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Microbiome metabolite quantification methods enabling insights into human health and disease

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ABSTRACT

Many of the health-associated impacts of the microbiome are mediated by its chemical activity, producing and modifying small molecules (metabolites). Thus, microbiome metabolite quantification has a central role in efforts to elucidate and measure microbiome function. In this review, we cover general considerations when designing experiments to quantify microbiome metabolites, including sample preparation, data acquisition and data processing, since these are critical to downstream data quality. We then discuss data analysis and experimental steps to demonstrate that a given metabolite feature is of microbial origin. We further discuss techniques used to quantify common microbial metabolites, including short-chain fatty acids (SCFA), secondary bile acids (BAs), tryptophan derivatives, *N*-acyl amides and trimethylamine N-oxide (TMAO). Lastly, we conclude with challenges and future directions for the field.

1. Introduction

Metabolomics is defined as the study of small molecules, including those modified, created and consumed by metabolism. Metabolism is affected by factors like diet and activity level, but in various species like humans, the microorganisms that inhabit the host–collectively called the microbiome–also have a critical role [1]. Bacteria are the best-studied members of the microbiome on a metabolic level, but the microbiome also includes eukaryotes (helminths, protozoa, yeasts, fungi), archaea and viruses [2]. The microbiome may alter small molecules and their properties, including how long they remain at a given site in the body, and their effects [3]. The microbiome also produces entirely unique metabolites that do not arise without its presence [4]. Certain bacteria in the gut produce signaling molecules that affect host metabolic gene expression, as in the case of secondary bile acids [5]. The microbiome and their effects on metabolism have long-term and wide-ranging consequences for the host [6]. For instance, decreased levels of gastrointestinal short-chain fatty acids (SCFAs), a major group of gut microbiome-derived metabolites, has been associated with Alzheimer's disease [7]. Similarly, stool samples from colorectal cancer patients have markedly lower levels of butyrate compared to those from healthy people [8]. As such, the examination of microbial metabolism is relevant to applications in human health.

Microbial metabolomics can involve annotation of detected molecules as well as quantification of these molecules. Quantification is often performed relative to reference samples, but absolute measurements may be taken if needed (for example in the case of a toxicity assessment of a certain compound, Table 1). Annotation may be required to gain biological insight, like in examining metabolic pathways. In contrast, mass spectrometry (MS)-based methods are able to use a combination of mass and retention time (or other chromatographic characteristics) to quantify a biomarker in clinical samples, even in the absence of annotation or structural elucidation.

The purpose of this review is to serve as a primer for researchers new

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Table 1
Example scenarios of microbial metabolite quantification.

Project goal	Absolute or relative quantification needed?	Annotation needed?	Example references
Determine which metabolites come from the microbiome	Relative quantification may be sufficient	Yes, for biological insight	[4,11]
Identify microorganisms producing a specific metabolite, or conditions in which it is produced	Relative quantification may be sufficient (even binary detected/not-detected information may suffice, being aware of limit of detection caveats)	No	[12,13]
Correlate specific microbial metabolites with clinical outcomes; biomarker identification, validation and implementation	Initial studies may use relative quantification, but often absolute quantification is needed for subsequent studies	Not necessarily	[14–16]
Determine the mechanistic role of a microbial metabolite in disease pathogenesis	Initial studies may use relative quantification, but often absolute quantification is needed for subsequent studies	Yes	[17]
Analyze metabolism of a given drug or food product by the microbiome	Relative quantification initially, which may be followed by absolute quantification, for example to assess toxicity	Yes	[18–20]
Assess the toxicity of a specific microbial metabolite	Absolute quantification	Yes	[21]

to metabolomics who are seeking to discover and quantify novel microbial metabolites or understand the role of well-studied microbial metabolites in human disease through the use of human studies or mouse models. We begin by discussing critical considerations for sample collection and analysis of microbial metabolites, so that quality data may be generated. Additionally, we will discuss some of the most common analytical methods used to study metabolites that are directly produced or chemically modified by the gut microbiome. From there, we will introduce means for identifying whether a metabolite is microbial in origin or produced by the mammalian host cells, though we acknowledge that some metabolites will be commonly produced by both microbiome and host, and may broadly distribute throughout the body [9]. After this, we highlight methods for determining the chemical structure of novel metabolites or the annotation of metabolites that have been previously studied. We then discuss specific quantitative methods for a wide variety of chemical families associated with the microbiome, with tools for relative or absolute quantification. Although thousands of metabolites have a microbial origin [10], we focus here on some of the most-studied classes (Fig. 1), with the strongest current knowledge base with regards to human health. We conclude by introducing some of the challenges and opportunities for innovation currently associated with microbial metabolite quantification.

2. Critical considerations when setting up microbial metabolite quantification studies

a. Impact of sample collection and metabolite extraction methods.

Microbes can produce a wide variety of metabolites, ranging from small branched-chain amino acids to more complicated molecules like bile acids. Additionally, some metabolites are only found in specific tissues, or at greater abundance in one tissue compared to another. For example, the bile acid tyrosocholic acid can be below the limit of detection in cecum, colon, and fecal samples and thus would be missed if small intestine samples aren't collected [11]. Thus, it is crucial to consider what sample type, sample collection method, and metabolite extraction method would work best for the metabolite(s) of interest prior to beginning an experiment.

Sample storage immediately following sample collection can have a dramatic impact on metabolite stability. Only a minority of fecal metabolites are unaffected after 24 h at room temperature, with strong effects observed on microbial metabolic activity [22,23]. Higher stability is observed in urine and serum kept at room temperature, but microbially produced branched-chain amino acids show a significant change in concentration in less than 24 h [24,25]. Thus, immediate quenching of metabolism, for example through snap-freezing samples, is usually advisable, though these methods should be designed to minimize metabolite leakage from cells [26–28]. Nevertheless, immediate snap-freezing may not be practical under field conditions. Storing urine and whole blood samples on ice can help reduce changes to metabolite

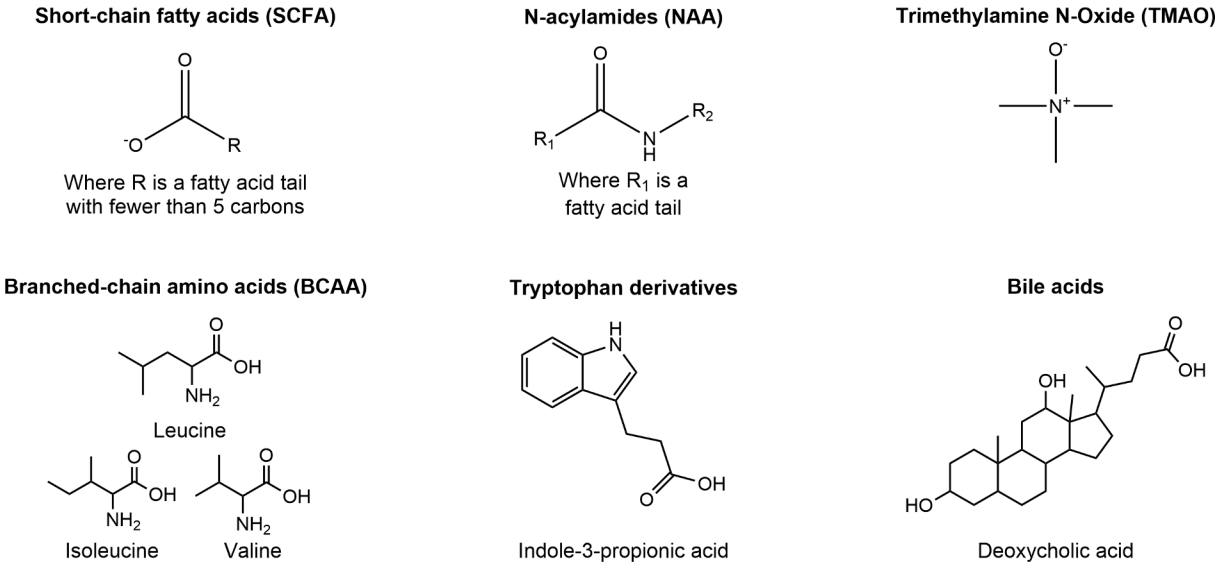

Fig. 1. Example microbial metabolites. Figure generated using ChemDraw software.

concentrations until they can be processed or stored into a −80°C freezer [24,29]. For fecal samples, 95 % ethanol has the largest overlap of metabolites with freshly frozen samples for up to four days at room temperature [30,31]. Thus, this is a suitable alternative to snap-freezing during field collection. Fecal occult blood test (FOBT) cards have also shown acceptable performance [30]. Although RNAlater has commonly been used to preserve fecal samples for sequencing, it is usually considered incompatible with mass spectrometry due to the high salt content [30–32], and is likely to decrease Nuclear Magnetic Resonance (NMR) spectroscopy sensitivity [33]. Commercial sampling kits designed for fecal metabolomics analysis are currently being developed [34]. For biofluids, volumetric absorptive microsampling (VAMS) serves as a viable means of sample collection at room temperature, with the majority of studies reporting stable metabolite concentrations for at least a few days following drying, though some metabolite levels are affected by 2 h [35–40].

Sample storage prior to metabolite extraction and data acquisition should minimize freeze–thaw cycles, which can have major impact on multiple microbial metabolite classes, including branched-chain amino acids [22,24]. Sample lyophilization can likewise lead to significant changes in the relative abundance of microbial metabolite classes such as short-chain fatty acids and branched chain amino acids [41]. Long-term storage (even without freeze–thaw) can still impact the metabolome [42]. For example, 20 % of metabolite signals in dried blood spots stored at −20°C showed differential abundance by storage time, with some changes suggestive of degradation [43]. Likewise, some molecules such as lipids may oxidize during storage, unless stored under nitrogen gas [44].

b. Analytical instrumentation and data acquisition best practices.

As a consequence of the variety of chemical classes produced by the microbiome, no single analytical method can analyze all microbial products. For instance, liquid chromatography-mass spectrometry (LC-MS), one of the most prolific analytical instruments in metabolomics research, is not well-suited to analyze microbially-produced short chain fatty acids without additional processing steps prior to analysis. Therefore, methods of data acquisition, which consequently affect sample preparation as well, must be considered prior to sample collection.

Data acquisition following metabolite extraction uses one of two main workflows: untargeted or targeted. Untargeted metabolomics aims to detect and quantify as many known and unknown metabolites as possible within a biological system, given experimental decisions like sample type and extraction solvent. Challenges include the high degree of diversity in chemical structures and varying concentration ranges between metabolites. In contrast, targeted metabolomics focuses on the precise and accurate measurement of a predefined set of metabolites. This is useful when studying a specific metabolic pathway or validating biomarkers identified by untargeted approaches. Other strengths include high sensitivity and specificity, quantitative and reproducible results, faster data processing and analyses, and suitability for validation studies. However, targeted approaches require prior knowledge of the metabolites to be measured and are therefore limited to the detection and quantification of known metabolites. In all cases, data acquisition in randomized order is critical to prevent the introduction of systematic effects that would confound data interpretation.

LC-MS is one of the most used methods for metabolomics analysis in microbiome research, allowing for the detection of a wide range of compounds, including polar compounds that may be challenging to detect using other methods [45–47]. Additionally, LC-MS based methods have large libraries of public reference data which can help with putatively annotating molecules in untargeted analysis, although these libraries tend to be composed of data collected from positive electrospray ionization, making annotation of data collected via negative electrospray ionization more challenging. While a powerful technique, LC-MS analysis can be complicated by the high degree of sample complexity, which can lead to issues with ion suppression and interference, as well as inter-run and inter-site variability in chromatography retention times.

Gas chromatography-mass spectrometry (GC–MS) is another common instrument in microbiome metabolomics research (e.g. [48–53]), allowing for the identification of volatile and semi-volatile compounds in complex mixtures, or derivatized non-volatile compounds. This chemical class and size restriction is a major limitation of GC–MS. Furthermore, artifacts resulting from derivatization (or uneven derivatization between samples) could impair quantification [54]. On the other hand, a strength of GC–MS is the standardized electron ionization (EI) conditions, facilitating spectral comparisons between experiments over time and to reference libraries. Most GC–MS instruments currently in use or in facilities are low-mass-resolution, so they may not provide high-confidence annotations, though this is being addressed by technology such as GC-orbitrap instruments [55].

NMR can also be used for microbiome metabolomics due to its non-destructive nature, easy sample preparation in bulk material analyses, and lack of chemical derivatization requirements [56]. For example, short-chain fatty acids and branched-chain amino acids could be studied in feces by NMR using simple three-step sample preparation protocols [57,58]. NMR can also be advantageous in detecting and characterizing compounds that are challenging to analyze with LC-MS, such as sugars, organic acids, alcohols, polyols, and highly polar compounds [59–61]. Quantification by the addition of chemical standards, also known as quantitative NMR (qNMR), allows for the accurate measurement of NMR peak heights with the added internal standard [62]. However, qNMR can obscure the reliable measurement of peak intensities due to a high level of NMR signal overlap [63]. Low sensitivity is the inherent disadvantage and foremost challenge for the application of NMR compared with MS [64]. However, continuous developments to improve the sensitivity through the use of higher magnetic field strength [65], cryogenically cooled probes [66], and microprobes [67] have significantly enhanced the sensitivity of NMR.

Though less commonly implemented, other analytical methods such as capillary electrophoresis-MS (CE-MS) (e.g. [68,69]), direct infusion MS with no separation (e.g. [70,71]), matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)(e.g. [72]), or desorption electrospray ionization mass spectrometry (DESI-MS) (e.g. [70]) can also be used to quantify microbial metabolites.

Overall, key considerations guiding instrumental choices are the specific goals of the experiment (see Table 1 for some example scenarios), the analytes of interest, sensitivity, precision, and accuracy needed, and whether absolute versus relative quantification is required. However, in all cases, best practices will include quality assurance/quality control steps, which should include system suitability testing. While out of the scope of this review, readers are referred to recent reviews on best quality assurance/quality control practices for targeted [73] and untargeted metabolomics [74].

c. Data processing best practices.

There have been extensive reviews on data processing for metabolomics (e.g. [75–78]), and detailed protocols published on data processing tools and approaches (e.g. [79]), which naturally also apply to microbiome metabolite analysis. Here, we highlight critical points for consideration. LC-MS-based metabolomics data processing refers to "metabolite features" as a combination of chromatographic retention time and mass over charge (m/z). This recognizes that different isomers will have the same m/z but different structures, leading to different retention times. Likewise, one single structure can lead to multiple adducts or in-source fragments, so that one unique metabolite can have multiple m/z values, though at the same retention time. This complexity is one of the challenges of metabolomics data processing. Combining ion mobility with current chromatography and MS techniques is helping to distinguish between isomers (e.g. [80]).

Currently, there are many popular non-commercial platforms for untargeted analysis, such as MZmine [81], XCMS [82], OpenMS [83], and MS-DIAL [84]. For targeted analysis, example softwares include Skyline [85], MRMkit [86] and OpenMS [83]. One key strength of

MZmine is its modular design, which allows users to customize the software based on their needs [81]. XCMS has two versions: the XCMS R package or XCMS Online (cloud-based version). For XCMS Online, users can upload and process LC-MS data directly without installation of software or coding skills [87]. MS-DIAL is an alternative platform, which implements deconvolution algorithms for data-independent acquisition (DIA) data sets [84]. Skyline was designed for quantitative proteomics but was later expanded to small molecules; a strength is that it is not instrument-specific [85,88]. MRMkit is an MS-based targeted analysis software that can learn retention time offset patterns for different compound classes. This information is then used to provide recommendations for peak picking in multimodal ion chromatograms, which can be complex and difficult to interpret manually [86]. OpenMS is an open-source, cross-platform software designed for high-throughput targeted and untargeted MS data. It provides a set of over 180 tools and workflows [83].

Peak detection algorithms are used as a first data processing step to identify the true peaks in the data, and to differentiate them from background noise (Fig. 2). Deconvolution separates experimental data to create a pure spectrum for each component present in a sample [89,90]. MSHub is a newly developed machine learning deconvolution method that deconvolutes GC–MS data automatically and evaluates the consistency of fragmentation patterns across multiple samples [91]. DecoID is another new deconvolution method that seeks to address mixed fragmentation spectra using a LASSO regression method. This can help improve metabolite identification rates [92].

The prerequisite of quantitative metabolite analysis is to ensure that the same metabolites are being compared across all samples. Peak alignment, normalization and batch effect removal are important steps after peak detection. Without proper peak alignment, comparisons between samples can be misleading. The above-described software packages can perform alignment by themselves, or they can be aided by external tools such as neighbor-wise compound-specific Graphical Time Warping (ncGTW) [93]. Normalization is the process of adjusting metabolite peak areas to reduce technical variability between samples due to differences in sample preparation, instrument performance, or other factors that could lead to incorrect conclusions [94]. There are several normalization methods for MS data, such as internal standard normalization [95], total ion current (TIC) normalization [96] and probabilistic quotient normalization (PQN) [97]. PQN assumes that the alterations in concentration of one analyte will only affect part of the data, while changes in the sample concentration impact the complete data in each sample. WaveICA normalization is based on wavelet analysis, which can not only remove batch effects but also show better classification for untargeted metabolomics data [98]. hRUV is a batch effect removal tool which can remove batch effects for both intra-batch and inter-batch variation [99]. All normalization methods make assumptions, so it is important to pick a method that addresses technical variability within the specific dataset.

Most data processing workflows will remove background signals contributed by tubes, instruments, solvents, etc. This is best performed by generating an empty tube that will go through the same extraction and handling processes as actual samples (“process blanks”). Data acquired from that blank can then be used to process the acquired data. Blanks can also be added at the time of data acquisition but would not allow for the removal of contaminating features arising during the extraction steps. Common methods for background signal removal include removing metabolites that are shared with the blank (which may however remove real metabolite features in case of low-level carryover) or removing any metabolite that fails to meet a certain signal intensity fold difference threshold when compared to blanks (less affected by minor carryover effects). More complex, data-dependent approaches have also been developed [100]. Packages such as CROP [101], MetaClean [102], or NeatMS [103] can remove non-informative features contributed from the blank or false positive features introduced by data processing. Removing these figures will help minimize noise in the data and may help with statistical power and annotation.

3. Attributing microbial origin of candidate features: Important considerations

Following proper data-processing, non-biological features should be minimized in the sample, leaving mainly biological metabolic features. At this point, identifying whether each remaining feature is of microbial origin can be determined through further analyses. Resources like the Microbial Metabolites Database [10] provide known microbe-metabolite relationships, though these associations do not represent the full scope of microbial chemistry. Moreover, much of untargeted metabolomics data still cannot be easily annotated [104,105]. Moving from known microbial metabolites into unknown microbe-metabolite relationships requires additional steps to identify any microbial origins. Broadly, these approaches can be grouped into software-based methods and experimental methods.

a. Software-based approaches.

Correlations are among the most popular methods to estimate associations in microbiome-metabolome data. Typically, researchers select correlation techniques based on normality and/or fit of data, such as parametric (normally distributed) or nonparametric approaches. For example, Spearman’s rank correlation coefficient and Pearson’s rank correlation coefficient are both popular approaches for exploring microbiome-metabolome associations [106–109]. Correlations are especially useful for quickly identifying strong associations between microbes and metabolites, offering simple guidelines for determining linear relationships between these data [110]. However, such correlation approaches assume data are independent, which is not true of microbiome-metabolome data. Moreover, the compositional nature of microbiome and metabolome data further confound correlation analyses [111,112]. Thus, correlations can be useful for identifying basic patterns within the data, but should only be used cautiously, with additional validations to eliminate false associations.

Beyond general approaches like Spearman or Pearson, correlation approaches can be expanded to perform specialized comparisons. One such correlation-based technique is weighted gene co-expression network analysis (WGCNA). Briefly, WGCNA creates a network of co-expressed genes clustered by their similarity and uses this network to report associations with other paired data [113,114]. Through integration with microbial and metabolomic data, WGCNA can identify microbe-metabolite associations by focusing on bacterial genes correlated with the metabolites of interest. For example, WGCNA has been used to examine relationships between bacteria and metabolites associated with Chagas disease [115], revealing key metabolite-altering bacterial taxa involved in *Trypanosoma cruzi* infection. By itself, WGCNA is a useful technique for attributing microbe-metabolite relationships, but it also represents a broader data analysis approach

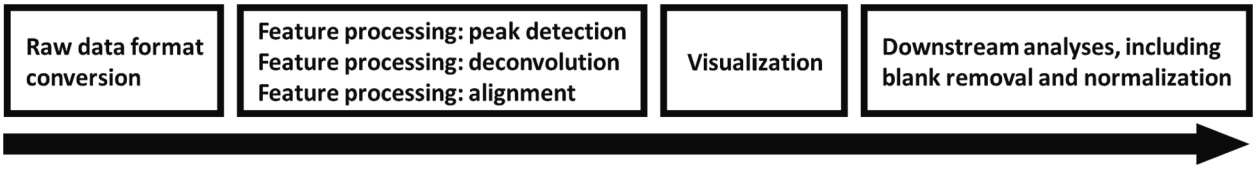

Fig. 2. Workflow for LC-MS data analysis.

involving correlations.

To overcome the hurdles seen in correlations, new software tools estimate microbe-metabolite relationships while considering the compositional and dependent nature of these data. One such tool is microbe-metabolite vectors, also known as 'mmvec' [116]. mmvec is a neural network pipeline that predicts microbe-metabolite relationships using microbial sequence and metabolomic data. Rather than identifying associations, mmvec examines the probability of co-occurrence between microbe-metabolite pairs derived from their paired abundances [116]. However, mmvec is computationally intensive and less suited for large datasets. Additionally, there are no validation or confidence measures built into mmvec; it simply outputs the conditional probability values for all microbe-metabolite pairs [116]. Despite these limitations, mmvec is an useful alternative to correlations for exploring microbe-metabolite relationships with integrated multi-omics data [117–120].

Other data analysis techniques can include the Mass Spectrometry Search Tool (MASST) [13]. Akin to a Basic Local Alignment Search Tool (BLAST) search for comparing sequence data, MASST is a web-based search tool that searches inputted MS/MS data against a range of public MS/MS spectra databases. MASST uses a spectral library containing tens of thousands of annotated mass spectra of small molecules to match the *m/z* values of user-submitted spectra against those in the library. The output contains multiple potential matches ranked by their similarity to the user's spectra. Importantly, sample information attributed to these public data are available when performing MASST searches, allowing researchers to identify reported sources of detected MS/MS spectra, like potential microbial sources. For example, Quinn and colleagues used MASST to run searches against public data to further confirm association between novel amino acid-conjugated bile acids and the microbiome [11].

An additional data analysis approach involves reference data-driven (RDD) analyses. RDD approaches use a controlled, standardized vocabulary when creating metadata of database references used to match against user-submitted MS/MS data. Based on these matches, researchers can get source information of their submitted MS/MS spectra through matches to these curated metadata reports of public data. Importantly, these RDD approaches maximize the utility of MS/MS spectra and allow matches against curated source information, as seen by Gauglitz et al. [121] While this particular study focuses on identifying food sources through RDD analyses, these approaches can be extended into broader and microbial contexts.

b. Experimental approaches.

These data analysis-centric approaches can be complemented through experimental techniques to report microbe-metabolite relationships. One predominant experimental approach for validating microbe-metabolite associations involves culturing the candidate-producing microbe and screening for metabolite(s) of interest, usually informed by prior hypotheses of a microbe-metabolite relationship. This process isolates the microbe from its natural environment and avoids host organism modification of the metabolite of interest. Thus, analyzing the metabolites produced in the bacterial culture reports a direct microbe-metabolite relationship. Previous studies have used this technique in varied ways, ranging from culturing bacteria to reconstructing genome-wide metabolic pathways of the detected metabolites [122–125] or tracing microbiome-dependent metabolites in colonized mice to metabolome profiles of cultured bacteria [126]. However, culturing conditions are meant to simulate, not recreate, the microbe's natural environment; the expected metabolites might not necessarily be produced in all culturing experiments.

Another experimental approach involves comparisons between germ-free and colonized animal models, such as mice. These approaches compare sterile and microbially-colonized animal metabolome data, where metabolites enriched in the colonized animals likely have some microbial association. Examining different abundance patterns of metabolites can highlight microbially-modulated metabolite enrichment or depletion. Such animal-based comparison approaches are particularly useful for confirming the microbiome's role in influencing specific metabolites, because it tests for the metabolites in the "natural" environment with the greater microbiome community. This concept can be mimicked in humans by investigating samples from humans with resected colons, as was the case with identifying the bacterial origin of 5-hydroxyindole [127], or through the administration of a poorly absorbed oral antibiotic cocktail [128–130]. However, these approaches only report broad microbe-metabolite relationships without providing specific microbial sources. For example, microbe-associated metabolites might be produced by the host responding to the bacterial presence, rather than being produced by the bacterium itself. These animal-model approaches simply indicate a trend and require further work to demonstrate any metabolites are indeed produced by the microbe in question. One method to address this shortcoming is through stable isotope labeling of bacteria, which enables differentiation with host-derived unlabeled metabolites by LC-MS [9]. Uchimura et al gavaged mice with ^{13}C-labeled *E. coli* to investigate the nature and spatial spread of microbiome-derived metabolites. After 2 h, an untargeted LC-MS analysis showed labeled amino acids, fatty acids and nucleotides, produced by the microbiome, penetrate organs throughout the body. Through this, the researchers were able to determine the ratio of microbiome-produced branched-chain amino acids to host-derived in each organ [9]. Another approach, as used in experiments to identify the microbial origin of indolepropionic acid, is to knock out crucial genes associated with the production of a metabolite in cultured bacteria and then inoculate germ-free mice with the mutant bacteria to identify if the metabolite of interest is still produced [131]. Unfortunately, sterile vs. colonized animal comparisons might not always detect host-produced antagonistic pathways influencing microbial activity, such as metabolic reabsorption in the ileum. Despite these drawbacks, animal models are popular for determining microbe-metabolite relationships, such as confirming microbial metabolism of novel amino acid-conjugated bile acids [11], identifying blood metabolomic responses to gut microbial activity [4], describing longitudinal analyses of urine metabolomes throughout gut bacterial colonization [132], and linking gut microbial activity to energy and lipid storing metabolites involved in modulating host metabolism [133], to name a few.

These approaches reflect varying ways of identifying microbial origins for metabolites, but there are important considerations to note when doing these analyses. The first major consideration is how sample type and preparation method can influence observed microbiome-metabolome associations. This is particularly important from an experimental side, as evidenced by Han and colleagues observing distinct metabolomes based on sample types of colonized mice [126]. Additionally, another important consideration includes spatial dynamics of the gut. For example, metabolites with reduced abundance in the lower gastrointestinal tract might be absorbed by the gastrointestinal tract, or their depleted abundance could be the result of microbial modulation. A third consideration when investigating microbial origins of metabolites is that a detected functional gene might not always produce a metabolite in all contexts. This consideration is especially prominent when identifying a microbe-metabolite relationship through culturing. Since the culture does not perfectly mimic the microbe's natural environment, some metabolites might not be produced in that cultured setting despite the metabolite being produced in the gut.

Ultimately, identifying a microbial origin of a metabolite requires careful consideration and should ideally involve multiple complementary analyses. The approaches discussed here are not an exhaustive list but represent common ways for investigating microbial origins of metabolites. Other growing areas for identifying microbe-metabolite relationships include machine learning tools [134], especially for determining chemical properties of such microbially-derived metabolites.

4. From microbial features to chemical structures of novel microbial metabolites

Having established that a novel feature is microbial in origin, the next step is structure elucidation. Most of the candidate microbe-metabolite associations described above rely on untargeted metabolomics. An accurate structure elucidation is critical to understand the biological role of the metabolite, and its biosynthesis pathway.

The first step in the structural identification involves acquiring the metabolites in reasonable quantities, typically in the microgram to milligram range. Unless native environments provide enough of the metabolites, culture conditions may be required to accrue more. This involves routine microbiology techniques, including cultivation of microbial strains on specific media with special nutrients, cocultivation of multiple strains together, and production of metabolites of interest in a specific growth phase [135–137]. However, as discussed above, it is important to note that the majority of microorganisms resist cultivation in the laboratory as the required conditions deviate too far from the organism's natural environment [138]. Furthermore, metabolite production is often context-dependent. As an alternative, isolation chip (iChip) incubation has been developed to facilitate growth in simulated natural environments [139]. For example, a variant of the iChip has been successfully used to cultivate anaerobic species of the human oral microbiome [140]. Another successful iteration is the gut-on-a-chip platform which enabled the coculture of 200 different aerobic and anaerobic species with human intestinal epithelium [141].

The isolation of microbial metabolites involves a wide range of LC methods based on separation capacities and selectivity [142]. Aside from isolation and separation of microbial metabolites, a major application of high-performance liquid chromatography (HPLC) methods is dereplication by hyphenated techniques including LC-NMR and LC-MS [143]. Dereplication involves identifying known compounds based on the reported data generated by MS or NMR. Most dereplication bioinformatics tools, including GNPS [144], Metlin [145], DNP [146], and NIST [147], work with integrated or online databases of accurate mass spectra, compound structures, structures of building blocks of molecules, and fragmentation routes or combination of multiple features.

After sample isolation and replication, the metabolite is ready for structural analysis. NMR is the core technique in structural elucidation, and can be supplemented with other analytical techniques such as high resolution MS, circular dichroism (vibrational and electronic), Raman optical activity, optical rotation measurements, and X-ray crystallography, depending on molecule properties [148]. ^{1}H and ^{13}C NMR spectra provide information about the quantitative composition of the unknown metabolite [149]. The interpretation of NMR data along with high resolution MS data is used for the determination of the molecular formula of the novel metabolite [149]. The assembly of the relative configuration is further supported by additional 2-dimensional NMR experiments such as HSQC (Heteronuclear Single Quantum Coherence), COSY (Correlation spectroscopy), and HMBC (Heteronuclear multiple bond correlation) [150]. The structure elucidation of novel metabolites may be a complicated process based on structural complexity. Spectroscopists may interpret different structures from the same initial NMR data [151], by misjudged interpretation of chemical shift annotations, misinterpretation of characteristic spectral features, and resonance overlap in NMR signals [152].

For labs without access to an NMR or without the expertise to interpret NMR data, or sometimes prior to NMR data acquisition, MS can also be used to understand the structure of an unknown metabolite. Example annotation tools for MS data include molecular networking, which groups features by MS2 spectral similarity, enabling annotation propagation to analogs with mass differences compared to library references [144,153]. MEtabolite SubStructure Auto-Recommender (MESSAR) automatically builds potential relationships between spectral data and substructure data, which are captured and learned from public spectral libraries. This approach allows MESSAR to provide multiple candidates for annotation with similar substructures, even if the exact molecule is not present in the reference database [154]. Small Molecule Accurate Recognition Technology (SMART 2.0) is a web-based tool with improved accuracy for mixture sample analysis [155]. While structures can be determined from MS/MS or MS/MS/MS data, they cannot usually provide stereochemical determination. Additionally, identification of the molecular structure from untargeted MS/MS data alone can be challenging, as multiple molecules may have the same m/z values and fragmentation patterns. Feature annotation is a very active area of metabolomics research, and new tools are regularly being developed [156].

In general, no single technique can unambiguously assign the structure of a complex unknown compound. The synergistic combination of information obtained from different techniques, like crystallography and NMR, combined with chemical and biosynthetic logic, lead to structure elucidation of novel metabolites with higher reliability [151].

5. Class-specific quantification methods for common microbial metabolites

After annotating the metabolite of interest (or choosing to forgo annotation should it not be necessary for the goal of the study), quantification should be performed next. Greater method validation is required when quantifying novel microbial metabolites that lack established methods. This would involve testing data acquisition methods using pure standards across a range of known concentrations, ideally of the same molecule or of molecules with similar functional groups, to ensure separation (if using chromatography techniques) and to assess the limit of detection/limit of quantification. Method validation with standards should be followed by assessment on representative biological samples, such as sample pools (pooled quality control [pooled QC]) or standardized biological samples (National Institute of Standards and Technology (NIST) standard reference materials). Quantification of frequently studied microbial metabolites will need less in-house method validation, since they generally rely on already-developed methods. Additionally, absolute quantification of known metabolites can be aided by diluting samples with a known amount of an isotopically labeled version of the metabolite [128,157]. In this section, we highlight quantification methods for highly studied microbial metabolites. Should a novel microbial metabolite share structural commonality with one of the metabolic classes listed below, the quantitative methods associated with that class can be used as a starting point for method development.

a. Short-chain fatty acids.

Fatty acids with fewer than 8 carbon atoms are classified as short-chain fatty acids (SCFAs). The most prevalent SCFAs include acetic acid, propionic acid, and butyric acid [158,159]. When dietary fibers undergo fermentation by bacterial hydrolytic enzymes, the primary products are SCFAs. SCFAs play a crucial role in promoting the proliferation of beneficial bacteria, supplying energy to colonocytes, modulating the gut immune system, preventing autoimmune diseases, and enhancing overall gastrointestinal health [160–164].

Feces, plasma, and tissue samples each offer unique information about SCFA production, metabolism, and distribution within the body [165]. Fecal samples are a conventional choice for evaluating SCFA production within the gastrointestinal tract. However, their SCFA concentrations may not accurately reflect the local rates and levels of SCFA production. This is because as much as 90 % of these metabolites are absorbed by colonocytes [166], and the concentration of SCFAs varies along the length of the gut. The highest levels are typically found in the cecum and proximal colon, gradually decreasing towards the distal colon [161,167,168]. Plasma samples are useful for evaluating systemic SCFA, despite their typically low concentrations. SCFAs produced in the gut can be transported via the portal vein to the liver, where they undergo metabolism before being released into the systemic circulation [167]. Tissue samples can provide insights into how SCFA are metabolized and used by specific organs or tissues. For instance, adipose tissue

is a significant site for SCFA uptake and metabolism, while the brain can also use SCFAs as an energy source [165,169,170] (Fig. 3).

To ensure accurate and precise quantification of SCFAs, it is essential to consider their chemical and physical properties, as these factors can significantly impact the accuracy and precision of measurements. Some important properties to take into consideration include [165,171,172]:

1) Volatility: SCFAs are volatile compounds that can evaporate during sample preparation and analysis.
2) Solubility: Some solvents have selectivity in extracting specific SCFAs. For instance, diethyl ether is suitable for extracting butyric acid and valeric acid, while hexane is commonly used for the extraction of less polar SCFAs, such as caproic acid and caprylic acid.
3) Ionization: SCFAs are weak acids that can ionize in solution, with the degree of ionization being dependent on the pH of the solution. The ionization state of SCFAs can influence their behavior in chromatography and their detectability using mass spectrometry.
4) Stability: SCFAs can be prone to degradation or chemical modifications during sample preparation and storage. It is crucial to employ optimized storage conditions (e.g., low temperature, acidification) and appropriate derivatization or stabilization methods that help overcome these challenges.
5) Matrix effects: SCFAs are often found in low concentrations within complex matrices like feces or plasma, which contain various other organic acids and metabolites. These matrix effects have the potential to interfere with the accurate quantification of SCFAs [173,174].

For reliable SCFA measurements, it's essential to consider the properties described above and use appropriate methods. Modern analytical methods, both qualitative and quantitative, are highly effective for analyzing SCFAs in a variety of sample types. These methods include approaches like GC, HPLC, and CE, often paired with detection methods such as mass spectrometry.

In the context of GC–MS analysis, a common procedure for extracting SCFAs from various matrices involves the addition of an acidifying agent, such as phosphoric or sulfuric acid, to the sample. Subsequently, this mixture undergoes liquid–liquid extraction (LLE) with an organic solvent, like ethyl acetate or diethyl ether. These extraction techniques are chosen to specifically address concerns related to solubility and stability, thereby enhancing the accuracy of the quantification process. It's worth noting that water extraction is discouraged for SCFA GC–MS analysis due to its detrimental impact on MS determination [175,176]. Despite being considered somewhat more prone to errors, the liquid–liquid extraction method (LLE) offers advantages such as straightforwardness and swiftness. Moreover, it obviates the need for specialized extraction filters, cartridges, or columns [48].

In LC-MS analysis, the extraction of SCFAs often employs solid-phase extraction (SPE). This technique uses a solid-phase sorbent, like C18 silica-based material, to selectively adsorb and retain the SCFAs from the sample matrix. Following this adsorption, the sorbent undergoes a washing process, and the SCFAs are subsequently eluted using solvents such as methanol, formic acid or acetonitrile. The resulting extract is then evaporated to dryness and reconstituted in a solvent compatible with downstream chromatography and MS methods, such as water or acetonitrile (Table 2) [174,177–180].

Derivatization involves the chemical modification of a compound's structure to optimize its suitability for analytical applications. In the context of SCFAs, this often includes introducing an alkyl or silyl group to the carboxylic acid functional group. This chemical modification improves the main challenges of the volatility and stability of SCFAs, rendering them more compatible with analysis methods such as GC–MS and LC-MS [172]. Derivatization reagents for SCFAs commonly include pentafluorobenzyl bromide (PFBBr) [165], trimethylsilyl (TMS) derivatization reagents [181], and chloroformate [182,183]. PFBBr derivatization is time-consuming, and silylation requires anhydrous conditions, which is cumbersome [184,185]. In contrast, chloroformate derivatization is widely preferred for SCFA derivatization due to its convenient operation, mild conditions, and reduced time consumption [182]. However, it's worth noting that derivatization involves organic solvents and other compounds that may be toxic, volatile, or flammable. Moreover, it extends processing time and introduces another potential source of variability, contamination, and errors [165,185].

GC–MS separation is often carried out using FFAP columns (polar nitroterephthalic-acid-modified polyethylene glycol). It has proven to be a robust method, albeit one that involves time-consuming sample preparation and derivatization processes. In contrast, LC-MS may require less time for sample preparation and analysis, while employing lower temperature conditions can also prevent the degradation of volatile SCFAs [186–188]. However, SCFAs pose challenges in terms of their chromatographic and ionization properties when using conventional LC-MS equipment [174]. To address these challenges, for certain types of matrices, derivatizing samples can enhance the chromatographic separation and ionizability of SCFAs. Some effective strategies include derivatization using 3-nitrophenylhydrazine and aniline [189,190]. Both strategies yield favorable results and allow for SCFA analysis on conventional C18 LC columns. Typical mobile phases consist of acidified HPLC-grade polar solvents (e.g., water) and non-polar solvents (such as acetonitrile or methanol) for analyte separation through isocratic or gradient elution profiles. These separations are conducted under relatively mild reaction conditions [189,191,192].

b. Bile acids.

Bile acids (BAs) occupy a central position in the absorption, excretion and metabolism of lipids within the body [197]. The gut microbiome modifies endogenous primary BAs to produce exogenous secondary BAs [198]. The best-studied secondary BAs are lithocholic acid (LCA) and deoxycholic acid (DOC) [199]. However, recent work has identified more than 140 new secondary BAs in existing datasets, including the

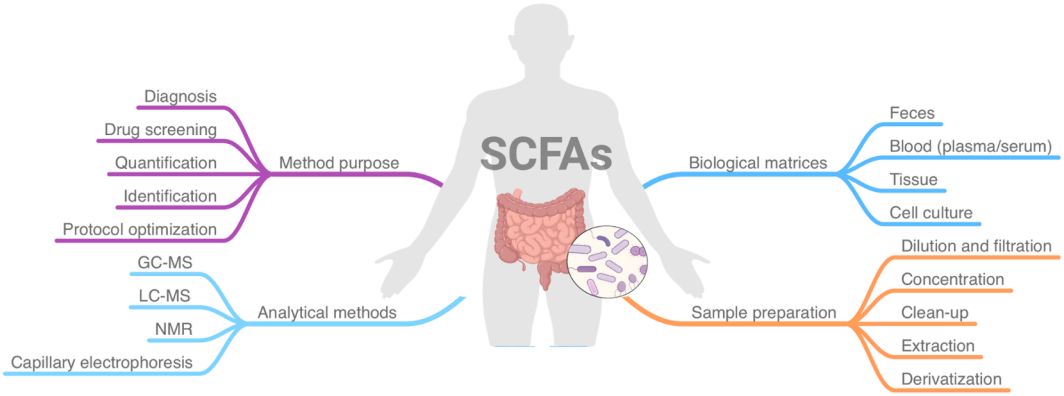

Fig. 3. Hallmarks of short-chain fatty acids quantification. Figure generated using BioRender.com.

Table 2
Short chain fatty acid quantification methods.

Quantification Method	Reference	Sample Type	Extraction	Column	Mobile Phase	Ionization Method	Molecule(s)
GC–MS	Zhu et al., 2022 [193]	Feces	Diethyl ether	DB-FFAP	Helium, flow rate 1.5 mL/min	EI	Acetic acid, formic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, 2-methylvaleric acid, 4-methylvaleric acid, hexanoic acid, heptanoic acid
	Fu et al., 2022 [194]	Feces	HS-SPME (Headspace Solid-Phase Microextraction). Derivatized with isobutyl chloroformate and isobutanol	Rxi-5MS	Helium, flow rate 1.0 mL/min	EI	Formic acid, acetic acid, propanoic acid, isobutyric acid, butyric acid, 2-methylbutyric acid, isovaleric acid, valeric acid, 3-methylvaleric acid, hexanoic acid, heptanoic acid
	Kim et al., 2022 [173]	Plasma, Serum, Feces, Cecum	Methyl *tert*-butyl ether	DB-FFAP	Helium, flow rate 1.0 mL/min	EI	Acetic acid, propionic acid, butyric acid, valeric acid
	Yao et al., 2022 [195]	Plasma	Methyl *tert*-butyl ether	DB-WAX	Helium, flow rate 1.2 mL/min	EI	Acetic acid, propionic acid, butyric acid
	Wang et al., 2020 [196]	Serum	Ethanol and n-hexane	DB-FFAP	Helium, flow rate 1.0 mL/min	EI	Acetic acid, propionic acid, isobutyric acid, pivalic acid, butyric acid, 3-methylbutyric acid, 2-methylbutyric acid, valeric acid, 2-ethylbutyric acid, 2-methylvaleric acid, 3-methylvaleric acid, 4-methyl-valeric acid, hexanoic acid, cyclopentanic acid
LC-MS	Shafaei et al., 2021 [177]	Serum	Methyl *tert*-butyl ether. Derivatized with 3-nitrophenylhydrazine hydrochloride and 1-ethyl-3-(3-dimethylaminopropyl)carbodii-mide hydrochloride	C18-AR	A: Water. B: Acetonitrile	ESI - Negative mode	Acetic acid, propionic acid, isobutyric acid, butyric, acid, isovaleric acid, valeric acid, 4-methyl valeric acid, hexanoic acid
	Bihan et al., 2022 [178]	Cecal contents	Water/acetonitrile (1:1. v/v). Derivatized with aniline and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride	C18	A: Water + 0.1 % formic acid. B: Methanol + 0.1 % formic acid	ESI - Positive mode	Acetate, propionate, butyrate
		Microbial cultures	Water/methanol (1:1. v/v). Derivatized with aniline and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride				
	Nagatomo et al., 2018 [179]	Feces	Methanol with QuEChERS method. Derivatized with 2-picolylamine in 2,2′-dipyridyl disulfide and triphenylphosphine in acetonitrile	BEH C18	A: Water + 0.1 % formic acid. B: Methanol + 0.1 % formic acid	ESI - Positive mode	Acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, hydroangelic acid, 4-methylvaleric, succinic acid
	Liebisch et al., 2019 [180]	Feces, Colon content	70 % isopropanol. Derivatized with 3-nitrophenylhydrazine hydrochloride and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride	XB-C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Negative mode	Acetate, propionate, butyrate, isobutyrate
	Saha et al., 2021 [174]	Feces, Liver, Kidney, Brain, Skeletal muscle, Spleen, Microbial media	0.5 % orthophosphoric acid	PGC C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive and negative mode	Acetate, butyrate, isobutyrate, isovalerate, valerate, lactate, propionate.

novel bile acids phenylalanocholic acid (Phe-chol), tyrosocholic acid (Tyr-chol) and leucocholic acid (Leu-chol) [11]. With credit to public metabolomic databases (GNPS, MassIVE) and the use of synthesis-based reverse metabolomics, even more novel bile conjugates are being discovered [200]. Secondary bile acids at increased levels promote higher chances of developing colorectal carcinomas, gallstone disease, cirrhosis of the liver and *Clostridioides difficile* infection [201–211]. BAs serve as signaling molecules with the ability to bind to receptors such as farnesoid X receptor (FXR), Takeda G protein-coupled receptor 5 (TGR5), constitutive androstane receptor (CAR), and more, with each BA being able to bind with multiple receptor types with varying degrees of effect [212,213]. Further adding to the complexity of BA signaling, bile acids can be conjugated with glycine, taurine, and to a lesser studied extent, other amino acids, which can affect the ability of BAs to act as substrates for specific receptors in the body [11,12,214]. In the context of cancer, the pro- or anticarcinogenic roles of specific BAs have been shown to be reliant on the type of neoplasia and the concentration of the BA, emphasizing the importance of accurate quantification of BAs to better understand their role in the body [215].

Regardless of the specific bile acid structure, quantification methods have relied predominantly on LC-MS approaches. Samples most commonly collected for BA analysis are blood, urine, feces, liver, and bile, depending on the BAs of interest. A C18 LC column coupled with MS/MS analysis is most commonly used for BA analysis [216,217] (Table 3). NMR is another common method to analyze BAs and distinguish stereochemical differences (Table 4). Although there is minimal sample preparation, the lower sensitivity of NMR compared to LC-MS make it less common for current BA research [218]. Enzyme-linked immunosorbent assay (ELISA) is another method used to measure BAs but can be limited by antibody cross-reactivity [219–221].

Separation and quantification of BAs and their conjugates are challenging because of marked differences in chemical properties, the

Table 3
MS-based bile acid quantification methods.

Quantification Method	Reference	Sample Type	Extraction	Column	Mobile Phase	Ionization Method	Molecule(s)
LC-MS	Sarafian et al., 2015 [224]	Plasma	Ice cold methanol	BEH C8	A: Acetonitrile/water (1:10. v/v) + 1 mM ammonium acetate. B: Acetonitrile/2-propanol (1:1. v/v)	ESI - Negative mode	Primary and secondary BAs
	Alnouti et al., 2008 [222]	Bile	Oasis-HLB SPE conditioned with MeOH. Washed twice with water and eluted with MeOH.	C18	A: 95:5 Methanol: acetonitrile. B: Water + 7.5 mM ammonium acetate, pH 4	ESI - Positive and negative mode	Positive - Glycine conjugated BAs. Negative - Taurine and unconjugated Bas
	Lucas et al., 2021 [12]	Bacterial culture	100 % water	BEH C18	A: Water + 10 mM ammonium acetate, pH 6. B: Methanol	ESI - Negative mode	Secondary BAs
	Gómez et al., 2020 [225]	Plasma, Serum	Diluted with water. Proteins precipitated with 2-propanol	BEH C18	A: 95:5 Water: acetonitrile. B: 5:95 Water: acetonitrile	ESI - Positive and negative mode	Unconjugated and conjugated BAs and secondary BAs
		Liver	Water:chloroform:methanol (1:1:3. v/v/v)	BEH C18	A: 95:5 Water: acetonitrile. B: 5:95 Water: acetonitrile	ESI - Positive and negative mode	Unconjugated and conjugated BAs and secondary BAs

Table 4
NMR-based bile acid quantification methods.

Quantitation Method	Reference	Sample Type	Solutions	NMR	Molecule(s)
1H NMR	Nagana Gowda et al., 2009 [226]	Bile	Double distilled water, D_2O, Trimethylsilylpropanoic acid (TSP)	800 MHz NMR spectrometer with 5-2mm CHN inverse probehead	Glycocholic acid, glycodeoxycholic acid, glycochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid
1H NMR/13C NMR (1H–1H, 1H–13C)	Nagana Gowda et al., 2006 [227]	Bile	Double distilled water, D_2O, Trimethylsilylpropanoic acid (TSP)	800 MHz NMR spectrometer with 5-mm CHN inverse probehead	Glycocholic acid, glycodeoxycholic acid, glycochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid

presence of isomeric forms, and their relatively low concentrations in biological samples [222]. Longer LC gradients allow for adequate separation and annotation of isomers from MS/MS analysis [223]. Current research continues to discover new derivatives of BAs, further complicating efforts to link the health of our gut's microbiome to the quantity and signaling properties of BAs that have relevant physiological effects.

c. Branched-chain amino acids.

Branched-chain amino acids (BCAAs) only comprise three amino acids: isoleucine, leucine, and valine. These molecules are essential, and primarily gained through host diet or synthesized by the microbiome. Many studies have linked the level of BCAAs with insulin resistance, obesity, and microbiome diversity [228–230]. There has been conflicting evidence on the benefits of elevated BCAA levels [231,232], and no comprehensive understanding of the relationship between BCAA and microbiome health is currently evident.

Fecal samples can be a non-invasive method to measure the BCAA content in the gut. However, considering BCAAs are often absorbed by intestinal tissues and transported throughout the body, fecal analysis may only give rough quantity estimates and no tissue or function-specific information. For this, BCAAs would need to be extracted from GI tract samples. BCAA levels have also been commonly measured in blood samples [230,233]. Due to the polar nature of these amino acids, extraction from any sample type would need to be done with a polar solvent like methanol, water, or isopropanol (Table 5).

Detection of these three BCAAs is relatively straightforward. They can be separated by LC and are ionized well in positive-mode MS. It is important to note that leucine and isoleucine are structural isomers, with the same precursor *m/z*. MS2 fragmentation patterns need to be collected to accurately differentiate the two molecules. Additionally, they tend to have very similar retention times, and often cannot be resolved by non-polar stationary phase columns. Using a more polar stationary phase column, like a HILIC column, BEH amide column, or a C18-PFP can help resolve these two molecules [46]. GC–MS methods have also been used for the detection of BCAAs, however they are less common and typically involve first derivatizing the BCAAs to propyl esters [234]. Beyond these MS instruments, other methods of BCAA

Table 5
Branched-chain amino acid quantification methods.

Quantification Method	Reference	Sample Type	Extraction	Column	Mobile Phase	Ionization Method	Molecule(s)
LC-MS	Fingerhut et al., 2016 [237]	Blood	Neobase test-kit from PerkinElmer	BEH C8	A: Water + 0.1 % formic acid + 0.01 % heptafluorobutyric acid. B: Acetonitrile + 0.1 % formic acid + 0.01 % heptafluorobutyric acid	ESI - Positive mode	All BCAAs
	Le et al., 2018 [46]	Serum	Acetonitrile	Tested multiple, but C18-PFP worked best	Tested multiple, but ended up using: A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	All BCAAs
GC–MS	Zheng et al., 2013 [238]	Feces, Plasma, Urine	Sodium hydroxide solutions	HP-5 ms capillary column	Helium, flow rate 1 mL/min	EI	Propyl-ester derived BCAAs
CE-MS	Piestanksky et al., 2021 [239]	Standards, Plasma	Acetonitrile	Fused silica capillary	50:50 Methanol:water + 5 mM ammonium acetate	ESI - Positive mode	All BCAAs

detection can also be done. BCAAs can be detected with NMR [235], and even more novel methods like a paper strip test have been developed for analysis [236].

d. Tryptophan Derivatives.

L-tryptophan is an essential amino acid that can be catabolized through one of three different metabolic pathways: the serotonin/5-hydroxytryptamine pathway, the indole-derivative pathways, or the kynurenine pathway, which makes up approximately 95 % of tryptophan metabolism [240–242]. The gut microbiome plays a crucial role in L-tryptophan metabolism. The primary direct role of the microbiome in tryptophan catabolism is through the direct and exclusive production of indole derivatives, although some species of bacteria are able to directly produce kynurenine as well [242–245]. Mouse studies have shown that the indole derivatives indole-3-propionic acid and indoxyl sulfate are not produced without the presence of gut microbes and that germ-free mice had higher levels of tryptophan (the precursor of indole-3-propionic acid and indoxyl sulfate) and lower levels of kynurenine in their serum compared to the germ free mice [4,246]. Due to the complex variety of molecules produced from tryptophan in the gut, it is important to select sample types, extraction methods, and detection methods that will allow for the detection of metabolites of interest.

The majority of tryptophan derivatives are present in multiple sample types, but urine generally offers the largest number of unique tryptophan derivatives when compared to feces and plasma, with higher overall concentrations of almost all tryptophan derivatives when compared to plasma [45,247,248]. Additionally, some tryptophan derivatives can't be detected in urine, such as 5-hydroxyindole (present in feces) and 3,4-dihydroxyphenyl acetic acid (present in plasma) [45,248]. For the broadest possible analyses of tryptophan derivatives, collection of both fecal and urine samples is recommended.

For extraction of tryptophan derivatives, biofluids like urine, plasma, and cerebrospinal fluid can be easily extracted using a "dilute-and-shoot" strategy of a 10 fold dilution with water [248,249] or by a simple protein precipitation procedure where ice cold methanol or acetonitrile is added to the biofluid samples and centrifuged, followed by collection of the supernatant [250,251] (Table 6). Tissue and fecal samples tend to follow a similar protein precipitation method, but they are homogenized first.

Tryptophan and its derivatives are primarily quantified using LC-MS. C18 columns tend to be favored since tryptophan derivatives have large non-polar indole groups or rings; in contrast, tryptophan derivatives were not retained well on a BEH amide HILIC column and HILIC columns did not provide reliable separation and peak shape for indole tryptophan derivatives [248,252]. For low volume samples or samples with high levels of matrix effects, tryptophan and its metabolites can be derivatized to increase sensitivity and improve ionization, but this naturally adds extra steps, which can be time-consuming for large sample batches or add additional variation [253,254]. Chromatography is most commonly performed with water and acetonitrile as mobile phases, both of which contain 0.1 % formic acid to aid in the formation of ions. Tryptophan derivatives are almost universally ionized in positive mode; however, indoxyl sulfate ionizes better in negative mode [248,249]. Instead of using MS for detection, some studies have elected to use a fluorometer for detection after LC separation due to the native fluorescence of tryptophan [255–258]. This provides increased sensitivity for tryptophan derivatives but does limit the ability to investigate non-fluorescent molecules without some form of derivatization. GC–MS has been used for the quantification of tryptophan derivatives but is traditionally more challenging due to the need for chemical derivatization [51–53].

Due to the broad range of molecules that can be derived from tryptophan, quantifying several types of tryptophan derivatives simultaneously can be difficult. Tryptophan derivatives concentrations can cover 4 magnitudes of concentrations, each with their own ranges of linearity, making it challenging to find a sample concentration that will work for all potential metabolites of interest [247,248,252]. Additionally, tryptophan derivatives can have a variety of functional groups each with their own pKas, impacting the polarity of the molecule based on the pH of the solution it is in [247,252].

e. Trimethylamine N-oxide (TMAO).

Trimethylamine N-oxide (TMAO) is generated through oxidation of trimethylamine (TMA), a metabolite generated in the gut by the microbiome from carnitine, choline, and betaine that is transferred to the liver. In the liver, host flavin-dependent monooxygenase (FMO)

Table 6
Tryptophan microbial metabolites quantification methods.

Quantification Method	Reference	Sample Type	Extraction	Column	Mobile Phase	Ionization Method	Molecule(s)
LC-MS	Marcobal et al., 2013 [45]	*Bifidobacterium longum* NCC2705	Methanol	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	Indole-3-lactic acid
		Urine	Water + 10 mM ammonium formate	C18	A: Water + 10 mm ammonium formate. B: Methanol + 10 mm ammonium formate	ESI - Positive and negative mode	Indole derivatives
			Water + 10 mM ammonium formate and ACN	HILIC	A: 10:90 Water:acetonitrile + 15 mm ammonium formate. B: 90:10 Water:acetonitrile + 15 mm ammonium formate		
	Anesi et al., 2019 [248]	Plasma	Ostro 96 well plate – 3:1 ACN with 0.1 % formic acid	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive and negative mode	Tryptophan-kynurenine pathway, serotonin pathway, indole derivatives
		Urine	Water with 0.1 % formic acid	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive and negative mode	Tryptophan-kynurenine pathway, serotonin pathway, indole derivatives
	Lai et al., 2021 [247]	Brain Tissue	Methanol	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	Indole derivatives
		Feces	Methanol/water (1:1. v/v)	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	Indole derivatives
LC-Fluorescence	Desbonnet et al., 2008 [255]	Plasma	4 M perchloric acid	C18	97:3 Water:acetonitrile + 50 mM acetic acid + 100 mM Zinc acetate	N/A	Tryptophan-Kynurenine pathway
GC–MS	Zhao et al., 2017 [51]	Serum, Urine	Methanol - Derivatized with alkyl chloroformate	DB-5 MS	Helium, flow rate 1.0 mL/min	EI	Indole derivatives

isoforms 1 and 3 convert TMA into TMAO. The level of TMAO in the body is dependent upon factors such as diet, gender, natural flora in the gut, age, and medications [259]. High levels of TMAO are strongly correlated to the development of cardiovascular diseases, such as atherosclerosis, stroke, heart failure, and kidney disease [260].

The types of samples analyzed for TMAO quantification include plasma or urine [261,262]. A key consideration when quantifying TMAO is that levels differ between males and females, with males generally having lower TMAO levels than females [263].

TMAO is typically analyzed using LC-MS, with GC–MS and NMR methods used less commonly (Table 7, Table 8) [46,264]. One could even use colorimetric array-based sensors, which is newer and not as explored as other methods [265]. For LC-MS, the type of column used to quantify TMAO is typically a C18 column, though Boyce et al (2019) suggested higher resolution using a C18-PFP (pentafluorophenyl) column [46,266,267]. Another column commonly used is a HILIC column [262].

f. *N*-acyl-amides.

The microbiome creates *N*-acyl amides (NAAs) that closely mimic the function of endogenously produced signaling molecules, particularly endocannabinoids. The microbiome is capable of producing 6 families of *N*-acyl amides: *N*-acyl glycine, *N*-acyloxyacyl lysine, *N*-acyloxyacyl glutamine, *N*-acyl lysine/ornithine, *N*-acyl alanine, and *N*-acyl serinol [269]. These NAA mimics can serve as agonists or antagonists of G-protein-coupled receptors (GPCR). For instance, *Neisseria meningitidis*, the bacteria responsible for meningococcal disease, produces *N*-3-hydroxypalmitoyl-ornithine which serves selectively as an agonist for sphingosine-1-phosphate receptor 4 (S1PR4), a GPCR that has a role in human inflammatory responses [269,270].

Sample type can impact the NAAs detected in a study. While the majority of detected *N*-acyl homoserine molecules are present in serum, cecal content, and liver samples, some *N*-acyl homoserines are detected exclusively in one sample type, such as 3OH-C10-HSL only being detected in serum and C12-HSL only being detected in liver and cecal content [47]. As a result, when collecting in vivo samples, it may be beneficial to collect at least one biofluid and one tissue sample based on the NAA of interest.

NAAs can be detected from bacterial cultures or from tissue samples. For in vitro studies, a simple extraction can be performed using equal volumes of ethyl acetate followed by aspiration of ethyl acetate upon separation [269,271] or using n-butanol [272] (Table 9). For serum and tissues, samples are typically extracted and homogenized using cold methanol or acetonitrile and then purified using a C18 solid phase extraction column to reduce matrix effects [47,273,274]. After extraction and vacuum drying, NAA samples should be resuspended with 50 % to 100 % methanol. 100 % MeOH was the most commonly used extraction solvent, but different NAAs favor different ratios of MeOH to water [275]. Additionally, some researchers elect to acidify their resuspension solvent [276,277]. Acidification can help with the dissolution and ionization of charged NAAs as well as with sample stability, but can cause hydrolysis of any ester bonds present in molecules of interest if left at acidic conditions for prolonged periods [278].

NAAs have long lipid chains, so they have historically been analyzed using methods that are suitable for the detection of lipids, particularly LC-MS. For the LC separation, NAAs are analyzed using a C18 column due to the long acyl chains that are characteristic of the class. To correct for the effects of extraction, resuspension, and ESI ionization on quantification, labeled internal standards for the NAAs of interest should be suspended in the earliest extraction solvent (ng/mL scale [277,279,280]). Due to the large variety of NAAs, a labeled version of the NAA of interest might not always be available. In that case, the extraction solvent should contain at least one labeled NAA from each of the 6 families mentioned previously.

NAAs can be challenging to quantify. Some NAAs have shown clear signs of matrix suppression, making solid phase extraction a crucial step in the preparation of many NAAs [47]. Additionally, NAA concentration can be as low as pg/g of sample, making sensitive techniques or purification required in order to detect them [273,281]. NAAs are a broad class of molecules, as there are a large variety of acyl chains that each molecule can use as their acyl group. Some acyl groups may be unsaturated. While MS can demonstrate the presence of a double bond, elucidating the location of the double bond is challenging and better suited for a method like NMR. No extraction method will universally extract all NAAs well, so it is important to carefully consider which NAAs are of primary interest before selecting an extraction method.

Table 8
NMR-based trimethylamine N-oxide quantification methods.

Quantitation Method	Reference	Sample Type	Solutions	NMR	Molecule (s)
1H NMR	Garcia et al., 2017 [264]	Plasma	Aqueous citrate buffer (pH = 4.4)	400 MHz with a 4-mm indirect detection probe	TMAO

6. Conclusion

The microbiome has a vast role in host metabolism through its production and modification of small molecules. Growing knowledge of this role has led to increased needs for quantification of microbial metabolism, though challenges remain (Box 1). Samples should be acquired using a method that minimizes contaminants and stored to prevent their degradation, but the chosen methods may depend on the specific molecules being investigated. Many instruments and types of analysis are available for quantification, each with its own use cases and limitations. SCFAs and bile acids are some of the most commonly analyzed molecules, but other classes have biological relevance of their own. Furthermore, increasing implementation of untargeted approaches is

Table 7
MS-based trimethylamine N-oxide quantification methods.

Quantification Method	Reference	Sample Type	Extraction	Column	Mobile Phase	Ionization Method	Molecule (s)
LC-MS	Le et al., 2018 [46]	Serum	Acetonitrile	C18, C18-PFP, HILIC	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	TMAO
	Hefni et al., 2021 [262]	Urine	Water. Derivatizatized with iodoacetonitrile in acetonitrile:methanol (9:1. v/v)	HILIC	30:70 Water:acetonitrile + 25 mM ammonium formate	ESI - Positive mode	TMAO
		Plasma	Not diluted. Derivatizatized with iodoacetonitrile in acetonitrile:methanol (9:1. v/v)				
GC–MS	Bain et al., 2004 [268]	Plasma	Acidification with 0.01 M HCl before adding water. Addition of 5 M KOH to improve volatilization	SPB-1	Helium, flow rate 60 mL/min	EI	TMAO

Table 9
N-acyl amide quantification methods.

Quantification Method	Reference	Sample Type	Extraction	Column	Mobile Phase	Ionization Method	Molecule(s)
LC-MS	Cohen et al., 2017 [269]	Bacterial cultures	Ethyl acetate	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive and negative mode	*N*-acyl glycines, *N*-acyloxyacyl lysines, *N*-acyloxyacyl glutamines, *N*-acyl lysines/ornithines, *N*-acyl alanines, *N*-acyl serinols
	Tørring et al., 2017 [272]	Bacterial cultures	n-butanol	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	*N*-acyl histidines
	Xue et al., 2021 [47]	Serum	Methanol + 0.1 % formic acid. Mixture preconditioned with a C18 solid phase extraction column.	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	*N*-acyl homoserines
		Liver, Cecum	Methanol, followed by dichloromethane:methanol (1:1. v/v). Mixture preconditioned with a C18 solid phase extraction column.	C18	A: Water + 0.1 % formic acid. B: Acetonitrile + 0.1 % formic acid	ESI - Positive mode	*N*-acyl homoserines
	Tan et al., 2009 [273]	Brain	Methanol followed by water. Preconditioned with a C18 solid phase extraction column.	C18	A: 80:20 Water:methanol + 1 mM ammonium acetate. B: 100 % methanol + 1 mM ammonium acetate	ESI - Positive and negative mode	*N*-acyl amino acids

leading to the discovery of novel microbial metabolites.

This review focused on molecular classes produced by microbiome bacteria, but more is being discovered about metabolites produced by other microbiome members. For instance, the presence of the protozoa *Blastocystis* has been associated with higher abundance of archaea like *Methanobrevibacter smithii; M. smithii*, in turn, supports human health by removing end products of bacterial fermentation which supports glycan digestion and helps to increase butyrate production [282].

Microbial metabolism is an expansive topic given the complexities of its composition and methods of analysis. Indeed, there are many unique specialized metabolites that we were regretfully unable to cover here due to a lack of space, which include, but are not limited to piericidins [283,284], p-cresol [285,286], and exopolysaccharides [287,288]. While methods for quantifying these classes and others are not explicitly

Box 1
Challenges and opportunities

a) Coverage. Even though 'omics' pipelines aim to cover all analytes, this is not achievable in metabolomics. Combining approaches, including extraction and instrumental methods, will increase coverage but also cost and complexity of acquisition and processing.
b) Lack of consensus about methods. In parallel with the necessary requirement for diverse methods for different metabolite classes and for broad metabolite coverage, there is also sometimes considerable variation in methods implemented between research groups, even for the same metabolite class (see the multiple approaches described in this review's tables). This can make comparisons between studies and between research groups challenging, especially for untargeted studies or studies that implement only relative quantification in a targeted workflow.
c) Improved automation for sample preparation and metabolite extraction. Sample preparation for metabolomics experiments as well as metabolomics data acquisition steps lag behind sequencing preparation workflows in terms of automation. Improved automation at this step would increase reproducibility. This is a particular need with regards to tissue analysis.
d) Lack of consensus about data processing pipelines. In addition to variability in sample preparation, there are often considerable differences in data processing between groups.
e) Spatial distribution. Microbiome composition will differ between organs, sites, and even on the microscopic level. Likewise, microbial metabolism is context-dependent and will differ from the microscopic to the macroscopic scale. This represents a challenge, in that spatial analysis is required to obtain comprehensive information about microbiota metabolism. However, this spatiality is also an opportunity to better understand regulators of metabolism and how they affect health.
f) Annotation challenges. The discovery of novel microbial metabolites is hampered by database biases, which may favor mammalian metabolites to the detriment of microbial metabolites. However, there is currently considerable effort being expended to address this issue. Structure elucidation is often labor-intensive and costly, but this is critical to go beyond known molecules and increase biological insight.
g) Challenges of confirming a microbial source for a novel metabolite. Microbial metabolite production may be context-dependent. Thus, production may not always be observed in the artificial monoculture settings that are often used to confirm the producing organism. New culture techniques are helping address this issue.
h) Better integration between microbiome metabolomics and the natural product field, especially with regards to workflows for the discovery of novel microbial metabolites. A major strength of natural product chemistry is its focus on de novo elucidation of novel structures, including those produced by microorganisms. However, there is sometimes a lack of interaction between metabolome researchers and natural product chemists.
i) Lack of commercially available standards. This can hamper confirmation of structural annotations, as well as data normalization and cross-study comparisons.
j) Method cost. Most microbial metabolite quantification techniques are costly and may be labor-intensive. Cheaper methods including point of care approaches, test strips, and at-home options will be critical to ensure personalized medicine applications.

mentioned here, quantitative methods can be inferred from this review based on structural similarities. For example, in the case of p-cresol, methods highlighted for kynurenine and its derivatives would likely work well.

Tools at each step of the process are continuing to be developed, which will lead to improved application to medicine, especially with the growing need to tailor health approaches to the individual. As this area of research expands, more light will be shed on the best practices for microbiome metabolism quantification and its applicability to human health. A key ongoing and future step will be to go from quantification to functional insight, and to the development of new interventions.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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References

[1] F.-P.-J. Martin, M.-E. Dumas, Y. Wang, C. Legido-Quigley, I.K.S. Yap, H. Tang, S. Zirah, G.M. Murphy, O. Cloarec, J.C. Lindon, N. Sprenger, L.B. Fay, S. Kochhar, P. van Bladeren, E. Holmes, J.K. Nicholson, A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model, Mol. Syst. Biol. 3 (2007) 112.

[2] R. Vemuri, E.M. Shankar, M. Chieppa, R. Eri, K. Kavanagh, Beyond Just Bacteria: Functional Biomes in the Gut Ecosystem Including Virome, Mycobiome, Archaeome and Helminths, Microorganisms. 8 (2020), https://doi.org/10.3390/microorganisms8040483.

[3] N. Koppel, V. Maini Rekdal, E.P. Balskus, Chemical transformation of xenobiotics by the human gut microbiota, Science. 356 (2017), https://doi.org/10.1126/science.aag2770.

[4] W.R. Wikoff, A.T. Anfora, J. Liu, P.G. Schultz, S.A. Lesley, E.C. Peters, G. Siuzdak, Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites, Proc. Natl. Acad. Sci. u. s. a. 106 (2009) 3698–3703.

[5] J. Ueyama, M. Nadai, H. Kanazawa, M. Iwase, H. Nakayama, K. Hashimoto, T. Yokoi, K. Baba, K. Takagi, K. Takagi, T. Hasegawa, Endotoxin from various gram-negative bacteria has differential effects on function of hepatic cytochrome P450 and drug transporters, Eur. J. Pharmacol. 510 (2005) 127–134.

[6] J.F. Cryan, S.M. O'Mahony, The microbiome-gut-brain axis: from bowel to behavior, Neurogastroenterol. Motil. 23 (2011) 187–192.

[7] L. Zhang, Y. Wang, X. Xiayu, C. Shi, W. Chen, N. Song, X. Fu, R. Zhou, Y.-F. Xu, L. Huang, H. Zhu, Y. Han, C. Qin, Altered Gut Microbiota in a Mouse Model of Alzheimer's Disease, J. Alzheimers. Dis. 60 (2017) 1241–1257.

[8] T.L. Weir, D.K. Manter, A.M. Sheflin, B.A. Barnett, A.L. Heuberger, E.P. Ryan, Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults, PLoS One. 8 (2013) e70803.

[9] Y. Uchimura, T. Fuhrer, H. Li, M.A. Lawson, M. Zimmermann, B. Yilmaz, J. Zindel, F. Ronchi, M. Sorribas, S. Hapfelmeier, S.C. Ganal-Vonarburg, M. Gomez de Agüero, K.D. McCoy, U. Sauer, A.J. Macpherson, Antibodies Set Boundaries Limiting Microbial Metabolite Penetration and the Resultant Mammalian Host Response, Immunity. 49 (2018) 545–559.e5.

[10] D.S. Wishart, E. Oler, H. Peters, A. Guo, S. Girod, S. Han, S. Saha, V.W. Lui, M. LeVatte, V. Gautam, R. Kaddurah-Daouk, N. Karu, MiMeDB: the Human Microbial Metabolome Database, Nucleic Acids Res. 51 (2023) D611–D620.

[11] R.A. Quinn, A.V. Melnik, A. Vrbanac, T. Fu, K.A. Patras, M.P. Christy, Z. Bodai, P. Belda-Ferre, A. Tripathi, L.K. Chung, M. Downes, R.D. Welch, M. Quinn, G. Humphrey, M. Panitchpakdi, K.C. Weldon, A. Aksenov, R. da Silva, J. Avila-Pacheco, C. Clish, S. Bae, H. Mallick, E.A. Franzosa, J. Lloyd-Price, R. Bussell, T. Thron, A.T. Nelson, M. Wang, E. Leszczynski, F. Vargas, J.M. Gauglitz, M. J. Meehan, E. Gentry, T.D. Arthur, A.C. Komor, O. Poulsen, B.S. Boland, J. T. Chang, W.J. Sandborn, M. Lim, N. Garg, J.C. Lumeng, R.J. Xavier, B. I. Kazmierczak, R. Jain, M. Egan, K.E. Rhee, D. Ferguson, M. Raffatellu, H. Vlamakis, G.G. Haddad, D. Siegel, C. Huttenhower, S.K. Mazmanian, R. M. Evans, V. Nizet, R. Knight, P.C. Dorrestein, Global chemical effects of the microbiome include new bile-acid conjugations, Nature. 579 (2020) 123–129.

[12] L.N. Lucas, K. Barrett, R.L. Kerby, Q. Zhang, L.E. Cattaneo, D. Stevenson, F.E. Rey, D. Amador-Noguez, Dominant Bacterial Phyla from the Human Gut Show Widespread Ability To Transform and Conjugate Bile Acids, mSystems. (2021) e0080521.

[13] M. Wang, A.K. Jarmusch, F. Vargas, A.A. Aksenov, J.M. Gauglitz, K. Weldon, D. Petras, R. da Silva, R. Quinn, A.V. Melnik, J.J.J. van der Hooft, A.M. Caraballo-Rodríguez, L.F. Nothias, C.M. Aceves, M. Panitchpakdi, E. Brown, F. Di Ottavio, N. Sikora, E.O. Elijah, L. Labarta-Bajo, E.C. Gentry, S. Shalapour, K.E. Kyle, S. P. Puckett, J.D. Watrous, C.S. Carpenter, A. Bouslimani, M. Ernst, A.D. Swafford, E.I. Zúñiga, M.J. Balunas, J.L. Klassen, R. Loomba, R. Knight, N. Bandeira, P. C. Dorrestein, Mass spectrometry searches using MASST, Nat. Biotechnol. 38 (2020) 23–26.

[14] Z. Wang, E. Klipfell, B.J. Bennett, R. Koeth, B.S. Levison, B. Dugar, A.E. Feldstein, E.B. Britt, X. Fu, Y.-M. Chung, Y. Wu, P. Schauer, J.D. Smith, H. Allayee, W.H. W. Tang, J.A. DiDonato, A.J. Lusis, S.L. Hazen, Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease, Nature. 472 (2011) 57–63.

[15] C.A. Cason, K.T. Dolan, G. Sharma, M. Tao, R. Kulkarni, I.B. Helenowski, B. M. Doane, M.J. Avram, M.M. McDermott, E.B. Chang, C.K. Ozaki, K.J. Ho, Plasma microbiome-modulated indole- and phenyl-derived metabolites associate with advanced atherosclerosis and postoperative outcomes, J. Vasc. Surg. 68 (2018) 1552–1562.

[16] S. Cussotto, I. Delgado, A. Anesi, S. Dexpert, A. Aubert, C. Beau, D. Forestier, P. Ledaguenel, E. Magne, F. Mattivi, L. Capuron, Tryptophan Metabolic Pathways Are Altered in Obesity and Are Associated With Systemic Inflammation, Front. Immunol. 11 (2020) 557.

[17] M.R. Wilson, Y. Jiang, P.W. Villalta, A. Stornetta, P.D. Boudreau, A. Carrá, C. A. Brennan, E. Chun, L. Ngo, L.D. Samson, B.P. Engelward, W.S. Garrett, S. Balbo, E.P. Balskus, The human gut bacterial genotoxin colibactin alkylates DNA, Science. 363 (2019), https://doi.org/10.1126/science.aar7785.

[18] R.S. Mehta, J.R. Mayers, Y. Zhang, A. Bhosle, N.R. Glasser, L.H. Nguyen, W. Ma, S. Bae, T. Branck, K. Song, L. Sebastian, J.A. Pacheco, H.-S. Seo, C. Clish, S. Dhe-Paganon, A.N. Ananthakrishnan, E.A. Franzosa, E.P. Balskus, A.T. Chan, C. Huttenhower, Gut microbial metabolism of 5-ASA diminishes its clinical efficacy in inflammatory bowel disease, Nat. Med. 29 (2023) 700–709.

[19] P. Spanogiannopoulos, T.S. Kyaw, B.G.H. Guthrie, P.H. Bradley, J.V. Lee, J. Melamed, Y.N.A. Malig, K.N. Lam, D. Gempis, M. Sandy, W. Kidder, E.L. Van Blarigan, C.E. Atreya, A. Venook, R.R. Gerona, A. Goga, K.S. Pollard, P. J. Turnbaugh, Host and gut bacteria share metabolic pathways for anti-cancer drug metabolism, Nat Microbiol. 7 (2022) 1605–1620.

[20] Z. He, L. Chen, J. Catalan-Dibene, G. Bongers, J.J. Faith, C. Suebsuwong, R. J. DeVita, Z. Shen, J.G. Fox, J.J. Lafaille, G.C. Furtado, S.A. Lira, Food colorants metabolized by commensal bacteria promote colitis in mice with dysregulated expression of interleukin-23, Cell Metab. 33 (2021) 1358–1371.

[21] M. Zimmermann, M. Zimmermann-Kogadeeva, R. Wegmann, A.L. Goodman, Separating host and microbiome contributions to drug pharmacokinetics and toxicity, Science. 363 (2019), https://doi.org/10.1126/science.aat9931.

[22] J. Gratton, J. Phetcharaburanin, B.H. Mullish, H.R.T. Williams, M. Thursz, J. K. Nicholson, E. Holmes, J.R. Marchesi, J.V. Li, Optimized Sample Handling Strategy for Metabolic Profiling of Human Feces, Anal. Chem. 88 (2016) 4661–4668.

[23] L.C. Phua, P.K. Koh, P.Y. Cheah, H.K. Ho, E.C.Y. Chan, Global gas chromatography/time-of-flight mass spectrometry (GC/TOFMS)-based metabonomic profiling of lyophilized human feces, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 937 (2013) 103–113.

[24] M. Rotter, S. Brandmaier, C. Prehn, J. Adam, S. Rabstein, K. Gawrych, T. Brüning, T. Illig, H. Lickert, J. Adamski, R. Wang-Sattler, Stability of targeted metabolite profiles of urine samples under different storage conditions, Metabolomics. 13 (2017) 4.

[25] G. Anton, R. Wilson, Z.-H. Yu, C. Prehn, S. Zukunft, J. Adamski, M. Heier, C. Meisinger, W. Römisch-Margl, R. Wang-Sattler, K. Hveem, B. Wolfenbuttel, A. Peters, G. Kastenmüller, M. Waldenberger, Pre-analytical sample quality: metabolite ratios as an intrinsic marker for prolonged room temperature exposure of serum samples, PLoS One. 10 (2015) e0121495.

[26] C.J. Bolten, P. Kiefer, F. Letisse, J.-C. Portais, C. Wittmann, Sampling for Metabolome Analysis of Microorganisms, Analytical Chemistry. 79 (2007) 3843–3849, https://doi.org/10.1021/ac0623888.

[27] T. Fuhrer, D. Heer, B. Begemann, N. Zamboni, High-Throughput, Accurate Mass Metabolome Profiling of Cellular Extracts by Flow Injection–Time-of-Flight Mass Spectrometry, Analytical Chemistry. 83 (2011) 7074–7080, https://doi.org/10.1021/ac201267k.

[28] J.C. Ewald, S. Heux, N. Zamboni, High-Throughput Quantitative Metabolomics: Workflow for Cultivation, Quenching, and Analysis of Yeast in a Multiwell Format, Analytical Chemistry. 81 (2009) 3623–3629, https://doi.org/10.1021/ac900002u.

[29] P. Yin, A. Peter, H. Franken, X. Zhao, S.S. Neukamm, L. Rosenbaum, M. Lucio, A. Zell, H.-U. Häring, G. Xu, R. Lehmann, Preanalytical aspects and sample quality assessment in metabolomics studies of human blood, Clin. Chem. 59 (2013) 833–845.

[30] E. Loftfield, E. Vogtmann, J.N. Sampson, S.C. Moore, H. Nelson, R. Knight, N. Chia, R. Sinha, Comparison of Collection Methods for Fecal Samples for Discovery Metabolomics in Epidemiologic Studies, Cancer Epidemiol. Biomarkers Prev. 25 (2016) 1483–1490.

[31] Z. Wang, C.P. Zolnik, Y. Qiu, M. Usyk, T. Wang, H.D. Strickler, C.R. Isasi, R. C. Kaplan, I.J. Kurland, Q. Qi, R.D. Burk, Comparison of Fecal Collection Methods

for Microbiome and Metabolomics Studies, Front. Cell. Infect. Microbiol. 8 (2018) 301.
[32] R. Sinha, J. Chen, A. Amir, E. Vogtmann, J. Shi, K.S. Inman, R. Flores, J. Sampson, R. Knight, N. Chia, Collecting Fecal Samples for Microbiome Analyses in Epidemiology Studies, Cancer Epidemiol. Biomarkers Prev. 25 (2016) 407–416.
[33] A.E. Kelly, H.D. Ou, R. Withers, V. Dötsch, Low-conductivity buffers for high-sensitivity NMR measurements, J. Am. Chem. Soc. 124 (2002) 12013–12019.
[34] S. Ramamoorthy, S. Levy, M. Mohamed, A. Abdelghani, A.M. Evans, L.A. D. Miller, L. Mehta, S. Moore, E. Freinkman, S.K. Hourigan, An ambient-temperature storage and stabilization device performs comparably to flash-frozen collection for stool metabolomics in infants, BMC Microbiol. 21 (2021) 59.
[35] P. Denniff, N. Spooner, Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis, Anal. Chem. 86 (2014) 8489–8495.
[36] Y. Qu, K. Brady, R. Apilado, T. O'Malley, S. Reddy, P. Chitkara, C. Ibarra, R. V. Alexander, T. Dervieux, Capillary blood collected on volumetric absorptive microsampling (VAMS) device for monitoring hydroxychloroquine in rheumatoid arthritis patients, J. Pharm. Biomed. Anal. 140 (2017) 334–341.
[37] C. Volani, G. Caprioli, G. Calderisi, B.B. Sigurdsson, J. Rainer, I. Gentilini, A. A. Hicks, P.P. Pramstaller, G. Weiss, S.V. Smarason, G. Paglia, Pre-analytic evaluation of volumetric absorptive microsampling and integration in a mass spectrometry-based metabolomics workflow, Anal. Bioanal. Chem. 409 (2017) 6263–6276.
[38] M.G.M. Kok, C. Nix, G. Nys, M. Fillet, Targeted metabolomics of whole blood using volumetric absorptive microsampling, Talanta. 197 (2019) 49–58.
[39] M. Protti, R. Mandrioli, L. Mercolini, Tutorial: Volumetric absorptive microsampling (VAMS), Anal. Chim. Acta. 1046 (2019) 32–47.
[40] M. Protti, M.C. Catapano, B.G. Samolsky Dekel, J. Rudge, G. Gerra, L. Somaini, R. Mandrioli, L. Mercolini, Determination of oxycodone and its major metabolites in haematic and urinary matrices: Comparison of traditional and miniaturised sampling approaches, J. Pharm. Biomed. Anal. 152 (2018) 204–214.
[41] J. Saric, Y. Wang, J. Li, M. Coen, J. Utzinger, J.R. Marchesi, J. Keiser, K. Veselkov, J.C. Lindon, J.K. Nicholson, E. Holmes, Species variation in the fecal metabolome gives insight into differential gastrointestinal function, J. Proteome Res. 7 (2008) 352–360.
[42] V.L. Stevens, E. Hoover, Y. Wang, K.A. Zanetti, Pre-Analytical Factors that Affect Metabolite Stability in Human Urine, Plasma, and Serum: A Review, Metabolites. 9 (2019), https://doi.org/10.3390/metabo9080156.
[43] F. Ottosson, F. Russo, A. Abrahamsson, N. MacSween, J. Courraud, Z.K. Nielsen, D.M. Hougaard, A.S. Cohen, M. Ernst, Effects of Long-Term Storage on the Biobanked Neonatal Dried Blood Spot Metabolome, J. Am. Soc. Mass Spectrom. 34 (2023) 685–694.
[44] C.Z. Ulmer, J.P. Koelmel, C.M. Jones, T.J. Garrett, J.J. Aristizabal-Henao, H. W. Vesper, J.A. Bowden, A Review of Efforts to Improve Lipid Stability during Sample Preparation and Standardization Efforts to Ensure Accuracy in the Reporting of Lipid Measurements, Lipids. 56 (2021) 3–16.
[45] A. Marcobal, P.C. Kashyap, T.A. Nelson, P.A. Aronov, M.S. Donia, A. Spormann, M.A. Fischbach, J.L. Sonnenburg, A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice, ISME J. 7 (2013) 1933–1943.
[46] T.T. Le, A. Shafaei, A. Genoni, C. Christophersen, A. Devine, J. Lo, P.L. Wall, M. C. Boyce, Development and validation of a simple LC-MS/MS method for the simultaneous quantitative determination of trimethylamine-N-oxide and branched chain amino acids in human serum, Anal. Bioanal. Chem. 411 (2018) 1019–1028.
[47] J. Xue, L. Chi, P. Tu, Y. Lai, C.-W. Liu, H. Ru, K. Lu, Detection of gut microbiota and pathogen produced N-acyl homoserine in host circulation and tissues, NPJ Biofilms Microbiomes. 7 (2021) 53.
[48] S. De Baere, V. Eeckhaut, M. Steppe, C. De Maesschalck, P. De Backer, F. Van Immerseel, S. Croubels, Development of a HPLC–UV method for the quantitative determination of four short-chain fatty acids and lactic acid produced by intestinal bacteria during in vitro fermentation, J. Pharm. Biomed. Anal. 80 (2013) 107–115.
[49] C. Zhang, P. Tang, H. Xu, Y. Weng, Q. Tang, H. Zhao, Analysis of Short-Chain Fatty Acids in Fecal Samples by Headspace-Gas Chromatography, Chromatographia. 81 (2018) 1317–1323.
[50] R. Zhao, L. Chu, Y. Wang, Y. Song, P. Liu, C. Li, J. Huang, X. Kang, Application of packed-fiber solid-phase extraction coupled with GC–MS for the determination of short-chain fatty acids in children's urine, Clin. Chim. Acta. 468 (2017) 120–125.
[51] L. Zhao, Y. Ni, M. Su, H. Li, F. Dong, W. Chen, R. Wei, L. Zhang, S.P. Guiraud, F.-P. Martin, C. Rajani, G. Xie, W. Jia, High Throughput and Quantitative Measurement of Microbial Metabolome by Gas Chromatography/Mass Spectrometry Using Automated Alkyl Chloroformate Derivatization, Anal. Chem. 89 (2017) 5565–5577.
[52] F. Dong, F. Hao, I.A. Murray, P.B. Smith, I. Koo, A.M. Tindall, P.M. Kris-Etherton, K. Gowda, S.G. Amin, A.D. Patterson, G.H. Perdew, Intestinal microbiota-derived tryptophan metabolites are predictive of Ah receptor activity, Gut Microbes. 12 (2020) 1–24.
[53] I. Sadok, A. Gamian, M.M. Staniszewska, Chromatographic analysis of tryptophan metabolites, J. Sep. Sci. 40 (2017) 3020–3045.
[54] D.J. Beale, F.R. Pinu, K.A. Kouremenos, M.M. Poojary, V.K. Narayana, B. A. Boughton, K. Kanojia, S. Dayalan, O.A.H. Jones, D.A. Dias, Review of recent developments in GC-MS approaches to metabolomics-based research, Metabolomics. 14 (2018) 152.
[55] K. Xu, Y. Guo, Y. Wang, Y. Ren, V. Low, S. Cho, L. Ping, K. Peng, X. Li, Y. Qiu, Q. Liu, Z. Li, Z. Wang, Decreased Enterobacteriaceae translocation due to gut microbiota remodeling mediates the alleviation of premature aging by a high-fat diet, Aging Cell. 22 (2023) e13760.
[56] I.P. Gerothanassis, A. Troganis, V. Exarchou, K. Barbarossou, NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY: BASIC PRINCIPLES AND PHENOMENA, AND THEIR APPLICATIONS TO CHEMISTRY, BIOLOGY AND MEDICINE, Chem. Educ. Res. Pract. 3 (2002) 229–252.
[57] L. Bervoets, J.H. Ippel, A. Smolinska, N. van Best, P.H.M. Savelkoul, M.A. H. Mommers, J. Penders, Practical and Robust NMR-Based Metabolic Phenotyping of Gut Health in Early Life, J. Proteome Res. 20 (2021) 5079–5087.
[58] S. Lamichhane, C.C. Yde, M.S. Schmedes, H.M. Jensen, S. Meier, H.C. Bertram, Strategy for Nuclear-Magnetic-Resonance-Based Metabolomics of Human Feces, Anal. Chem. 87 (2015) 5930–5937.
[59] Y. Iwasaki, T. Sawada, K. Hatayama, A. Ohyagi, Y. Tsukuda, K. Namekawa, R. Ito, K. Saito, H. Nakazawa, Separation technique for the determination of highly polar metabolites in biological samples, Metabolites. 2 (2012) 496–515.
[60] G.A. Nagana Gowda, Y.N. Gowda, D. Raftery, Expanding the limits of human blood metabolite quantitation using NMR spectroscopy, Anal. Chem. 87 (2015) 706–715.
[61] M. Mora-Ortiz, P. Nuñez Ramos, A. Oregioni, S.P. Claus, NMR metabolomics identifies over 60 biomarkers associated with Type II Diabetes impairment in db/db mice, Metabolomics. 15 (2019) 1–16.
[62] S.K. Bharti, R. Roy, Quantitative 1H NMR spectroscopy, Trends, Analyt. Chem. 35 (2012) 5–26.
[63] S. Kostidis, R.D. Addie, H. Morreau, O.A. Mayboroda, M. Giera, Quantitative NMR analysis of intra- and extracellular metabolism of mammalian cells: A tutorial, Anal. Chim. Acta. 980 (2017) 1–24.
[64] A.-H.-M. Emwas, The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research, Methods Mol. Biol. 1277 (2015) 161–193.
[65] R. Gruetter, S.A. Weisdorf, V. Rajanayagan, M. Terpstra, H. Merkle, C.L. Truwit, M. Garwood, S.L. Nyberg, K. Ugurbil, Resolution Improvements in in Vivo 1H NMR Spectra with Increased Magnetic Field Strength, J. Magn. Reson. 135 (1998) 260–264.
[66] H.C. Keun, O. Beckonert, J.L. Griffin, C. Richter, D. Moskau, J.C. Lindon, J. K. Nicholson, Cryogenic probe 13C NMR spectroscopy of urine for metabonomic studies, Anal. Chem. 74 (2002) 4588–4593.
[67] J.H. Grimes, T.M. O'Connell, The application of micro-coil NMR probe technology to metabolomics of urine and serum, J. Biomol. NMR. 49 (2011) 297–305.
[68] M. Ferrer, B.A. Raczkowska, M. Martínez-Martínez, C. Barbas, D. Rojo, Phenotyping of gut microbiota: Focus on capillary electrophoresis, Electrophoresis. 38 (2017) 2275–2286.
[69] E. Mishima, S. Fukuda, C. Mukawa, A. Yuri, Y. Kanemitsu, Y. Matsumoto, Y. Akiyama, N.N. Fukuda, H. Tsukamoto, K. Asaji, H. Shima, K. Kikuchi, C. Suzuki, T. Suzuki, Y. Tomioka, T. Soga, S. Ito, T. Abe, Evaluation of the impact of gut microbiota on uremic solute accumulation by a CE-TOFMS-based metabolomics approach, Kidney Int. 92 (2017) 634–645.
[70] P. Pruski, G.D.S. Correia, H.V. Lewis, K. Capuccini, P. Inglese, D. Chan, R. G. Brown, L. Kindinger, Y.S. Lee, A. Smith, J. Marchesi, J.A.K. McDonald, S. Cameron, K. Alexander-Hardiman, A.L. David, S.J. Stock, J.E. Norman, V. Terzidou, T.G. Teoh, L. Sykes, P.R. Bennett, Z. Takats, D.A. MacIntyre, Direct on-swab metabolic profiling of vaginal microbiome host interactions during pregnancy and preterm birth, Nat. Commun. 12 (2021) 5967.
[71] C. Ayala-Ortiz, N. Graf-Grachet, V. Freire-Zapata, J. Fudyma, G. Hildebrand, R. AminiTabrizi, C. Howard-Varona, Y.E. Corilo, N. Hess, M.B. Duhaime, M. B. Sullivan, M.M. Tfaily, MetaboDirect: an analytical pipeline for the processing of FT-ICR MS-based metabolomic data, Microbiome. 11 (2023) 28.
[72] W.J. Moree, V.V. Phelan, C.-H. Wu, N. Bandeira, D.S. Cornett, B.M. Duggan, P. C. Dorrestein, Interkingdom metabolic transformations captured by microbial imaging mass spectrometry, Proc. Natl. Acad. Sci. u. s. a. 109 (2012) 13811–13816.
[73] S. Sarmad, M.R. Viant, W.B. Dunn, R. Goodacre, I.D. Wilson, K.E. Chappell, J. L. Griffin, V.B. O'Donnell, B. Naicker, M.R. Lewis, T. Suzuki, A proposed framework to evaluate the quality and reliability of targeted metabolomics assays from the UK Consortium on Metabolic Phenotyping (MAP/UK), Nat. Protoc. 18 (2023) 1017–1027.
[74] J.A. Kirwan, H. Gika, R.D. Beger, D. Bearden, W.B. Dunn, R. Goodacre, G. Theodoridis, M. Witting, L.-R. Yu, I.D. Wilson, metabolomics Quality Assurance and Quality Control Consortium (mQACC), Quality assurance and quality control reporting in untargeted metabolic phenotyping: mQACC recommendations for analytical quality management, Metabolomics. 18 (2022) 70.
[75] A. Bauermeister, H. Mannochio-Russo, L.V. Costa-Lotufo, A.K. Jarmusch, P. C. Dorrestein, Mass spectrometry-based metabolomics in microbiome investigations, Nat. Rev. Microbiol. 20 (2022) 143–160.
[76] J. Debik, M. Sangermani, F. Wang, T.S. Madssen, G.F. Giskeødegård, Multivariate analysis of NMR-based metabolomic data, NMR Biomed. 35 (2022) e4638.
[77] S. Alseekh, A. Aharoni, Y. Brotman, K. Contrepois, J. D'Auria, J. Ewald, J. C. Ewald, P.D. Fraser, P. Giavalisco, R.D. Hall, M. Heinemann, H. Link, J. Luo, S. Neumann, J. Nielsen, L. Perez de Souza, K. Saito, U. Sauer, F.C. Schroeder, S. Schuster, G. Siuzdak, A. Skirycz, L.W. Sumner, M.P. Snyder, H. Tang, T. Tohge, Y. Wang, W. Wen, S. Wu, G. Xu, N. Zamboni, A.R. Fernie, Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices, Nat. Methods. 18 (2021) 747–756.
[78] J. Van Der Hooft, K. Hanhineva, Metabolomics Data Processing and Data Analysis-Current Best Practices, Mdpi AG, 2021.

[79] Z. Pang, G. Zhou, J. Ewald, L. Chang, O. Hacariz, N. Basu, J. Xia, Using MetaboAnalyst 5.0 for LC-HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data, Nat. Protoc. 17 (2022) 1735–1761.
[80] J.C. Poland, K.L. Leaptrot, S.D. Sherrod, C.R. Flynn, J.A. McLean, Collision Cross Section Conformational Analyses of Bile Acids via Ion Mobility-Mass Spectrometry, J. Am. Soc. Mass Spectrom. (2020), https://doi.org/10.1021/jasms.0c00015.
[81] R. Schmid, S. Heuckeroth, A. Korf, A. Smirnov, O. Myers, T.S. Dyrlund, R. Bushuiev, K.J. Murray, N. Hoffmann, M. Lu, A. Sarvepalli, Z. Zhang, M. Fleischauer, K. Dührkop, M. Wesner, S.J. Hoogstra, E. Rudt, O. Mokshyna, C. Brungs, K. Ponomarov, L. Mutabdžija, T. Damiani, C.J. Pudney, M. Earll, P. O. Helmer, T.R. Fallon, T. Schulze, A. Rivas-Ubach, A. Bilbao, H. Richter, L.-F. Nothias, M. Wang, M. Orešič, J.-K. Weng, S. Böcker, A. Jeibmann, H. Hayen, U. Karst, P.C. Dorrestein, D. Petras, X. Du, T. Pluskal, Integrative analysis of multimodal mass spectrometry data in MZmine 3, Nat. Biotechnol. (2023), https://doi.org/10.1038/s41587-023-01690-2.
[82] R. Tautenhahn, C. Böttcher, S. Neumann, Highly sensitive feature detection for high resolution LC/MS, BMC Bioinformatics. 9 (2008) 504.
[83] H.L. Röst, T. Sachsenberg, S. Aiche, C. Bielow, H. Weisser, F. Aicheler, S. Andreotti, H.-C. Ehrlich, P. Gutenbrunner, E. Kenar, X. Liang, S. Nahnsen, L. Nilse, J. Pfeuffer, G. Rosenberger, M. Rurik, U. Schmitt, J. Veit, M. Walzer, D. Wojnar, W.E. Wolski, O. Schilling, J.S. Choudhary, L. Malmström, R. Aebersold, K. Reinert, O. Kohlbacher, OpenMS: a flexible open-source software platform for mass spectrometry data analysis, Nat. Methods. 13 (2016) 741–748.
[84] H. Tsugawa, T. Cajka, T. Kind, Y. Ma, B. Higgins, K. Ikeda, M. Kanazawa, J. VanderGheynst, O. Fiehn, M. Arita, MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis, Nat. Methods. 12 (2015) 523–526.
[85] L.K. Pino, B.C. Searle, J.G. Bollinger, B. Nunn, B. MacLean, M.J. MacCoss, The Skyline ecosystem: Informatics for quantitative mass spectrometry proteomics, Mass Spectrom. Rev. 39 (2020) 229–244.
[86] G. Teo, W.S. Chew, B.J. Burla, D. Herr, E.S. Tai, M.R. Wenk, F. Torta, H. Choi, MRMkit: Automated Data Processing for Large-Scale Targeted Metabolomics Analysis, Anal. Chem. 92 (2020) 13677–13682.
[87] R. Tautenhahn, G.J. Patti, D. Rinehart, G. Siuzdak, X.C.M.S. Online, a web-based platform to process untargeted metabolomic data, Anal. Chem. 84 (2012) 5035–5039.
[88] K.J. Adams, B. Pratt, N. Bose, L.G. Dubois, L. St John-Williams, K.M. Perrott, K. Ky, P. Kapahi, V. Sharma, M.J. MacCoss, M.A. Moseley, C.A. Colton, B. X. MacLean, B. Schilling, J.W. Thompson, Alzheimer's Disease Metabolomics Consortium, Skyline for Small Molecules: A Unifying Software Package for Quantitative Metabolomics, J. Proteome Res. 19 (2020) 1447–1458.
[89] B.N. Colby, Spectral deconvolution for overlapping GC/MS components, J. Am. Soc. Mass Spectrom. 3 (1992) 558–562.
[90] R.G. Dromey, M.J. Stefik, T.C. Rindfleisch, A.M. Duffield, Extraction of Mass Spectra Free of Background and Neighboring Component Contributions from Gas Chromatography/mass Spectrometry Data, Anal. Chem. 48 (1976) 1368–1375.
[91] A.A. Aksenov, I. Laponogov, Z. Zhang, S.L.F. Doran, I. Belluomo, D. Veselkov, W. Bittremieux, L.F. Nothias, M. Nothias-Esposito, K.N. Maloney, B.B. Misra, A. V. Melnik, A. Smirnov, X. Du, K.L. Jones 2nd, K. Dorrestein, M. Panitchpakdi, M. Ernst, J.J.J. van der Hooft, M. Gonzalez, C. Carazzone, A. Amézquita, C. Callewaert, J.T. Morton, R.A. Quinn, A. Bouslimani, A.A. Orio, D. Petras, A. M. Smania, S.P. Couvillion, M.C. Burnet, C.D. Nicora, E. Zink, T.O. Metz, V. Artaev, E. Humston-Fulmer, R. Gregor, M.M. Meijler, I. Mizrahi, S. Eyal, B. Anderson, R. Dutton, R. Lugan, P.L. Boulch, Y. Guitton, S. Prevost, A. Poirier, G. Dervilly, B. Le Bizec, A. Fait, N.S. Persi, C. Song, K. Gashu, R. Coras, M. Guma, J. Manasson, J.U. Scher, D.K. Barupal, S. Alseekh, A.R. Fernie, R. Mirnezami, V. Vasiliou, R. Schmid, R.S. Borisov, L.N. Kulikova, R. Knight, M. Wang, G. B. Hanna, P.C. Dorrestein, K. Veselkov, Auto-deconvolution and molecular networking of gas chromatography-mass spectrometry data, Nat. Biotechnol. 39 (2021) 169–173.
[92] E. Stancliffe, M. Schwaiger-Haber, M. Sindelar, G.J. Patti, DecoID improves identification rates in metabolomics through database-assisted MS/MS deconvolution, Nat. Methods. 18 (2021) 779–787.
[93] C.-T. Wu, Y. Wang, Y. Wang, T. Ebbels, I. Karaman, G. Graça, R. Pinto, D. M. Herrington, Y. Wang, G. Yu, Targeted realignment of LC-MS profiles by neighbor-wise compound-specific graphical time warping with misalignment detection, Bioinformatics. 36 (2020) 2862–2871.
[94] T. Välikangas, T. Suomi, L.L. Elo, A systematic evaluation of normalization methods in quantitative label-free proteomics, Brief. Bioinform. 19 (2018) 1–11.
[95] M. Sysi-Aho, M. Katajamaa, L. Yetukuri, M. Oresic, Normalization method for metabolomics data using optimal selection of multiple internal standards, BMC Bioinformatics. 8 (2007) 93.
[96] S.-O. Deininger, D.S. Cornett, R. Paape, M. Becker, C. Pineau, S. Rauser, A. Walch, E. Wolski, Normalization in MALDI-TOF imaging datasets of proteins: practical considerations, Anal. Bioanal. Chem. 401 (2011) 167–181.
[97] F. Dieterle, A. Ross, G. Schlotterbeck, H. Senn, Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics, Anal. Chem. 78 (2006) 4281–4290.
[98] K. Deng, F. Zhao, Z. Rong, L. Cao, L. Zhang, K. Li, Y. Hou, Z.-J. Zhu, WaveICA 2.0: a novel batch effect removal method for untargeted metabolomics data without using batch information, Metabolomics. 17 (2021) 87.
[99] T. Kim, O. Tang, S.T. Vernon, K.A. Kott, Y.C. Koay, J. Park, D.E. James, S. M. Grieve, T.P. Speed, P. Yang, G.A. Figtree, J.F. O'Sullivan, J.Y.H. Yang, A hierarchical approach to removal of unwanted variation for large-scale metabolomics data, Nat. Commun. 12 (2021) 4992.
[100] C. Schiffman, L. Petrick, K. Perttula, Y. Yano, H. Carlsson, T. Whitehead, C. Metayer, J. Hayes, S. Rappaport, S. Dudoit, Filtering procedures for untargeted LC-MS metabolomics data, BMC Bioinformatics. 20 (2019) 334.
[101] Š. Kouřil, J. de Sousa, J. Václavík, D. Friedecký, T. Adam, CROP: correlation-based reduction of feature multiplicities in untargeted metabolomic data, Bioinformatics. 36 (2020) 2941–2942.
[102] K. Chetnik, L. Petrick, G. Pandey, MetaClean: a machine learning-based classifier for reduced false positive peak detection in untargeted LC-MS metabolomics data, Metabolomics. 16 (2020) 117.
[103] Y. Gloaguen, J.A. Kirwan, D. Beule, Deep Learning-Assisted Peak Curation for Large-Scale LC-MS Metabolomics, Anal. Chem. 94 (2022) 4930–4937.
[104] B.Y. Loulou Peisl, E.L. Schymanski, P. Wilmes, Dark matter in host-microbiome metabolomics: Tackling the unknowns–A review, Anal. Chim. Acta. 1037 (2018) 13–27.
[105] R.R. da Silva, P.C. Dorrestein, R.A. Quinn, Illuminating the dark matter in metabolomics, Proc. Natl. Acad. Sci. u. s. a. 112 (2015) 12549–12550.
[106] R. Liu, J. Hong, X. Xu, Q. Feng, D. Zhang, Y. Gu, J. Shi, S. Zhao, W. Liu, X. Wang, H. Xia, Z. Liu, B. Cui, P. Liang, L. Xi, J. Jin, X. Ying, X. Wang, X. Zhao, W. Li, H. Jia, Z. Lan, F. Li, R. Wang, Y. Sun, M. Yang, Y. Shen, Z. Jie, J. Li, X. Chen, H. Zhong, H. Xie, Y. Zhang, W. Gu, X. Deng, B. Shen, X. Xu, H. Yang, G. Xu, Y. Bi, S. Lai, J. Wang, L. Qi, L. Madsen, J. Wang, G. Ning, K. Kristiansen, W. Wang, Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention, Nat. Med. 23 (2017) 859–868.
[107] M.L. Santoru, C. Piras, A. Murgia, V. Palmas, T. Camboni, S. Liggi, I. Ibba, M. A. Lai, S. Orrù, S. Blois, A.L. Loizedda, J.L. Griffin, P. Usai, P. Caboni, L. Atzori, A. Manzin, Cross sectional evaluation of the gut-microbiome metabolome axis in an Italian cohort of IBD patients, Sci. Rep. 7 (2017) 9523.
[108] Y. Sun, Y. Su, W. Zhu, Microbiome-Metabolome Responses in the Cecum and Colon of Pig to a High Resistant Starch Diet, Front. Microbiol. 7 (2016) 779.
[109] F. Zhang, W. Zheng, Y. Xue, W. Yao, Suhuai suckling piglet hindgut microbiome-metabolome responses to different dietary copper levels, Appl. Microbiol. Biotechnol. 103 (2019) 853–868.
[110] J. Chong, J. Xia, Computational Approaches for Integrative Analysis of the Metabolome and Microbiome, Metabolites. 7 (2017), https://doi.org/10.3390/metabo7040062.
[111] G.B. Gloor, J.M. Macklaim, V. Pawlowsky-Glahn, J.J. Egozcue, Microbiome Datasets Are Compositional: And This Is Not Optional, Front. Microbiol. 8 (2017) 2224.
[112] A. Kalivodová, K. Hron, P. Filzmoser, L. Najdekr, H. Janečková, T. Adam, PLS-DA for compositional data with application to metabolomics, J. Chemom. 29 (2015) 21–28.
[113] B. Zhang, S. Horvath, A general framework for weighted gene co-expression network analysis, Stat. Appl. Genet. Mol. Biol. 4 (2005), https://doi.org/10.2202/1544-6115.1128.
[114] J. Li, D. Zhou, W. Qiu, Y. Shi, J.-J. Yang, S. Chen, Q. Wang, H. Pan, Application of Weighted Gene Co-expression Network Analysis for Data from Paired Design, Sci. Rep. 8 (2018) 622.
[115] L.-I. McCall, A. Tripathi, F. Vargas, R. Knight, P.C. Dorrestein, J.L. Siqueira-Neto, Experimental Chagas disease-induced perturbations of the fecal microbiome and metabolome, PLoS Negl. Trop. Dis. 12 (2018) e0006344.
[116] J.T. Morton, A.A. Aksenov, L.F. Nothias, J.R. Foulds, R.A. Quinn, M.H. Badri, T. L. Swenson, M.W. Van Goethem, T.R. Northen, Y. Vazquez-Baeza, M. Wang, N. A. Bokulich, A. Watters, S.J. Song, R. Bonneau, P.C. Dorrestein, R. Knight, Learning representations of microbe–metabolite interactions, Nature Methods. 16 (2019) 1306–1314, https://doi.org/10.1038/s41592-019-0616-3.
[117] P. Jasbi, A.E. Mohr, X. Shi, T. Mahmood, Q. Zhu, M. Bruening, H. Gu, C. Whisner, Microbiome and metabolome profiles of high screen time in a cohort of healthy college students, Sci. Rep. 12 (2022) 3452.
[118] N.A. Bokulich, P. Łaniewski, A. Adamov, D.M. Chase, J.G. Caporaso, M.M. Herbst-Kralovetz, Multi-omics data integration reveals metabolome as the top predictor of the cervicovaginal microenvironment, PLoS Comput. Biol. 18 (2022) e1009876.
[119] B.C. Taylor, F. Lejzerowicz, M. Poirel, J.P. Shaffer, L. Jiang, A. Aksenov, N. Litwin, G. Humphrey, C. Martino, S. Miller-Montgomery, P.C. Dorrestein, P. Veiga, S.J. Song, D. McDonald, M. Derrien, R. Knight, Consumption of Fermented Foods Is Associated with Systematic Differences in the Gut Microbiome and Metabolome, mSystems. 5 (2020), https://doi.org/10.1128/mSystems.00901-19.
[120] J.J. Haffner, M. Katemauswa, T.S. Kagone, E. Hossain, D. Jacobson, K. Flores, A. R. Parab, A.J. Obregon-Tito, R.Y. Tito, L.M. Reyes, L. Troncoso-Corzo, E. Guija-Poma, N. Meda, H. Carabin, T.P. Honap, K. Sankaranarayanan, C.M. Lewis Jr, L.-I. McCall, Untargeted Fecal Metabolomic Analyses across an Industrialization Gradient Reveal Shared Metabolites and Impact of Industrialization on Fecal Microbiome-Metabolome Interactions, mSystems. 7 (2022) e0071022.
[121] J.M. Gauglitz, K.A. West, W. Bittremieux, C.L. Williams, K.C. Weldon, M. Panitchpakdi, F. Di Ottavio, C.M. Aceves, E. Brown, N.C. Sikora, A. K. Jarmusch, C. Martino, A. Tripathi, M.J. Meehan, K. Dorrestein, J.P. Shaffer, R. Coras, F. Vargas, L.D. Goldasich, T. Schwartz, M. Bryant, G. Humphrey, A. J. Johnson, K. Spengler, P. Belda-Ferre, E. Diaz, D. McDonald, Q. Zhu, E.O. Elijah, M. Wang, C. Marotz, K.E. Sprecher, D. Vargas-Robles, D. Withrow, G. Ackermann, L. Herrera, B.J. Bradford, L.M.M. Marques, J.G. Amaral, R.M. Silva, F.P. Veras, T. M. Cunha, R.D.R. Oliveira, P. Louzada-Junior, R.H. Mills, P.K. Piotrowski, S. L. Servetas, S.M. Da Silva, C.M. Jones, N.J. Lin, K.A. Lippa, S.A. Jackson, R. K. Daouk, D. Galasko, P.S. Dulai, T.I. Kalashnikova, C. Wittenberg, R. Terkeltaub,

M.M. Doty, J.H. Kim, K.E. Rhee, J. Beauchamp-Walters, K.P. Wright Jr, M. G. Dominguez-Bello, M. Manary, M.F. Oliveira, B.S. Boland, N.P. Lopes, M. Guma, A.D. Swafford, R.J. Dutton, R. Knight, P.C. Dorrestein, Enhancing untargeted metabolomics using metadata-based source annotation, Nat. Biotechnol. 40 (2022) 1774–1779.
[122] C. Noecker, J. Sanchez, J.E. Bisanz, V. Escalante, M. Alexander, K. Trepka, A. Heinken, Y. Liu, D. Dodd, I. Thiele, B. DeFelice, P.J. Turnbaugh, Systems biology illuminates alternative metabolic niches in the human gut microbiome, bioRxiv. (2022) 2022.09.19.508335. https://doi.org/10.1101/2022.09.19.508335.
[123] M. Maansson, N.G. Vynne, A. Klitgaard, J.L. Nybo, J. Melchiorsen, D.D. Nguyen, L.M. Sanchez, N. Ziemert, P.C. Dorrestein, M.R. Andersen, L. Gram, An Integrated Metabolomic and Genomic Mining Workflow To Uncover the Biosynthetic Potential of Bacteria, mSystems. 1 (2016), https://doi.org/10.1128/mSystems.00028-15.
[124] S.R. Elsden, M.G. Hilton, J.M. Waller, The end products of the metabolism of aromatic amino acids by Clostridia, Arch. Microbiol. 107 (1976) 283–288.
[125] E.A. Smith, G.T. Macfarlane, Formation of Phenolic and Indolic Compounds by Anaerobic Bacteria in the Human Large Intestine, Microb. Ecol. 33 (1997) 180–188.
[126] S. Han, W. Van Treuren, C.R. Fischer, B.D. Merrill, B.C. DeFelice, J.M. Sanchez, S. K. Higginbottom, L. Guthrie, L.A. Fall, D. Dodd, M.A. Fischbach, J.L. Sonnenburg, A metabolomics pipeline for the mechanistic interrogation of the gut microbiome, Nature. 595 (2021) 415–420.
[127] P.A. Aronov, F.-J.-G. Luo, N.S. Plummer, Z. Quan, S. Holmes, T.H. Hostetter, T. W. Meyer, Colonic contribution to uremic solutes, J. Am. Soc. Nephrol. 22 (2011) 1769–1776.
[128] I. Nemet, X.S. Li, A. Haghikia, L. Li, J. Wilcox, K.A. Romano, J.A. Buffa, M. Witkowski, I. Demuth, M. König, E. Steinhagen-Thiessen, F. Bäckhed, M. A. Fischbach, W.H.W. Tang, U. Landmesser, S.L. Hazen, Atlas of gut microbe-derived products from aromatic amino acids and risk of cardiovascular morbidity and mortality, Eur. Heart J. 44 (2023) 3085–3096.
[129] R.A. Koeth, Z. Wang, B.S. Levison, J.A. Buffa, E. Org, B.T. Sheehy, E.B. Britt, X. Fu, Y. Wu, L. Li, J.D. Smith, J.A. DiDonato, J. Chen, H. Li, G.D. Wu, J.D. Lewis, M. Warrier, J.M. Brown, R.M. Krauss, W.H.W. Tang, F.D. Bushman, A.J. Lusis, S. L. Hazen, Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis, Nat. Med. 19 (2013) 576–585.
[130] W.H.W. Tang, Z. Wang, B.S. Levison, R.A. Koeth, E.B. Britt, X. Fu, Y. Wu, S. L. Hazen, Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk, N. Engl. J. Med. 368 (2013) 1575–1584.
[131] D. Dodd, M.H. Spitzer, W. Van Treuren, B.D. Merrill, A.J. Hryckowian, S. K. Higginbottom, A. Le, T.M. Cowan, G.P. Nolan, M.A. Fischbach, J. L. Sonnenburg, A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites, Nature. 551 (2017) 648–652.
[132] A. Marcobal, T. Yusufaly, S. Higginbottom, M. Snyder, J.L. Sonnenburg, G.I. Mias, Metabolome progression during early gut microbial colonization of gnotobiotic mice, Sci. Rep. 5 (2015) 11589.
[133] V.R. Velagapudi, R. Hezaveh, C.S. Reigstad, P. Gopalacharyulu, L. Yetukuri, S. Islam, J. Felin, R. Perkins, J. Borén, M. Oresic, F. Bäckhed, The gut microbiota modulates host energy and lipid metabolism in mice, J. Lipid Res. 51 (2010) 1101–1112.
[134] I. Subramanian, S. Verma, S. Kumar, A. Jere, K. Anamika, Multi-omics Data Integration, Interpretation, and Its Application, Bioinform. Biol. Insights. 14 (2020) 11779322–19899051.
[135] J. Park, A. Kerner, M.A. Burns, X.N. Lin, Microdroplet-enabled highly parallel co-cultivation of microbial communities, PLoS One. 6 (2011) e17019.
[136] Engineering Robust Production Microbes for Large-Scale Cultivation, Trends Microbiol. 27 (2019) 524–537.
[137] T. Clavel, J.C. Gomes-Neto, I. Lagkouvardos, A.E. Ramer-Tait, Deciphering interactions between the gut microbiota and the immune system via microbial cultivation and minimal microbiomes, Immunol. Rev. 279 (2017) 8–22.
[138] D. Nichols, N. Cahoon, E.M. Trakhtenberg, L. Pham, A. Mehta, A. Belanger, T. Kanigan, K. Lewis, S.S. Epstein, Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species, Appl. Environ. Microbiol. 76 (2010) 2445–2450.
[139] B. Berdy, A.L. Spoering, L.L. Ling, S.S. Epstein, In situ cultivation of previously uncultivable microorganisms using the ichip, Nat. Protoc. 12 (2017) 2232–2242.
[140] M.V. Sizova, T. Hohmann, A. Hazen, B.J. Paster, S.R. Halem, C.M. Murphy, N. S. Panikov, S.S. Epstein, New approaches for isolation of previously uncultivated oral bacteria, Appl. Environ. Microbiol. 78 (2012) 194–203.
[141] Y. Xiang, H. Wen, Y. Yu, M. Li, X. Fu, S. Huang, Gut-on-chip: Recreating human intestine in vitro, J. Tissue Eng. 11 (2020) 20417314–20965318.
[142] R.E. Higgs, J.A. Zahn, J.D. Gygi, M.D. Hilton, Rapid Method To Estimate the Presence of Secondary Metabolites in Microbial Extracts, Appl. Environ. Microbiol. 67 (2001), https://doi.org/10.1128/AEM.67.1.371-376.2001.
[143] S.P. Gaudêncio, F. Pereira, Dereplication: racing to speed up the natural products discovery process, Nat. Prod. Rep. 32 (2015) 779–810.
[144] M. Wang, J.J. Carver, V.V. Phelan, L.M. Sanchez, N. Garg, Y. Peng, D.D. Nguyen, J. Watrous, C.A. Kapono, T. Luzzatto-Knaan, C. Porto, A. Bouslimani, A.V. Melnik, M.J. Meehan, W.-T. Liu, M. Crüsemann, P.D. Boudreau, E. Esquenazi, M. Sandoval-Calderón, R.D. Kersten, L.A. Pace, R.A. Quinn, K.R. Duncan, C.-C. Hsu, D.J. Floros, R.G. Gavilan, K. Kleigrewe, T. Northen, R.J. Dutton, D. Parrot, E.E. Carlson, B. Aigle, C.F. Michelsen, L. Jelsbak, C. Sohlenkamp, P. Pevzner, A. Edlund, J. McLean, J. Piel, B.T. Murphy, L. Gerwick, C.-C. Liaw, Y.-L. Yang, H.-U. Humpf, M. Maansson, R.A. Keyzers, A.C. Sims, A.R. Johnson, A.M. Sidebottom, B. E. Sedio, A. Klitgaard, C.B. Larson, C.A.B. P, D. Torres-Mendoza, D.J. Gonzalez, D. B. Silva, L.M. Marques, D.P. Demarque, E. Pociute, E.C. O'Neill, E. Briand, E.J.N. Helfrich, E.A. Granatosky, E. Glukhov, F. Ryffel, H. Houson, H. Mohimani, J.J. Kharbush, Y. Zeng, J.A. Vorholt, K.L. Kurita, P. Charusanti, K.L. McPhail, K.F. Nielsen, L. Vuong, M. Elfeki, M.F. Traxler, N. Engene, N. Koyama, O.B. Vining, R. Baric, R.R. Silva, S.J. Mascuch, S. Tomasi, S. Jenkins, V. Macherla, T. Hoffman, V. Agarwal, P.G. Williams, J. Dai, R. Neupane, J. Gurr, A.M.C. Rodríguez, A. Lamsa, C. Zhang, K. Dorrestein, B.M. Duggan, J. Almaliti, P.-M. Allard, P. Phapale, L.-F. Nothias, T. Alexandrov, M. Litaudon, J.-L. Wolfender, J.E. Kyle, T.O. Metz, T. Peryea, D.-T. Nguyen, D. VanLeer, P. Shinn, A. Jadhav, R. Müller, K.M. Waters, W. Shi, X. Liu, L. Zhang, R. Knight, P.R. Jensen, B.O. Palsson, K. Pogliano, R.G. Linington, M. Gutiérrez, N.P. Lopes, W.H. Gerwick, B.S. Moore, P.C. Dorrestein, N. Bandeira, Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking, Nat. Biotechnol. 34 (2016) 828–837.
[145] C.A. Smith, G. O'Maille, E.J. Want, C. Qin, S.A. Trauger, T.R. Brandon, D. E. Custodio, R. Abagyan, G. Siuzdak, METLIN: a metabolite mass spectral database, Ther. Drug Monit. 27 (2005) 747–751.
[146] Dictionary of Natural Products, (n.d.). https://dnp.chemnetbase.com/chemical/ChemicalSearch.xhtml?dswid=-6075 (accessed May 26, 2023).
[147] NIST Standard Reference Database, (n.d.). https://www.nist.gov/srd (accessed May 26, 2023).
[148] D.A. Dias, O.A.H. Jones, D.J. Beale, B.A. Boughton, D. Benheim, K. A. Kouremenos, J.-L. Wolfender, D.S. Wishart, Current and Future Perspectives on the Structural Identification of Small Molecules in Biological Systems, Metabolites. 6 (2016), https://doi.org/10.3390/metabo6040046.
[149] M. Elyashberg, Identification and structure elucidation by NMR spectroscopy, Trends, Analyt. Chem. 69 (2015) 88–97.
[150] Z. Huang, M.S. Chen, C.P. Woroch, T.E. Markland, M.W. Kanan, A framework for automated structure elucidation from routine NMR spectra, Chem. Sci. 12 (2021) 15329–15338.
[151] S.-M. Shen, G. Appendino, Y.-W. Guo, Pitfalls in the structural elucidation of small molecules. A critical analysis of a decade of structural misassignments of marine natural products, Nat. Prod. Rep. 39 (2022) 1803–1832.
[152] R. Kreis, Issues of spectral quality in clinical 1H-magnetic resonance spectroscopy and a gallery of artifacts, NMR Biomed. 17 (2004) 361–381.
[153] L.-F. Nothias, D. Petras, R. Schmid, K. Dührkop, J. Rainer, A. Sarvepalli, I. Protsyuk, M. Ernst, H. Tsugawa, M. Fleischauer, F. Aicheler, A.A. Aksenov, O. Alka, P.-M. Allard, A. Barsch, X. Cachet, A.M. Caraballo-Rodriguez, R.R. Da Silva, T. Dang, N. Garg, J.M. Gauglitz, A. Gurevich, G. Isaac, A.K. Jarmusch, Z. Kameník, K.B. Kang, N. Kessler, I. Koester, A. Korf, A. Le Gouellec, M. Ludwig, C. Martin H, L.-I. McCall, J. McSayles, S.W. Meyer, H. Mohimani, M. Morsy, O. Moyne, S. Neumann, H. Neuweger, N.H. Nguyen, M. Nothias-Esposito, J. Paolini, V.V. Phelan, T. Pluskal, R.A. Quinn, S. Rogers, B. Shrestha, A. Tripathi, J.J.J. van der Hooft, F. Vargas, K.C. Weldon, M. Witting, H. Yang, Z. Zhang, F. Zubeil, O. Kohlbacher, S. Böcker, T. Alexandrov, N. Bandeira, M. Wang, P.C. Dorrestein, Feature-based molecular networking in the GNPS analysis environment, Nat. Methods. 17 (2020) 905–908.
[154] Y. Liu, A. Mrzic, P. Meysman, T. De Vijlder, E.P. Romijn, D. Valkenborg, W. Bittremieux, K. Laukens, MESSAR: Automated recommendation of metabolite substructures from tandem mass spectra, PLoS One. 15 (2020) e0226770.
[155] C. Zhang, Y. Idelbayev, N. Roberts, Y. Tao, Y. Nannapaneni, B.M. Duggan, J. Min, E.C. Lin, E.C. Gerwick, G.W. Cottrell, W.H. Gerwick, Author Correction: Small Molecule Accurate Recognition Technology (SMART) to Enhance Natural Products Research, Sci. Rep. 10 (2020) 4724.
[156] L. Cao, M. Guler, A. Tagirdzhanov, Y.-Y. Lee, A. Gurevich, H. Mohimani, MolDiscovery: learning mass spectrometry fragmentation of small molecules, Nat. Commun. 12 (2021) 3718.
[157] H.-F. Schött, S. Krautbauer, M. Höring, G. Liebisch, S. Matysik, A. Validated, Fast Method for Quantification of Sterols and Gut Microbiome Derived 5α/β-Stanols in Human Feces by Isotope Dilution LC-High-Resolution MS, Anal. Chem. 90 (2018) 8487–8494.
[158] G. den Besten, K. van Eunen, A.K. Groen, K. Venema, D.-J. Reijngoud, B. M. Bakker, The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism, J. Lipid Res. 54 (2013) 2325–2340.
[159] L.A. Marques, C.B.B. Cazarin, J. Bicas, M.R. Maróstica, E. Carrilho, S. Bogusz, Determination of Short Chain Fatty Acids in Mice Feces by Capillary Electrophoresis, J. Braz. Chem. Soc. 30 (2019) 1326–1334.
[160] G. den Besten, A. Bleeker, A. Gerding, K. van Eunen, R. Havinga, T.H. van Dijk, M. H. Oosterveer, J.W. Jonker, A.K. Groen, D.-J. Reijngoud, B.M. Bakker, Short-Chain Fatty Acids Protect Against High-Fat Diet-Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation, Diabetes. 64 (2015) 2398–2408.
[161] A. Koh, F. De Vadder, P. Kovatcheva-Datchary, F. Bäckhed, From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites, Cell. 165 (2016) 1332–1345.
[162] P. Liu, Y. Wang, G. Yang, Q. Zhang, L. Meng, Y. Xin, X. Jiang, The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis, Pharmacol. Res. 165 (2021) 105420.
[163] E.N. Bergman, Energy contributions of volatile fatty acids from the gastrointestinal tract in various species, Physiol. Rev. 70 (1990) 567–590.
[164] Y.P. Silva, A. Bernardi, R.L. Frozza, The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication, Front. Endocrinol. 11 (2020) 25.
[165] L.R. Hoving, M. Heijink, V. van Harmelen, K.W. van Dijk, M. Giera, GC-MS Analysis of Short-Chain Fatty Acids in Feces, Cecum Content, and Blood Samples, in: M. Giera (Ed.), Clinical Metabolomics: Methods and Protocols, Springer, New York, New York, NY, 2018, pp. 247–256.

[166] J. de la Cuesta-Zuluaga, N.T. Mueller, R. Álvarez-Quintero, E.P. Velásquez-Mejía, J.A. Sierra, V. Corrales-Agudelo, J.A. Carmona, J.M. Abad, J.S. Escobar, Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors, Nutrients. 11 (2018), https://doi.org/10.3390/nu11010051.
[167] J.H. Cummings, E.W. Pomare, W.J. Branch, C.P. Naylor, G.T. Macfarlane, Short chain fatty acids in human large intestine, portal, hepatic and venous blood, Gut. 28 (1987) 1221–1227.
[168] D. Parada Venegas, M.K. De la Fuente, G. Landskron, M.J. González, R. Quera, G. Dijkstra, H.J.M. Harmsen, K.N. Faber, M.A. Hermoso, Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases, Front. Immunol. 10 (2019) 277.
[169] D.L. Topping, P.M. Clifton, Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides, Physiol. Rev. 81 (2001) 1031–1064.
[170] A. Haghikia, S. Jörg, A. Duscha, J. Berg, A. Manzel, A. Waschbisch, A. Hammer, D.-H. Lee, C. May, N. Wilck, A. Balogh, A.I. Ostermann, N.H. Schebb, D.A. Akkad, D.A. Grohme, M. Kleinewietfeld, S. Kempa, J. Thöne, S. Demir, D.N. Müller, R. Gold, R.A. Linker, Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine, Immunity. 43 (2015) 817–829.
[171] N. Trivedi, H.E. Erickson, V. Bala, Y.S. Chhonker, D.J. Murry, A Concise Review of Liquid Chromatography-Mass Spectrometry-Based Quantification Methods for Short Chain Fatty Acids as Endogenous Biomarkers, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms232113486.
[172] M.R.V. Bertolo, S. Bogusz Junior, Quantification of Short-Chain Fatty Acids in Feces, in: C.B. Betim Cazarin (Ed.), Basic Protocols in Foods and Nutrition, Springer US, New York, NY, 2022: pp. 73–92.
[173] K.-S. Kim, Y. Lee, W. Chae, J.-Y. Cho, An Improved Method to Quantify Short-Chain Fatty Acids in Biological Samples Using Gas Chromatography-Mass Spectrometry, Metabolites. 12 (2022), https://doi.org/10.3390/metabo12060525.
[174] S. Saha, P. Day-Walsh, E. Shehata, P.A. Kroon, Development and validation of a LC-MS/MS technique for the analysis of short chain fatty acids in tissues and biological fluids without derivatisation using isotope labelled internal standards, Molecules. 26 (2021) 6444.
[175] X. Han, J. Guo, Y. You, M. Yin, C. Ren, J. Zhan, W. Huang, A fast and accurate way to determine short chain fatty acids in mouse feces based on GC–MS, Journal of Chromatography b. 1099 (2018) 73–82.
[176] A. Banel, A. Jakimska, M. Wasielewska, L. Wolska, B. Zygmunt, Determination of SCFAs in water using GC-FID. Selection of the separation system, Anal. Chim. Acta. 716 (2012) 24–27.
[177] A. Shafaei, V. Vamathevan, J. Pandohee, N.G. Lawler, D. Broadhurst, M.C. Boyce, Sensitive and quantitative determination of short-chain fatty acids in human serum using liquid chromatography mass spectrometry, Anal. Bioanal. Chem. 413 (2021) 6333–6342.
[178] D.G. Bihan, T. Rydzak, M. Wyss, K. Pittman, K.D. McCoy, I.A. Lewis, Method for absolute quantification of short chain fatty acids via reverse phase chromatography mass spectrometry, PLoS One. 17 (2022) e0267093.
[179] R. Nagatomo, Y. Okada, M. Ichimura, K. Tsuneyama, K. Inoue, Application of 2-Picolylamine Derivatized Ultra-high Performance Liquid Chromatography Tandem Mass Spectrometry for the Determination of Short-chain Fatty Acids in Feces Samples, Anal. Sci. 34 (2018) 1031–1036.
[180] G. Liebisch, J. Ecker, S. Roth, S. Schweizer, V. Öttl, H.-F. Schött, H. Yoon, D. Haller, E. Holler, R. Burkhardt, S. Matysik, Quantification of Fecal Short Chain Fatty Acids by Liquid Chromatography Tandem Mass Spectrometry—Investigation of Pre-Analytic Stability, Biomolecules. 9 (2019) 121.
[181] S. Zhang, H. Wang, M.-J. Zhu, A sensitive GC/MS detection method for analyzing microbial metabolites short chain fatty acids in fecal and serum samples, Talanta. 196 (2019) 249–254.
[182] M. Li, R. Zhu, X. Song, Z. Wang, H. Weng, J. Liang, A sensitive method for the quantification of short-chain fatty acids by benzyl chloroformate derivatization combined with GC-MS, Analyst. 145 (2020) 2692–2700.
[183] T. Furuhashi, K. Sugitate, T. Nakai, Y. Jikumaru, G. Ishihara, Rapid profiling method for mammalian feces short chain fatty acids by GC-MS, Anal. Biochem. 543 (2018) 51–54.
[184] X. Tao, Y. Liu, Y. Wang, Y. Qiu, J. Lin, A. Zhao, M. Su, W. Jia, GC-MS with ethyl chloroformate derivatization for comprehensive analysis of metabolites in serum and its application to human uremia, Anal. Bioanal. Chem. 391 (2008) 2881–2889.
[185] L. He, M.A.I. Prodhan, F. Yuan, X. Yin, P.K. Lorkiewicz, X. Wei, W. Feng, C. McClain, X. Zhang, Simultaneous quantification of straight-chain and branched-chain short chain fatty acids by gas chromatography mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1092 (2018) 359–367.
[186] M. Dei Cas, R. Paroni, A. Saccardo, E. Casagni, S. Arnoldi, V. Gambaro, M. Saresella, C. Mario, F. La Rosa, I. Marventano, F. Piancone, G. Roda, A straightforward LC-MS/MS analysis to study serum profile of short and medium chain fatty acids, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1154 (2020) 121982.
[187] X. Feng, Z. Tang, B. Chen, S. Feng, Y. Liu, Q. Meng, A high-efficiency quantitation method for fatty aldehyde based on chemical isotope-tagged derivatisation, J. Food Compost. Anal. 107 (2022) 104381.
[188] S.-R. Ma, Q. Tong, Z.-X. Zhao, L. Cong, J.-B. Yu, J. Fu, P. Han, L.-B. Pan, R. Gu, R. Peng, Z.-W. Zhang, Y. Wang, J.-D. Jiang, Determination of berberine-upregulated endogenous short-chain fatty acids through derivatization by 2-bromoacetophenone, Anal. Bioanal. Chem. 411 (2019) 3191–3207.
[189] J. Han, K. Lin, C. Sequeira, C.H. Borchers, An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography-tandem mass spectrometry, Anal. Chim. Acta. 854 (2015) 86–94.
[190] J.C.Y. Chan, D.Y.Q. Kioh, G.C. Yap, B.W. Lee, E.C.Y. Chan, A novel LCMSMS method for quantitative measurement of short-chain fatty acids in human stool derivatized with 12C- and 13C-labelled aniline, J. Pharm. Biomed. Anal. 138 (2017) 43–53.
[191] W. Xu, D. Chen, N. Wang, T. Zhang, R. Zhou, T. Huan, Y. Lu, X. Su, Q. Xie, L. Li, L. Li, Development of high-performance chemical isotope labeling LC-MS for profiling the human fecal metabolome, Anal. Chem. 87 (2015) 829–836.
[192] M. Zeng, H. Cao, Fast quantification of short chain fatty acids and ketone bodies by liquid chromatography-tandem mass spectrometry after facile derivatization coupled with liquid-liquid extraction, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1083 (2018) 137–145.
[193] J.-H. Zhu, Q. Mao, S.-Y. Wang, H. Liu, S.-S. Zhou, W. Zhang, M. Kong, H. Zhu, S.-L. Li, Optimization and validation of direct gas chromatography-mass spectrometry method for simultaneous quantification of ten short-chain fatty acids in rat feces, J. Chromatogr. a. 1669 (2022) 462958.
[194] Z. Fu, Q. Jia, H. Zhang, L. Kang, X. Sun, M. Zhang, Y. Wang, P. Hu, Simultaneous quantification of eleven short-chain fatty acids by derivatization and solid phase microextraction-Gas chromatography tandem mass spectrometry, J. Chromatogr. a. 1661 (2022) 462680.
[195] L. Yao, E.A. Davidson, M.W. Shaikh, C.B. Forsyth, J.E. Prenni, C.D. Broeckling, Quantitative analysis of short-chain fatty acids in human plasma and serum by GC-MS, Anal. Bioanal. Chem. 414 (2022) 4391–4399.
[196] R. Wang, C. Fan, X. Fan, Y. Zhao, Y. Wang, P. Li, T. Tang, H. Yao, S. Chen, D. Chen, K. Qi, A Fast and Accurate Way to Determine Short Chain Fatty Acids in Human Serum by GC–MS and Their Distribution in Children with Digestive Diseases, Chromatographia. 83 (2020) 273–286.
[197] T.C. Northfield, P. Zentler-Munro, R. Jazwari, Bile Acids in Health and Disease: Update on Cholesterol Gallstones and Bile Acid Diarrhoea, Springer Science & Business Media, 2012.
[198] J. Zhang, L.-Z. Gao, Y.-J. Chen, P.-P. Zhu, S.-S. Yin, M.-M. Su, Y. Ni, J. Miao, W.-L. Wu, H. Chen, K.L.R. Brouwer, C.-X. Liu, L. Xu, W. Jia, K. Lan, Continuum of Host-Gut Microbial Co-metabolism: Host CYP3A4/3A7 are Responsible for Tertiary Oxidations of Deoxycholate Species, Drug Metab. Dispos. 47 (2019) 283–294.
[199] H. Ajouz, D. Mukherji, A. Shamseddine, Secondary bile acids: an underrecognized cause of colon cancer, World J. Surg. Oncol. 12 (2014) 164.
[200] P. Dorrestein, E. Gentry, S. Collins, M. Panitchpakdi, P. Belda-Ferre, A. Stewart, M. Wang, A. Jarmusch, J. Avila-Pacheco, D. Plichta, A. Aron, H. Vlamakis, A. Ananthakrishnan, C. Clish, R. Xavier, E. Baker, A. Patterson, R. Knight, D. Siegel, A Synthesis-Based Reverse Metabolomics Approach for the Discovery of Chemical Structures from Humans and Animals, (2021). https://doi.org/10.21203/rs.3.rs-820302/v1.
[201] B. Jia, Commentary: Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells, Front. Immunol. 10 (2019) 282.
[202] S. Yoshimoto, T.M. Loo, K. Atarashi, H. Kanda, S. Sato, S. Oyadomari, Y. Iwakura, K. Oshima, H. Morita, M. Hattori, K. Honda, Y. Ishikawa, E. Hara, N. Ohtani, Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome, Nature. 499 (2013) 97–101.
[203] R. Pai, A.S. Tarnawski, T. Tran, Deoxycholic acid activates beta-catenin signaling pathway and increases colon cell cancer growth and invasiveness, Mol. Biol. Cell. 15 (2004) 2156–2163.
[204] J.M. Ridlon, P.G. Wolf, H.R. Gaskins, Taurocholic acid metabolism by gut microbes and colon cancer, Gut Microbes. 7 (2016) 201–215.
[205] J.E. Wells, F. Berr, L.A. Thomas, R. Hermon Dowling, P.B. Hylemon, Isolation and characterization of cholic acid 7α-dehydroxylating fecal bacteria from cholesterol gallstone patients, Journal of Hepatology. 32 (2000) 4–10, https://doi.org/10.1016/s0168-8278(00)80183-x.
[206] F. Berr, G.A. Kullak-Ublick, G. Paumgartner, W. Münzing, P.B. Hylemon, 7 alpha-dehydroxylating bacteria enhance deoxycholic acid input and cholesterol saturation of bile in patients with gallstones, Gastroenterology. 111 (1996) 1611–1620.
[207] G. Kakiyama, P.B. Hylemon, H. Zhou, W.M. Pandak, D.M. Heuman, D.J. Kang, H. Takei, H. Nittono, J.M. Ridlon, M. Fuchs, E.C. Gurley, Y. Wang, R. Liu, A. J. Sanyal, P.M. Gillevet, J.S. Bajaj, Colonic inflammation and secondary bile acids in alcoholic cirrhosis, Am. J. Physiol. Gastrointest. Liver Physiol. 306 (2014) G929–G937.
[208] G. Kakiyama, W.M. Pandak, P.M. Gillevet, P.B. Hylemon, D.M. Heuman, K. Daita, H. Takei, A. Muto, H. Nittono, J.M. Ridlon, M.B. White, N.A. Noble, P. Monteith, M. Fuchs, L.R. Thacker, M. Sikaroodi, J.S. Bajaj, Modulation of the fecal bile acid profile by gut microbiota in cirrhosis, J. Hepatol. 58 (2013) 949–955.
[209] C.G. Buffie, V. Bucci, R.R. Stein, P.T. McKenney, L. Ling, A. Gobourne, D. No, H. Liu, M. Kinnebrew, A. Viale, E. Littmann, M.R.M. van den Brink, R.R. Jenq, Y. Taur, C. Sander, J.R. Cross, N.C. Toussaint, J.B. Xavier, E.G. Pamer, Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile, Nature. 517 (2015) 205–208.
[210] J.A. Sorg, A.L. Sonenshein, Bile Salts and Glycine as Cogerminants for *Clostridium difficile* Spores, Journal of Bacteriology. 190 (2008) 2505–2512, https://doi.org/10.1128/jb.01765-07.
[211] C.M. Theriot, A.A. Bowman, V.B. Young, Antibiotic-Induced Alterations of the Gut Microbiota Alter Secondary Bile Acid Production and Allow for Clostridium

difficile Spore Germination and Outgrowth in the Large Intestine, mSphere. 1 (2016), https://doi.org/10.1128/mSphere.00045-15.
[212] M. Makishima, A.Y. Okamoto, J.J. Repa, H. Tu, R.M. Learned, A. Luk, M.V. Hull, K.D. Lustig, D.J. Mangelsdorf, B. Shan, Identification of a nuclear receptor for bile acids, Science. 284 (1999) 1362–1365.
[213] V. Keitel, M. Donner, S. Winandy, R. Kubitz, D. Häussinger, Expression and function of the bile acid receptor TGR5 in Kupffer cells, Biochem. Biophys. Res. Commun. 372 (2008) 78–84.
[214] T. Suga, H. Yamaguchi, T. Sato, M. Maekawa, J. Goto, N. Mano, Preference of Conjugated Bile Acids over Unconjugated Bile Acids as Substrates for OATP1B1 and OATP1B3, PLoS One. 12 (2017) e0169719.
[215] T. Režen, D. Rozman, T. Kovács, P. Kovács, A. Sipos, P. Bai, E. Mikó, The role of bile acids in carcinogenesis, Cell. Mol. Life Sci. 79 (2022) 243.
[216] Z. Peng, Q. Zhang, Z. Mao, J. Wang, C. Liu, X. Lin, X. Li, W. Ji, J. Fan, M. Wang, C. Su, A rapid quantitative analysis of bile acids, lysophosphatidylcholines and polyunsaturated fatty acids in biofluids based on ultraperformance liquid chromatography coupled with triple quadrupole tandem massspectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1068–1069 (2017) 343–351.
[217] C. John, P. Werner, A. Worthmann, K. Wegner, K. Tödter, L. Scheja, S. Rohn, J. Heeren, M. Fischer, A liquid chromatography-tandem mass spectrometry-based method for the simultaneous determination of hydroxy sterols and bile acids, J. Chromatogr. a. 1371 (2014) 184–195.
[218] M. Dutta, J. Cai, W. Gui, A.D. Patterson, A review of analytical platforms for accurate bile acid measurement, Anal. Bioanal. Chem. 411 (2019) 4541–4549.
[219] S. Liu, Y. Zhang, B. Qu, G. Qin, J. Cheng, F. Lu, H. Qu, Y. Zhao, Detection of total bile acids in biological samples using an indirect competitive ELISA based on four monoclonal antibodies, Anal. Methods. 9 (2017) 625–633.
[220] S. Baldofski, H. Hoffmann, A. Lehmann, S. Breitfeld, L.-A. Garbe, R.J. Schneider, Enzyme-linked immunosorbent assay (ELISA) for the anthropogenic marker isolithocholic acid in water, J. Environ. Manage. 182 (2016) 612–619.
[221] X. Cui, N. Vasylieva, D. Shen, B. Barnych, J. Yang, Q. He, Z. Jiang, S. Zhao, B. D. Hammock, Biotinylated single-chain variable fragment-based enzyme-linked immunosorbent assay for glycocholic acid, Analyst. 143 (2018) 2057–2065.
[222] Y. Alnouti, I.L. Csanaky, C.D. Klaassen, Quantitative-profiling of bile acids and their conjugates in mouse liver, bile, plasma, and urine using LC-MS/MS, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 873 (2008) 209–217.
[223] L. Humbert, M.A. Maubert, C. Wolf, H. Duboc, M. Mahé, D. Farabos, P. Seksik, J. M. Mallet, G. Trugnan, J. Masliah, D. Rainteau, Bile acid profiling in human biological samples: comparison of extraction procedures and application to normal and cholestatic patients, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 899 (2012) 135–145.
[224] M.H. Sarafian, M.R. Lewis, A. Pechlivanis, S. Ralphs, M.J.W. McPhail, V.C. Patel, M.-E. Dumas, E. Holmes, J.K. Nicholson, Bile acid profiling and quantification in biofluids using ultra-performance liquid chromatography tandem mass spectrometry, Anal. Chem. 87 (2015) 9662–9670.
[225] C. Gómez, S. Stücheli, D.V. Kratschmar, J. Bouitbir, A. Odermatt, Development and Validation of a Highly Sensitive LC-MS/MS Method for the Analysis of Bile Acids in Serum, Plasma, and Liver Tissue Samples, Metabolites. 10 (2020), https://doi.org/10.3390/metabo10070282.
[226] G.A. Nagana Gowda, N. Shanaiah, A. Cooper, M. Maluccio, D. Raftery, Bile acids conjugation in human bile is not random: new insights from (1)H-NMR spectroscopy at 800 MHz, Lipids. 44 (2009) 527–535.
[227] G.A.N. Gowda, O.B. Ijare, B.S. Somashekar, A. Sharma, V.K. Kapoor, C. L. Khetrapal, Single-step analysis of individual conjugated bile acids in human bile using 1H NMR spectroscopy, Lipids. 41 (2006) 591–603.
[228] M. Zhou, J. Shao, C.-Y. Wu, L. Shu, W. Dong, Y. Liu, M. Chen, R.M. Wynn, J. Wang, J. Wang, W.-J. Gui, X. Qi, A.J. Lusis, Z. Li, W. Wang, G. Ning, X. Yang, D. T. Chuang, Y. Wang, H. Sun, Targeting BCAA Catabolism to Treat Obesity-Associated Insulin Resistance, Diabetes. 68 (2019) 1730–1746.
[229] J. Gojda, M. Cahova, Gut Microbiota as the Link between Elevated BCAA Serum Levels and Insulin Resistance, Biomolecules. 11 (2021), https://doi.org/10.3390/biom11101414.
[230] A. Agus, K. Clément, H. Sokol, Gut microbiota-derived metabolites as central regulators in metabolic disorders, Gut. 70 (2021) 1174–1182.
[231] M.A.B. Siddik, A.C. Shin, Recent Progress on Branched-Chain Amino Acids in Obesity, Diabetes, and Beyond, Endocrinol Metab (seoul). 34 (2019) 234–246.
[232] C.B. Newgard, J. An, J.R. Bain, M.J. Muehlbauer, R.D. Stevens, L.F. Lien, A. M. Haqq, S.H. Shah, M. Arlotto, C.A. Slentz, J. Rochon, D. Gallup, O. Ilkayeva, B. R. Wenner, W.S. Yancy Jr, H. Eisenson, G. Musante, R.S. Surwit, D.S. Millington, M.D. Butler, L.P. Svetkey, A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance, Cell Metab. 9 (2009) 311–326.
[233] L. Yu, Q. Zhu, Y. Li, P. Song, J. Zhang, Dietary Branched-Chain Amino Acids (BCAAs) and Risk of Dyslipidemia in a Chinese Population, Nutrients. 14 (2022), https://doi.org/10.3390/nu14091824.
[234] V. Ramos-Garcia, I. Ten-Doménech, A. Moreno-Giménez, L. Campos-Berga, A. Parra-Llorca, Á. Solaz-García, I. Lara-Cantón, A. Pinilla-Gonzalez, M. Gormaz, M. Vento, J. Kuligowski, G. Quintás, GC-MS analysis of short chain fatty acids and branched chain amino acids in urine and faeces samples from newborns and lactating mothers, Clin. Chim. Acta. 532 (2022) 172–180.
[235] A.M. Whipp, M. Heinonen-Guzejev, K.H. Pietiläinen, I. van Kamp, J. Kaprio, Branched-chain amino acids linked to depression in young adults, Front. Neurosci. 16 (2022) 935858.
[236] S. Teepoo, T. Sannok, S. Arsawiset, S. Sansenya, A portable device as a paper test strip platform with smartphone application for detection of branched-chain amino acids in edible insects, Food Chem. 405 (2023) 134560.
[237] R. Fingerhut, W. Röschinger, M. Heck, A rapid and sensitive UPLC-MS/MS-method for the separation and quantification of branched-chain amino acids from dried blood samples of patients with maple syrup urine disease (MSUD), Screening. 2 (2016) 2.
[238] X. Zheng, Y. Qiu, W. Zhong, S. Baxter, M. Su, Q. Li, G. Xie, B.M. Ore, S. Qiao, M. D. Spencer, S.H. Zeisel, Z. Zhou, A. Zhao, W. Jia, A targeted metabolomic protocol for short-chain fatty acids and branched-chain amino acids, Metabolomics. 9 (2013) 818–827.
[239] J. Piestansky, M. Matuskova, I. Cizmarova, D. Olesova, P. Mikus, Determination of Branched-Chain Amino Acids in Food Supplements and Human Plasma by a CE-MS/MS Method with Enhanced Resolution, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22158261.
[240] S. Haq, J.A. Grondin, W.I. Khan, Tryptophan-derived serotonin-kynurenine balance in immune activation and intestinal inflammation, FASEB J. 35 (2021) e21888.
[241] H.M. Roager, T.R. Licht, Microbial tryptophan catabolites in health and disease, Nat. Commun. 9 (2018) 3294.
[242] J. Gao, K. Xu, H. Liu, G. Liu, M. Bai, C. Peng, T. Li, Y. Yin, Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism, Front. Cell. Infect. Microbiol. 8 (2018) 13.
[243] H. Le Gouellec, B. Chaker, B. Polack, B. Guery, M.J. Toussaint, C. Stasia, Genestet, Scavenging of reactive oxygen species by tryptophan metabolites helps Pseudomonas aeruginosa escape neutrophil killing, Free Radical Biology and Medicine. 73 (2014) 400–410.
[244] G. Li, K.D. Young, Indole production by the tryptophanase TnaA in Escherichia coli is determined by the amount of exogenous tryptophan, Microbiology. 159 (2013) 402–410.
[245] I. Vujkovic-Cvijin, R.M. Dunham, S. Iwai, M.C. Maher, R.G. Albright, M. J. Broadhurst, R.D. Hernandez, M.M. Lederman, Y. Huang, M. Somsouk, S. G. Deeks, P.W. Hunt, S.V. Lynch, J.M. McCune, Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism, Sci. Transl. Med. 5 (2013) 193ra91.
[246] L. Desbonnet, G. Clarke, O. O'Sullivan, P.D. Cotter, T.G. Dinan, J.F. Cryan, Re: Gut microbiota depletion from early adolescence in mice: Implications for brain and behaviour, Brain Behav. Immun. 50 (2015) 335–336.
[247] Y. Lai, C.-W. Liu, L. Chi, H. Ru, K. Lu, High-Resolution Metabolomics of 50 Neurotransmitters and Tryptophan Metabolites in Feces, Serum, and Brain Tissues Using UHPLC-ESI-Q Exactive Mass Spectrometry, ACS, Omega. 6 (2021) 8094–8103.
[248] A. Anesi, J. Rubert, K. Oluwagbemigun, X. Orozco-Ruiz, U. Nöthlings, M.M. B. Breteler, F. Mattivi, Metabolic Profiling of Human Plasma and Urine, Targeting Tryptophan, Tyrosine and Branched Chain Amino Acid Pathways, Metabolites. 9 (2019), https://doi.org/10.3390/metabo9110261.
[249] D. Weber, P.J. Oefner, A. Hiergeist, J. Koestler, A. Gessner, M. Weber, J. Hahn, D. Wolff, F. Stämmler, R. Spang, W. Herr, K. Dettmer, E. Holler, Low urinary indoxyl sulfate levels early after transplantation reflect a disrupted microbiome and are associated with poor outcome, Blood. 126 (2015) 1723–1728.
[250] J. Zhen, P. Zhao, Y. Li, Y. Cai, W. Yu, W. Wang, L. Zhao, H. Wang, G. Huang, A. Xu, The Multiomics Analyses of Gut Microbiota, Urine Metabolome and Plasma Proteome Revealed Significant Changes in Allergy Featured with Indole Derivatives of Tryptophan, J. Asthma Allergy. 15 (2022) 117–131.
[251] M.G. Esperanza, K. Wrobel, A.G. Ojeda, M.E. Garay-Sevilla, A.R.C. Escobosa, E. Y. Barrientos, K. Wrobel, Liquid chromatography-mass spectrometry untargeted metabolomics reveals increased levels of tryptophan indole metabolites in urine of metabolic syndrome patients, Eur. J. Mass Spectrom. 26 (2020) 379–387.
[252] R. Fuertig, A. Ceci, S.M. Camus, E. Bezard, A.H. Luippold, B. Hengerer, LC-MS/MS-based quantification of kynurenine metabolites, tryptophan, monoamines and neopterin in plasma, cerebrospinal fluid and brain, Bioanalysis. 8 (2016) 1903–1917.
[253] H. Guo, Y. Jiao, X. Wang, T. Lu, Z. Zhang, F. Xu, Twins labeling-liquid chromatography/mass spectrometry based metabolomics for absolute quantification of tryptophan and its key metabolites, J. Chromatogr. a. 1504 (2017) 83–90.
[254] B. Tuka, A. Nyári, E.K. Cseh, T. Körtési, D. Veréb, F. Tömösi, G. Kecskeméti, T. Janáky, J. Tajti, L. Vécsei, Clinical relevance of depressed kynurenine pathway in episodic migraine patients: potential prognostic markers in the peripheral plasma during the interictal period, J. Headache Pain. 22 (2021) 1–19.
[255] L. Desbonnet, L. Garrett, G. Clarke, J. Bienenstock, T.G. Dinan, The probiotic Bifidobacteria infantis: An assessment of potential antidepressant properties in the rat, J. Psychiatr. Res. 43 (2008) 164–174.
[256] C. Hervé, P. Beyne, H. Jamault, E. Delacoux, Determination of tryptophan and its kynurenine pathway metabolites in human serum by high-performance liquid chromatography with simultaneous ultraviolet and fluorimetric detection, J. Chromatogr. B Biomed. Sci. Appl. 675 (1996) 157–161.
[257] M. Jaglin, M. Rhimi, C. Philippe, N. Pons, A. Bruneau, B. Goustard, V. Daugé, E. Maguin, L. Naudon, S. Rabot, Indole, a Signaling Molecule Produced by the Gut Microbiota, Negatively Impacts Emotional Behaviors in Rats, Front. Neurosci. 12 (2018), https://doi.org/10.3389/fnins.2018.00216.
[258] D. Favre, J. Mold, P.W. Hunt, B. Kanwar, P. 'ng Loke, L. Seu, J.D. Barbour, M.M. Lowe, A. Jayawardene, F. Aweeka, Y. Huang, D.C. Douek, J.M. Brenchley, J.N. Martin, F.M. Hecht, S.G. Deeks, J.M. McCune, Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease, Sci. Transl. Med. 2 (2010) 32ra36.
[259] P. Gatarek, J. Kaluzna-Czaplinska, Trimethylamine N-oxide (TMAO) in human health, EXCLI J. 20 (2021) 301.

[260] M.S. Thomas, M.L. Fernandez, Trimethylamine N-Oxide (TMAO), Diet and Cardiovascular Disease, Curr. Atheroscler. Rep. 23 (2021) 1–7.
[261] M. He, H. Yu, P. Lei, S. Huang, J. Ren, W. Fan, L. Han, H. Yu, Y. Wang, M. Ren, M. Jiang, Determination of Trimethylamine N-oxide and Betaine in Serum and Food by Targeted Metabonomics, Molecules. 26 (2021), https://doi.org/10.3390/molecules26051334.
[262] M.E. Hefni, M. Bergström, T. Lennqvist, C. Fagerström, C.M. Witthöft, Simultaneous quantification of trimethylamine N-oxide, trimethylamine, choline, betaine, creatinine, and propionyl-, acetyl-, and l-carnitine in clinical and food samples using HILIC-LC-MS, Anal. Bioanal. Chem. 413 (2021) 5349.
[263] A. Baranyi, A. Meinitzer, D. von Lewinski, H.-B. Rothenhäusler, O. Amouzadeh-Ghadikolai, H. Harpf, L. Harpf, H. Traninger, R. Hödl, B.M. Harb, B. Obermayer-Pietsch, M. Schweinzer, C.K. Braun, D. Enko, Sex-specific differences in trimethylamine N-oxide (TMAO) concentrations before and after cardiac rehabilitation in acute myocardial infarction patients, EXCLI J. 21 (2022) 1.
[264] E. Garcia, J. Wolak-Dinsmore, Z. Wang, X.S. Li, D.W. Bennett, M.A. Connelly, J. D. Otvos, S.L. Hazen, E.J. Jeyarajah, NMR quantification of trimethylamine-N-oxide in human serum and plasma in the clinical laboratory setting, Clin. Biochem. 50 (2017) 947–955.
[265] Z. Li, K.S. Suslick, Ultrasonic Preparation of Porous Silica-Dye Microspheres: Sensors for Quantification of Urinary Trimethylamine N-Oxide, ACS Appl. Mater. Interfaces. (2018), https://doi.org/10.1021/acsami.8b00995.
[266] Y. Kanemitsu, K. Asaji, Y. Matsumoto, H. Tsukamoto, D. Saigusa, C. Mukawa, T. Tachikawa, T. Abe, Y. Tomioka, Simultaneous quantitative analysis of uremic toxins by LC–MS/MS with a reversed-phase/cation-exchange/anion-exchange tri-modal mixed-mode column, Journal of Chromatography b. 1068–1069 (2017) 1–8.
[267] S. Veeravalli, K. Karu, I.R. Phillips, E.A. Shephard, A highly sensitive liquid chromatography electrospray ionization mass spectrometry method for quantification of TMA, TMAO and Creatinine in Mouse Urine, MethodsX. 4 (2017) 310–319.
[268] M.A. Bain, R. Faull, G. Fornasini, R.W. Milne, R. Schumann, A.M. Evans, Quantifying trimethylamine and trimethylamine-N-oxide in human plasma: interference from endogenous quaternary ammonium compounds, Anal. Biochem. 334 (2004) 403–405.
[269] L.J. Cohen, D. Esterhazy, S.-H. Kim, C. Lemetre, R.R. Aguilar, E.A. Gordon, A. J. Pickard, J.R. Cross, A.B. Emiliano, S.M. Han, J. Chu, X. Vila-Farres, J. Kaplitt, A. Rogoz, P.Y. Calle, C. Hunter, J.K. Bitok, S.F. Brady, Commensal bacteria make GPCR ligands that mimic human signalling molecules, Nature. 549 (2017) 48–53.
[270] W. Cho, A.G. York, R. Wang, T.P. Wyche, G. Piizzi, R.A. Flavell, J.M. Crawford, N-Acyl Amides from Neisseria meningitidis and Their Role in Sphingosine Receptor Signaling, Chembiochem. 23 (2022) e202200490.
[271] L.J. Cohen, H.-S. Kang, J. Chu, Y.-H. Huang, E.A. Gordon, B.V.B. Reddy, M. A. Ternei, J.W. Craig, S.F. Brady, Functional metagenomic discovery of bacterial effectors in the human microbiome and isolation of commendamide, a GPCR G2A/132 agonist, Proc. Natl. Acad. Sci. u. s. a. 112 (2015) E4825–E4834.
[272] T. Tørring, S.R. Shames, W. Cho, C.R. Roy, J.M. Crawford, Acyl Histidines: New N-Acyl Amides from Legionella pneumophila, Chembiochem. 18 (2017) 638–646.
[273] B. Tan, Y.W. Yu, M.F. Monn, H.V. Hughes, D.K. O'Dell, J.M. Walker, Targeted lipidomics approach for endogenous N-acyl amino acids in rat brain tissue, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 2890–2894.
[274] C. Landman, J.-P. Grill, J.-M. Mallet, P. Marteau, L. Humbert, E. Le Balc'h, M.-A. Maubert, K. Perez, W. Chaara, L. Brot, L. Beaugerie, H. Sokol, S. Thenet, D. Rainteau, P. Seksik, E. Quévrain, on behalf of the Saint Antoine IBD Network, Inter-kingdom effect on epithelial cells of the N-Acyl homoserine lactone 3-oxo-C12:2, a major quorum-sensing molecule from gut microbiota, PLoS One. 13 (2018) e0202587.
[275] H.B. Bradshaw, C.T. Johnson, Measuring the Content of Endocannabinoid-Like Compounds in Biological Fluids: A Critical Overview of Sample Preparation Methodologies, Endocannabinoid Signaling. (2023) 21–40.
[276] S.K. Syed, H.H. Bui, L.S. Beavers, T.B. Farb, J. Ficorilli, A.K. Chesterfield, M.-S. Kuo, K. Bokvist, D.G. Barrett, A.M. Efanov, Regulation of GPR119 receptor activity with endocannabinoid-like lipids, Am. J. Physiol. Endocrinol. Metab. 303 (2012) E1469–E1478.
[277] M.G.J. Balvers, K.C.M. Verhoeckx, R.F. Witkamp, Development and validation of a quantitative method for the determination of 12 endocannabinoids and related compounds in human plasma using liquid chromatography–tandem mass spectrometry, Journal of Chromatography b. 877 (2009) 1583–1590.
[278] R.K. Saini, P. Prasad, X. Shang, Y.-S. Keum, Advances in Lipid Extraction Methods-A Review, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms222413643.
[279] W. Röhrig, S. Achenbach, B. Deutsch, M. Pischetsrieder, Quantification of 24 circulating endocannabinoids, endocannabinoid-related compounds, and their phospholipid precursors in human plasma by UHPLC-MS/MS, J. Lipid Res. 60 (2019) 1475–1488.
[280] C. Sempio, J. Klawitter, M. Jackson, F. Freni, R. Shillingburg, K. Hutchison, L. C. Bidwell, U. Christians, J. Klawitter, Analysis of 14 endocannabinoids and endocannabinoid congeners in human plasma using column switching high-performance atmospheric pressure chemical ionization liquid chromatography–mass spectrometry, Anal. Bioanal. Chem. 413 (2021) 3381–3392.
[281] B. Tan, D.K. O'Dell, Y.W. Yu, M.F. Monn, H.V. Hughes, S. Burstein, J.M. Walker, Identification of endogenous acyl amino acids based on a targeted lipidomics approach, J. Lipid Res. 51 (2010) 112–119.
[282] M. Dubik, B. Pilecki, J.B. Moeller, Commensal Intestinal Protozoa—Underestimated Members of the Gut Microbial Community, Biology. 11 (2022) 1742.
[283] K. Li, Z. Su, Y. Gao, X. Lin, X. Pang, B. Yang, H. Tao, X. Luo, Y. Liu, X. Zhou, Cytotoxic Minor Piericidin Derivatives from the Actinomycete Strain Streptomyces psammoticus SCSIO NS126, Mar. Drugs. 19 (2021), https://doi.org/10.3390/md19080428.
[284] S. Shi, L. Cui, K. Zhang, Q. Zeng, Q. Li, L. Ma, L. Long, X. Tian, Streptomyces marincola sp. nov., a Novel Marine Actinomycete, and Its Biosynthetic Potential of Bioactive Natural Products, Front. Microbiol. 13 (2022) 860308.
[285] S. Guzmán-Salas, A. Weber, A. Malci, X. Lin, R. Herrera-Molina, W. Cerpa, C. Dorador, J. Signorelli, P. Zamorano, The metabolite p-cresol impairs dendritic development, synaptogenesis, and synapse function in hippocampal neurons: Implications for autism spectrum disorder, J. Neurochem. 161 (2022) 335–349.
[286] P. Bermudez-Martin, J.A.J. Becker, N. Caramello, S.P. Fernandez, R. Costa-Campos, J. Canaguier, S. Barbosa, L. Martinez-Gili, A. Myridakis, M.-E. Dumas, A. Bruneau, C. Cherbuy, P. Langella, J. Callebert, J.-M. Launay, J. Chabry, J. Barik, J. Le Merrer, N. Glaichenhaus, L. Davidovic, The microbial metabolite p-Cresol induces autistic-like behaviors in mice by remodeling the gut microbiota, Microbiome. 9 (2021) 157.
[287] H. Kawanabe-Matsuda, K. Takeda, M. Nakamura, S. Makino, T. Karasaki, K. Kakimi, M. Nishimukai, T. Ohno, J. Omi, K. Kano, A. Uwamizu, H. Yagita, I. G. Boneca, G. Eberl, J. Aoki, M.J. Smyth, K. Okumura, Dietary Lactobacillus-Derived Exopolysaccharide Enhances Immune-Checkpoint Blockade Therapy, Cancer Discov. 12 (2022) 1336–1355.
[288] C. Gaignard, C. Laroche, G. Pierre, P. Dubessay, C. Delattre, C. Gardarin, P. Gourvil, I. Probert, A. Dubuffet, P. Michaud, Screening of marine microalgae: Investigation of new exopolysaccharide producers, Algal Research. 44 (2019) 101711.