocerino, C. Di Scala, G. Della Gatta, Y. Maddalena, A. Buono, C. Bruno, L. Voto, D. Ercolini, *Front. Immunol.* **2019**, *10*, 191.

Marie-Christine Simon performed in-depth human clinical trials during her PhD at the German Diabetes Center, Duesseldorf. She has a very profound knowledge on diabetes-related metabolic and immunologic changes and brings her scientific understanding from her studies of nutrition sciences at the University of Bonn. Furthermore, she is certificated as Clinical Research Associate, University Frankfurt, Germany and as Registered Dietician. She has a strong background on microbiome research which she gained during her post-doctoral training at Fredrik Bäckheds Laboratory at the University Gothenburg. Thus, she aims for mechanistic understanding of the involvement of the microbiome in the human physiology and pathophysiology.

Christian Sina is a medical doctor and specialist in Internal Medicine and Gastroenterology. He is Chair & Professor of Nutritional Medicine, founding director of the Institute of Nutritional Medicine, and study program director "Nutritional Medicine" at the University of Lübeck. He serves as a gastroenterology and clinical nutrition consultant at the 1st Medical Department at the University Hospital Schleswig-Holstein, Campus Lübeck. Dr. Sina's current research interest is in digital therapeutics & personalized nutrition. Recently, Dr. Sina became Co-Founder of perfood GmbH, a company dedicated to developing digital nutritional therapeutics.

© 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Paola G. Ferrario is a biostatistician at the Department of Physiology and Biochemistry of Nutrition at the Max Rubner-Institut, the Federal Research Institute of Nutrition and Food in Germany. She serves as statistical editor in nutritional context. Dr. Ferrario received a best student paper award for her research in the field of nonparametric statistics and a best poster award for her research in the field of metabolomics. Her current research is focused on personalized nutrition.

Hannelore Daniel held the position of a Chair of Physiology of Human Nutrition at the Technical University of Munich (TUM), Germany. Her main research interests are control mechanisms in mammalian metabolism. Hannelore Daniel is a nutrition expert by training and worked in Germany, UK and USA. She is a member of the German National Academy of Sciences Leopoldina and an advisor for national and international funding agencies, ministries, and research centers. Hannelore Daniel has published around 460 scientific papers and reviews and has provided around 500 oral presentations as invited speaker. She received numerous scientific awards including the most prestigious science award in the state of Bavaria.

© 2022 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200476 by Cochrane Germany, Wiley Online Library on [19/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License