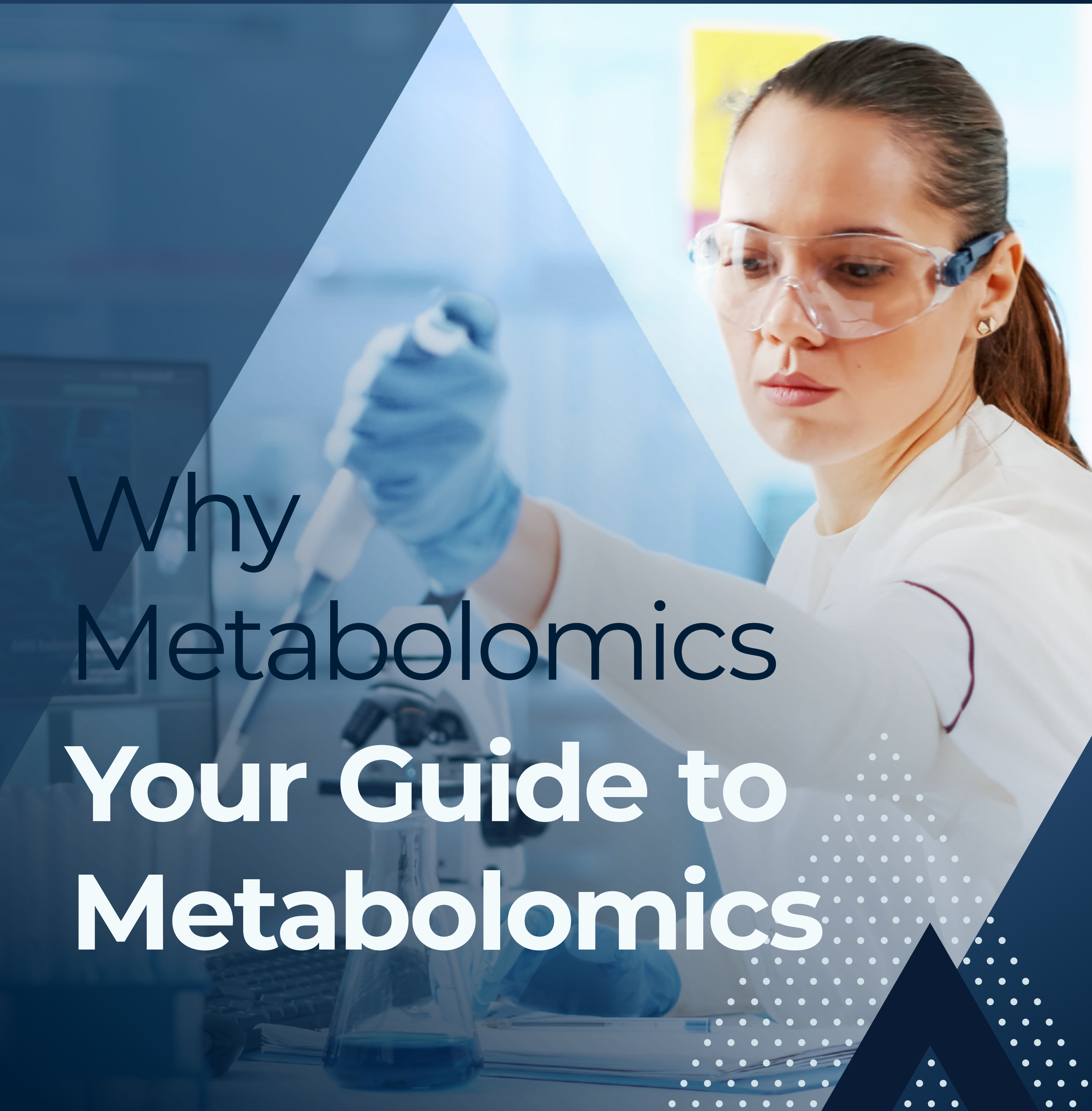
A GUIDE TO
METABOLOMICS

Metabolon



Why Metabolomics

Your Guide to Metabolomics

A GUIDE TO
METABOLOMICS

Contents

A GUIDE TO
METABOLOMICS

Chapter 1

Metabolomics, Metabolites, and the Metabolome

It's no secret—multi-omics research is quickly becoming the new standard in human disease research.

What is Metabolism?

Metabolism is the set of life-sustaining chemical reactions in organisms. The main purposes of metabolism are to: convert food or fuel to energy so that cellular processes can run; convert food to building blocks for proteins, lipids, nucleic acids, and carbohydrates; and eliminate metabolic wastes. These enzyme-catalyzed reactions allow organisms to grow, reproduce, maintain their structures, and respond to their environment.

What is Metabolomics?

Metabolomics is the identification of small molecules known as metabolites.[1] Metabolomics is a powerful companion to other commonly used omics techniques: genomics, transcriptomics, and proteomics. While these techniques provide information about genetic and functional potential, metabolomics goes a layer deeper by providing phenotypic information. Our genomes, transcriptomes, proteomes, and metabolomes don't exist in a vacuum—they impact and are impacted by one another as well as by the outside environment (Figure 1).

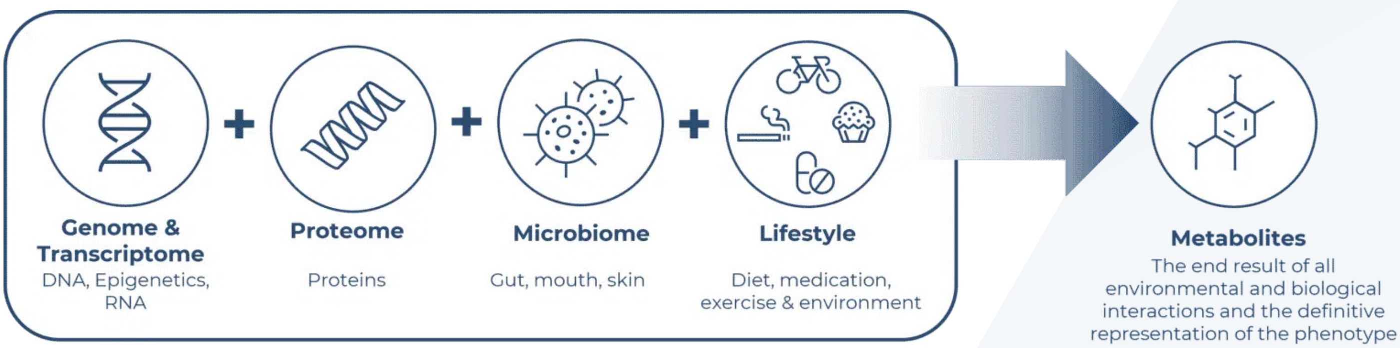


Figure 1.

The genome, studied via genomics, provides all of the information necessary for creating a functional organism. The transcriptome, studied via transcriptomics, reveals which genes are actually being turned into transcripts as well as which non-coding RNAs are present and can elucidate effects on the genome or even on the proteome and metabolome. The proteome, studied via proteomics, is the collection of proteins that have actually been translated from RNA transcripts. Finally, the metabolome, studied via metabolomics, includes all small molecules included that are present in an organism under a certain set of conditions. While each of these molecules can influence any of the others, environmental factors, such as therapeutic treatments, can also critically impact gene expression, regulation, protein folding, and metabolic function.

Figure 1. The complex interrelationship between genes, environment, and functional capacity.

The information gathered from genomics, transcriptomics, proteomics, and metabolomics, when combined (called multi-omics) provides a complete puzzle explaining the intricate regulatory and functional mechanisms behind living organisms. Think of it this way: other omics techniques—genomics, transcriptomics, and proteomics—can identify a tree as an apple tree, and even which kind of apple the tree produces. But metabolomics will tell you about the flavor profile of the apple, which antioxidants are present and how much, and how specific weather patterns have influenced those antioxidants. Such information can be useful for farmers to decide which plants to breed in the future and which might be the best candidates for commercialization. In the context of human health, metabolomics can help elucidate the molecular mechanisms behind the development of cancer (or any other disease) and why certain people respond to specific treatments but not others.[2]

What is a Metabolite?

Metabolites are small molecules less than 1.5 kDa in size.[3] The end products of metabolism, metabolites have a wide range of functions, including cell growth support, defense and inhibition, and stimulation. They include amino acids, alcohols, vitamins, polyols, organic acids, and many other types of molecules, and are often the building blocks for larger compounds.[2] Identifying metabolites and how they interact with one another—and how those interactions change under certain conditions—can aid in the discovery and understanding of how organisms work, why diseases develop (or not), why treatments are successful or not, and so much more.[2]

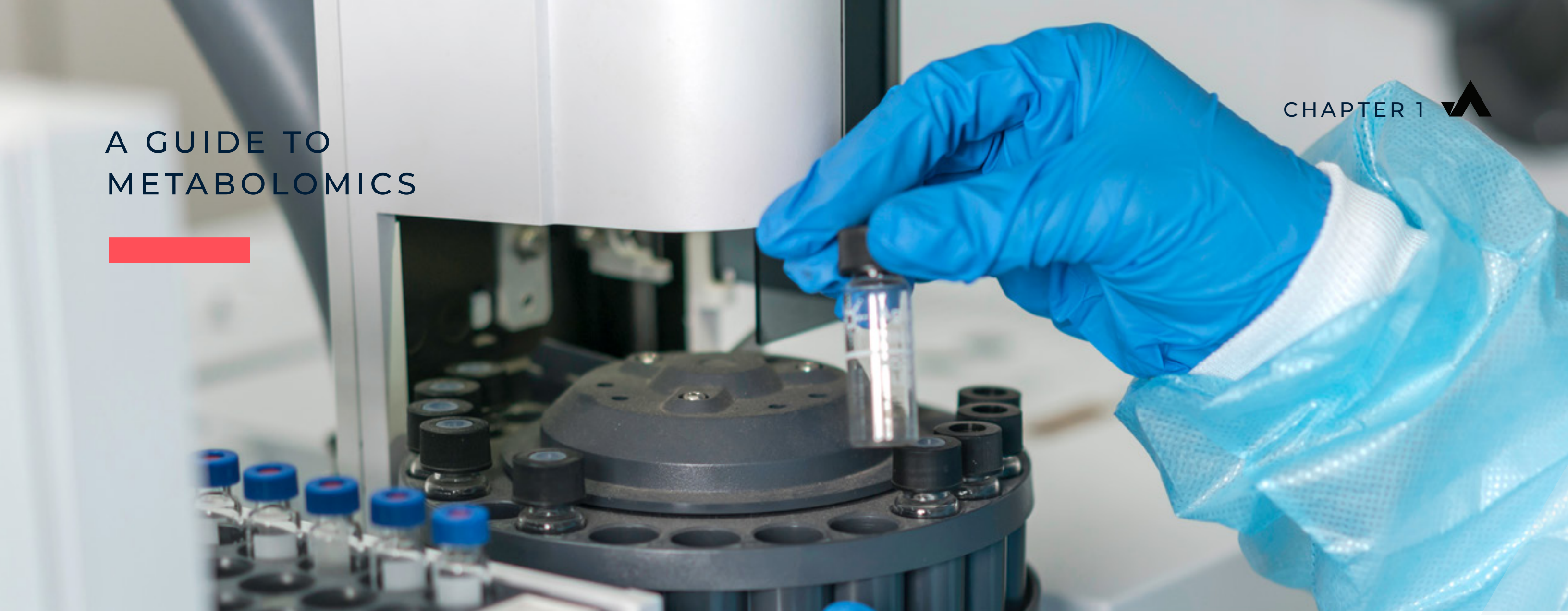
What is the Metabolome?

While the genome represents the entirety of genetic information encoded in DNA and the transcriptome all RNA transcripts, for example, the metabolome is the collection of all metabolites present in an organism. Metabolomes are incredibly fluid and can change drastically with differences in the outside environment, such as nutrient availability, medications, etc. Importantly, the metabolome doesn't just include metabolites produced natively by an organism, but also metabolites produced by the microbiome residing in and on that organism. Microbial metabolites, for example, can have a significant impact on the metabolism of therapeutics inside the host and can impact whether medications such as chemotherapy are effective.[2]

What is a Genotype?

Genotype refers to an individual's genes and DNA that determine its phenotype.

In other words, it is the "blueprint" for an organism's physical characteristics. The genotype is determined by the genes that are inherited from the organism's parents. The genotype is always present and will ultimately



determine the phenotype. Therefore, it is important to know the genotype of an organism in order to predict its phenotype.

What is a Phenotype?

Phenotype is the set of characteristics of an organism that are determined by a combination of genetics and the environment.

The term phenotype includes all physical and functional characteristics of an organism, from its morphology to its behavior. Many phenotypic traits are determined by a single gene, but most are the result of the interplay of multiple genes and the environment. The environment can influence phenotypic traits in many ways, including through nutrition, temperature, toxins, and stress. As a result, phenotype is often used as a general term for the overall appearance and function of an organism, rather than referring to a specific trait.

Many phenotypic traits are determined by a single gene, but most are the result of the interplay of multiple genes and the environment.

References

1. *Liu X, Locasale JW. Metabolomics: A Primer. Trends Biochem Sci. 2017;42(4):274-284. doi:10.1016/j.tibs.2017.01.004*

2. *Ashrafian H, Sounderajah V, Glen R, et al. Metabolomics: The Stethoscope for the Twenty-First Century. Med Princ Pract. 2021;30(4):301-310. doi:10.1159/000513545*

3. *Wishart DS, Tzur D, Knox C, et al. HMDB: the Human Metabolome Database. Nucleic Acids Res. 2007;35(Database issue):D521-D526. doi:10.1093/nar/gkl923*

Chapter 2

Other Omics Sciences and Metabolomics

In the first chapter of this guide, we provided a high-level overview of metabolites and metabolomics, the study of all the metabolites in a particular organism or system. In this chapter, we dive deeper into metabolomics by exploring how it fits into the overall omics landscape.

What are Omics?

Advances in scientific technology have made it possible to survey living systems at the tissue and cellular levels by measuring the thousands of molecules that comprise those systems. The high-throughput measurement of these molecules is collectively referred to as "omics."[1] While there are several types of omics approaches, there are four main omics disciplines that reflect the ever-increasing complexity of living systems, from genetic code to phenotype: genomics, transcriptomics, proteomics, and metabolomics.

Combining different omics disciplines through multi-omics studies provides a holistic picture of living organisms and systems.

Each of these omics disciplines can be studied individually, but biological systems don't act in a vacuum. Combining different omics disciplines through multi-omics studies[2] provides a holistic picture of living organisms and systems. Below, we define each of the four major omics disciplines, compare metabolomics to each of the other three, and discuss how studying them synergistically can drive scientific discovery.

Genomics and Metabolomics

Genomics and metabolomics sit at opposite ends of the biological spectrum. Genomics is the study, typically via sequencing, of all the genetic material in an organism, including single nucleotide polymorphisms (SNPs), indels, gene loss and amplification, and copy number variations (CNVs). In other words, genomics is the study of an organism's blueprint and any changes to that blueprint that impact the genetic potential. Metabolomics, on the other hand, is the complete set of small molecules present in an organism at a single point in time, measured via mass spectrometry (most commonly) or nuclear magnetic resonance (NMR). In essence, metabolomics is genetic potential in action and can be impacted by literally anything: food, drugs, medications, stress, etc.

Genomics is the study of an organism's blueprint and any changes to that blueprint that impact the genetic potential. Metabolomics, on the other hand, is the complete set of small molecules present in an organism at a single point in time, measured via mass spectrometry (most commonly) or nuclear magnetic resonance (NMR).

It isn't difficult to imagine how metabolomics and genomics datasets can add important contextual information to one another to elucidate why specific genetic anomalies are associated with specific phenotypes. The integration of genomic and metabolomic data has been extensively used to elucidate metabolite chemical structures, functions, and biosynthetic origins, and several tools have been developed[3] to aid such efforts. Genome-metabolome integrations have also proven particularly useful in oncology research, as cancer has critical genetic and metabolomic characteristics. One study[4] has even used genomics-metabolomics integration to help elucidate why genetic aberrations can be observed in both healthy and cancerous cells and why specific environmental and nutrient cues act as "selectors" during oncogenesis.

Transcriptomics and Metabolomics

While genomics represents an organism's genetic potential, not all of that potential is enacted. In fact, in humans, only 1.5% to 2% of the genome represents protein-coding genes. Cataloging the complete set of RNA transcripts (typically via microarrays or RNA sequencing) from DNA in a cell, tissue, or organism provides a better idea of which genetic elements have been activated.

Transcriptomics is a powerful tool for cataloging not just expressed genes, but also noncoding RNA elements (ribosomal RNA, messenger RNA, transfer RNA, microRNA, and long noncoding RNA) that may impact gene expression, and how these elements differ in healthy and diseased states. Transcripts often reveal signatures that cannot be detected through genomics approaches alone, such as RNA regulators of driver genes[5] in oncology. But the presence of a transcript doesn't always equate to mature, functional protein. Metabolomics, on the other hand, reveals critical information about which genetic instructions have not only been transcribed into RNA but have been converted into measurable phenotypes.

Transcripts often reveal signatures that cannot be detected through genomics approaches alone, such as RNA regulators of driver genes in oncology.

Integrated transcriptomics-metabolomics datasets can help demystify the bidirectional and multi-faceted interactions between DNA and RNA elements that lead to observable phenotypes and provide actionable insights into what is going on in a biological system. For example, combining transcriptomics and metabolomics data can make the molecular analysis of a blood sample even more comprehensive[6] when diagnosing disease and has been proposed as a powerful approach to drive personalized and precision medicine across a range of conditions.

Proteomics and Metabolomics

Proteomics is the most closely related omics technology to metabolomics. It adds another layer of complexity to genomics and transcriptomics by revealing not genetic potential, but which gene products (ie, proteins) have been synthesized by an organism. Several things can impact whether a mature, functional protein is produced by an organism, such as post-translational modifications or environmental toxins and nutrients, which is why combining genomic/transcriptomic and proteomic datasets can reveal important differences between what an organism might be doing and what an organism is actually doing.

Metabolomics is the study of the small molecules in an organism, and some of these are derived from proteins. Yet, there are some key differences between metabolomics and proteomics. First and foremost, proteomics captures information about all proteins produced by an organism, while metabolomics focuses on metabolites alone. Second, while proteomics seeks to understand and describe protein structure and function, metabolomics studies metabolites under given sets of conditions—for example, during drug treatment.

Combined, proteomics and metabolomics provide a complete phenotypic picture. Proteomics reveals which RNA transcripts have yielded mature, functional proteins while metabolomics drills deeper and explores how those proteins act differently under divergent circumstances. Integrated datasets combining proteomics and metabolomics have been used to identify cancer biomarkers[7] and characterize mechanisms of action[8] of anti-tumor agents.

Why Multi-omics Approaches Need Metabolomics

As more and more research combine various omic approaches to elucidate the basics of human biology and demystify disease progression, multi-omics is driving advances in human health and disease research. The U.S. market size of single-cell multi-omics alone is expected to exceed $7 billion USD by 2027—and metabolomics plays a critical role in enabling this rapid growth. Metabolomics is the study of specific phenotypes that result from the intricate and complex interaction between the genome, transcriptome, proteome, and environment. It is the final and critical piece of the puzzle because only it can reveal the result of these complex interactions.

Metabolomics is the study of specific phenotypes that result from the intricate and complex interaction between the genome, transcriptome, proteome, and environment.

Lipidomics—the large-scale study of pathways and networks of cellular lipids—and glycomics—the systematic study of all glycan (ie, sugar) structures of a given cell, tissue, or organism—are metabolomics "specializations" that are driving a significant proportion of metabolomics' influence on the overall contribution of multi-omics studies to advancing our understanding of health and disease. These specific metabolites often play critical roles in disease pathogenesis or serve as biomarkers. Sophisticated tools, such as the iKnife,[9] are being developed to aid in diagnosing, treating, and monitoring disease. For example, the iKnife can inform surgeons about the disease status of tissue during procedures by both heating the tissue then performing real-time lipidomics analysis of the resulting smoke.

The iKnife is just one very unique example of the many ways metabolomics is ushering in a new era in our understanding of health and disease. Multi-omics approaches centered on metabolomics as a key component are poised to revolutionize healthcare. Metabolon is positioned at the forefront of this new era in clinical research. The global metabolomics platform, which leverages Metabolon's unmatched in-house database of over 5,400 small molecules, helps researchers identify pharmacodynamic, efficacy and response biomarkers and reveals changes in key biological pathways. Many pharmaceutical companies have already leveraged this platform to improve their clinical trials and ensure successful trials of a variety of therapeutics.

References

1. *Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials; Board on Health Care Services; Board on Health Sciences Policy; Institute of Medicine; Micheel CM, Nass SJ, Omenn GS, editors. Evolution of Translational Omics: Lessons Learned and the Path Forward. Washington (DC): National Academies Press (US); 2012 Mar 23. 2, Omics-Based Clinical Discovery: Science, Technology, and Applications. Available from: https://www.ncbi.nlm.nih.gov/books/NBK202165/?report=classic*

2. *Subramanian I, Verma S, Jere A, Anamika K. Multi-omics Data Integration, Interpretation, and Its Application. Bioinformatics and Biology Insights. 01/31 2020;14:117793221989905. doi:10.1177/1177932219899051*

3. *Schorn MA, Verhoeven S, Ridder L, et al. A community resource for paired genomic and metabolomic data mining. Nature Chemical Biology. 2021/04/01 2021;17(4):363-368. doi:10.1038/s41589-020-00724-z*

4. *Gonçalves E, Frezza C. Genome and metabolome: chance and necessity. Genome Biology. 2021/09/23 2021;22(1):276. doi:10.1186/s13059-021-02501-0*

5. *Dai X, Kaushik AC, Zhang J. The Emerging Role of Major Regulatory RNAs in Cancer Control. Front Oncol. 2019;9:920. Published 2019 Sep 24. doi:10.3389/fonc.2019.00920*

6. *Li S, Todor A, Luo R. Blood transcriptomics and metabolomics for personalized medicine. Computational and Structural Biotechnology Journal. 2016/01/01/ 2016;14:1-7. doi:10.1016/j.csbj.2015.10.005*

7. *Ma Y, Zhang P, Wang F, Liu W, Yang J, Qin H. An Integrated Proteomics and Metabolomics Approach for Defining Oncofetal Biomarkers in the Colorectal Cancer. Annals of Surgery. 2012;255(4). doi:10.1097/SLA.0b013e31824a9a8b*

8. *Chen Y, Ni J, Gao Y, et al. Integrated proteomics and metabolomics reveals the comprehensive characterization of antitumor mechanism underlying Shikonin on colon cancer patient-derived xenograft model. Scientific Reports. 2020/08/24 2020;10(1):14092. doi:10.1038/s41598-020-71116-5*

9. *Tzafetas M, Mitra A, Paraskevaidi M, et al. The intelligent knife (iKnife) and its intraoperative diagnostic advantage for the treatment of cervical disease. Proc Natl Acad Sci U S A. Mar 31 2020;117(13):7338-7346. doi:10.1073/pnas.1916960117*

Chapter 3

Metabolite Identification and Detection

If you've studied the first two chapters of this comprehensive guide on metabolomics, you have a basic idea of what metabolomics is and how it fits into the greater omics landscape. In this chapter, we'll take a deeper dive into metabolomics, including the types of metabolomics-based analyses that you can do and what they tell you about the metabolic processes occurring in a biological host or environment.

What is Metabolomics?

As discussed in previous chapters, metabolomics is the identification and quantification of small molecules known as metabolites. Because these small molecules are necessary for cell growth, defense, inhibition, and stimulation—and because their function can be significantly impacted by a range of factors such as age, disease, drugs, and the environment—studying them is a powerful tool for understanding biological function.

Metabolites are sensitive to various factors, making the metabolome a fluid entity. Therefore, metabolomics, which can characterize these dynamic molecular changes, can be thought of as longitudinal phenotyping or a "study in motion." This makes metabolomics a particularly powerful tool for time series studies, such as responses to therapeutic regimens. Clinical applications like these will be discussed thoroughly in Chapter 5—Clinical Applications of Metabolomics.

It is important to recognize that the metabolome includes not just metabolites produced by the organism (such as amino acids, alcohols, and vitamins), but also exogenous metabolites[1] from the environment, including food, medication, or environmental toxins. A large proportion of studies also investigate the metabolome of the microbiome and how it, too, interacts with the host and environmental metabolomes to impact disease pathogenesis, drug response, nutrient acquisition, and more.

The Metabolome and Metabolic Reactions

Metabolomics-based analyses are nothing more than an observation of metabolic reactions within the host. Metabolites are the end products of those reactions, and identifying and quantifying them, especially over time, provides a good window into the biological function of the host under a defined set of conditions.

There are two basic types of metabolic reactions: catabolic, making energy from breaking down nutrients, and anabolic, building compounds.

But what, exactly, do we mean when we say metabolic reactions? Simply put, metabolic reactions are chemical reactions required to sustain life. There are two basic types of metabolic reactions: catabolic, making energy from breaking down nutrients, and anabolic, building compounds. Some examples of metabolic reactions are:

- Carbohydrates from foods are turned into glucose, which is then used to generate ATP (adenosine triphosphate, the energy currency of the cell).
- Drugs are broken down by the body into forms that are more easily excreted.
- Amino acids combine and form proteins, which have thousands of different forms and functions in the human body.
- Bacteria in the large intestine break down fiber into molecules called short-chain fatty acids, which then act as a source of energy for intestinal cells.

By sampling the metabolome frequently over a period of time, researchers can begin to understand how and why certain metabolic changes and patterns regulate or inform the development of disease, the efficacy of drugs, and other physiological processes. For example, researchers have found biomarkers that can inform certain cancers, and, going deeper, other biomarkers that can diagnose and prognosticate other cancers.[2,3] In some cases, the mechanism of action behind these different metabolite populations has been elucidated and can include molecular events such as altered gene expression, alternative splicing events, or impaired cellular processes.

Researchers have found biomarkers that can inform certain cancers, and, going deeper, other biomarkers that can diagnose and prognosticate other cancers.

Profiling Metabolomes

Mass spectrometry (MS) and nuclear magnetic resonance (NMR)-based techniques are most used to capture and analyze metabolomes.[4] In cases where investigators want to identify the metabolites in a set of samples, we use a global untargeted approach. When the aim is to quantify a set of known metabolites (such as in drug metabolism studies), we use targeted metabolomics. Untargeted metabolomics is used to generate hypotheses while targeted metabolomics is used to test hypotheses and better understand the findings of untargeted studies.

A wide range of sample types can be used for metabolomics analyses, including cells in culture or culture media, tissue, feces, blood, urine, and sweat. The sample source you use depends on what you'd like to study; for example, you'll select feces if you want to study microbial impacts on host nutrient acquisition, but tissue samples if you want to identify biomarkers that differentiate primary tumors and metastases. Samples must be carefully prepared according to the amount and type of sample and whether a targeted or untargeted approach is desired. Sample preparation will be discussed in greater detail in Chapter 9—Designing a Metabolomic Study.

References

1. *Liu X, Locasale JW. Metabolomics: A Primer. Trends Biochem Sci. 2017;42(4):274-284. doi:10.1016/j.tibs.2017.01.004*

2. *Perkins BA, Caskey CT, Brar P, et al. Precision medicine screening using whole-genome sequencing and advanced imaging to identify disease risk in adults. Proc Natl Acad Sci U S A. 2018;115(14):3686-3691. doi:10.1073/pnas.1706096114*

3. *Shen J, Ye Y, Chang DW, et al. Circulating metabolite profiles to predict overall survival in advanced non-small cell lung cancer patients receiving first-line chemotherapy. Lung Cancer. 2017;114:70-78. doi:10.1016/j.lungcan.2017.10.018*

4. *Ashrafian H, Sounderajah V, Glen R, et al. Metabolomics: The Stethoscope for the Twenty-First Century. Med Princ Pract. 2021;30(4):301-310. doi:10.1159/000513545*

Through Metabolon's end-to-end service, we ensure the maximum value, utility, and applicability of your data to advance your research objectives.

Chapter 4

The Importance of Metabolomics Insights

In the previous chapter of this guide, you learned a bit more about metabolites, the metabolome, and how to study the metabolome through metabolomics analyses. But why study the metabolome in the first place? In this chapter, we'll discuss why metabolomics is an attractive option on many levels, and provide a brief introduction to the academic, clinical, and commercial applications of metabolomics studies.

Metabolomics is an attractive option on many levels, and provides a brief introduction to the academic, clinical, and commercial applications of metabolomics studies.

What are Some of the Benefits of Metabolomic Studies?

Because metabolomics datasets provide phenotypic information, they are a powerful tool for adding context and direction to any research study. For example, enzyme kinetics studies (which are a critical component of drug development) can benefit from metabolomic profiling of the small molecules (ie, the cofactors and substrates) that interact with enzymes and impact their activity, helping troubleshoot studies that aren't working as expected and giving direction on where to go next.

Metabolomics datasets can also advance precision medicine research and development by shedding light on the person-to-person differences that impact how and why treatment strategies work or not. This can be something as simple as differential exposure to environmental components, such as pollution, that can impact drug effectiveness, or something as inherently unique as each individual metabolizes nutrients or medicines. Megan Showalter, PhD, Strategic Account Manager at Metabolon, provides an example we are probably all familiar with: "The globally accepted recommendation is that everyone take a certain dose of fish oil for heart and brain health, but we all metabolize it a bit differently. Metabolomics can help determine whether different people will benefit from different doses—or from fish oil, period."

"The globally accepted recommendation is that everyone take a certain dose of fish oil for heart and brain health, but we all metabolize it a bit differently. Metabolomics can help determine whether different people will benefit from different doses—or from fish oil, period."

Megan Showalter, PhD, Strategic Account Manager at Metabolon

Additional aspects of metabolomics studies that make them a great option for human medicine—personalized or not—are their application in next-generation disease diagnostics and their discovery potential for biomarkers. Several cancer biomarkers have already been identified using metabolomics, and the required samples can be collected in a non-invasive manner. Urine, breath, sweat, saliva, and fecal samples can all be collected painlessly and used to detect various diseases such as cancer, infectious diseases, neurodegenerative diseases, and more. These same samples can also be used for biomarker discovery, along with blood. And although blood collection isn't non-invasive per se, it is certainly far less invasive than traditional solid tissue biopsy.

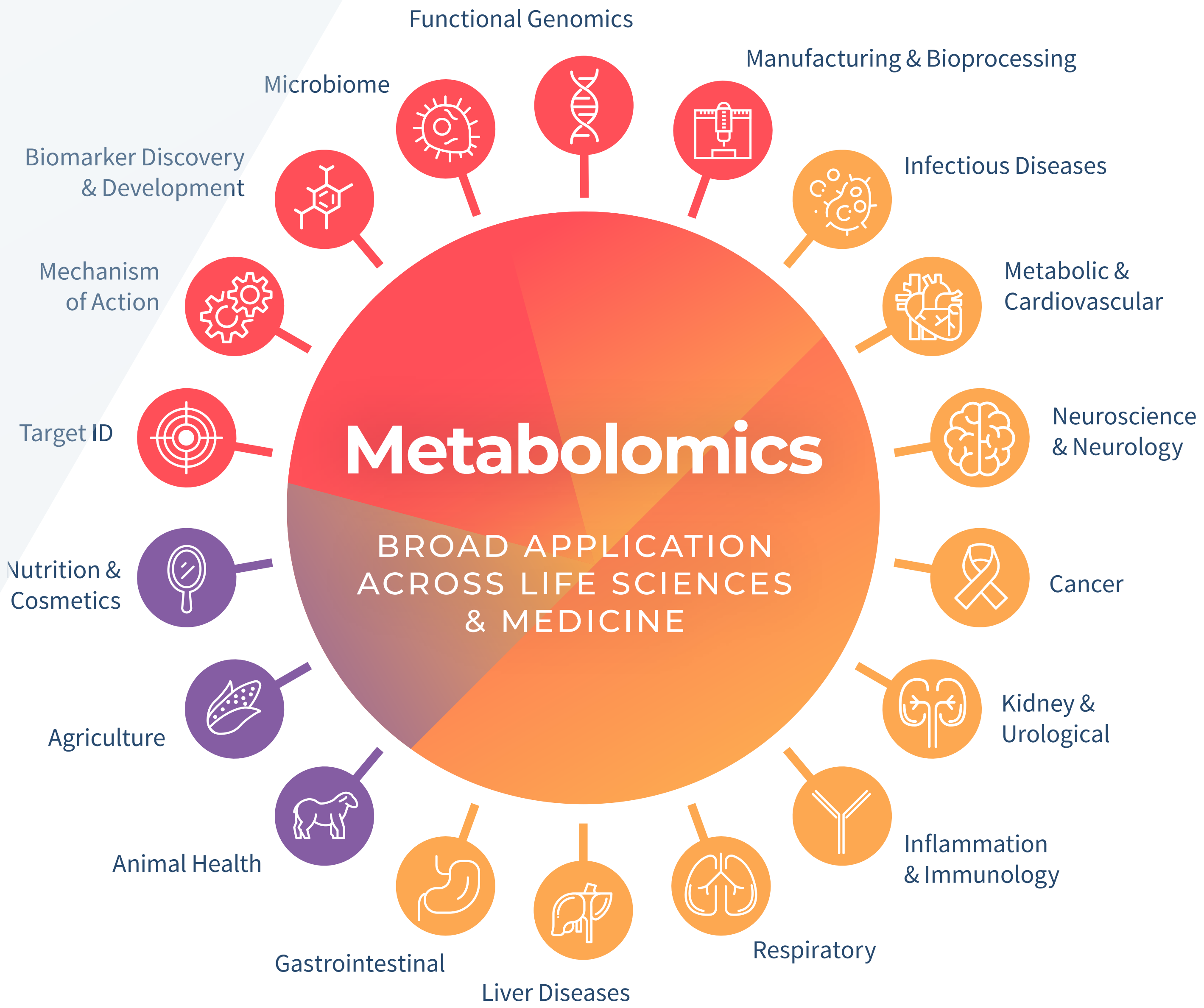
Metabolomics as Part of Multi-omics Approaches

Because the small molecules detected via metabolomics studies can be impacted by the genome, transcriptome, proteome, and environment, metabolomics is the only true reflection of phenotype at any given time. Any changes in or interactions between gene expression, protein expression, and the environment can be directly observed through the metabolome, making metabolomics the most complex and informative of all the omics techniques.

While each omic approach provides useful information, combining different approaches can reveal important biological and physiological signatures that can help elucidate the mechanisms behind specific disease etiologies, drug responses, and more. For a more in-depth discussion of metabolomics in the context of other omics approaches and why metabolomics is a critical component of multi-omics studies, refer to Chapter 2 of this guide.

Applications of Metabolomics

Metabolomics has many applications across the academic, clinical, and industrial sectors, from identifying disease biomarkers to accelerating pharmaceutical drug discovery to aiding farmers in selecting the most promising cultivars. Below we discuss some exciting capabilities realized through metabolomics in each of these sectors.



Academic

Academic research, which can also be called “bench science” or “basic science research” lays the foundational knowledge required to better understand biological systems and to make progress outside of the laboratory. The early stages of drug discovery, biomarker identification, microbial-host interactions in health and disease (for humans, plants, and animals), and more, typically start in the academic laboratory.

One of the hottest areas of research today is the human microbiome and its role in human health and disease. While the potential for leveraging the human microbiome to optimize human health has been recognized for several years, its therapeutic potential has been limited by knowledge gaps in precisely how the human microbiome interacts with its host. By adding metabolomics components to their (traditionally DNA-based) studies, researchers are beginning to understand how the microbiome impacts our immune systems,[1] digestive system,[2] metabolism,[3] skin, and brain function. These studies are laying the foundation for pre-clinical and, eventually, clinical studies that will make testing and addressing the microbiome in the clinic as common as measuring blood pressure.

Histone modification[4] is also a growing area of academic research, because it plays such an integral role in life—from basic biological processes like DNA repair to complex physiologies like disease etiology. Several metabolomics studies have reported crucial relationships between metabolites and histone modifications, and how fluctuations in these relationships can impact diseases such as cancer.[5] Basic research like this that provides mechanistic understanding often drives drug discovery work, eventually resulting in effective therapeutics used in clinical practice.

Clinical

Metabolic signatures have been used clinically to some capacity for over 30 years (and in some cases, even longer). For example: the heel-prick test done on babies after their birth detects certain inborn errors in metabolism; cholesterol tests measure lipid metabolites; and the Warburg effect[6] is a well-documented metabolic hallmark of cancer. But these are all tests or signatures applied to a global population—nothing is individualized about them. They are performed and read in the exact same way for everyone.

Metabolomics, by definition, is the high-throughput measurement of hundreds to thousands of metabolites at a time from multiple samples at once. This makes it the perfect tool for advancing precision medicine, which accounts for patient-specific variables that impact health and disease.

Many phenotypic traits are determined by a single gene, but most are the result of the interplay of multiple genes and the environment.

A GUIDE TO METABOLOMICS

Metabolomics provides a deep look into the metabolites present in any given sample at any given time, so it can be used to characterize metabolic anomalies associated with disease, discover and validate new therapeutic targets and biomarkers, and dictate personalized therapeutic approaches for patients based on their own unique metabolic profiles.

Metabolomics provides a deep look into the metabolites present in any given sample at any given time, so it can be used to characterize metabolic anomalies associated with disease, discover and validate new therapeutic targets and biomarkers, and dictate personalized therapeutic approaches for patients based on their own unique metabolic profiles.

Metabolomics can also help direct dosing strategies for clinical trials and help troubleshoot failed interventions. In one case study, investigators turned a failed phase II trial into a successful phase III trial using metabolomics to re-design the trial parameters. In another case study, metabolomics was integrated into a clinical trial design so investigators could gain insight on a precision treatment for pulmonary arterial hypertension. These are just two examples of many.

Commercial

There are several commercial applications for metabolomics, including pharmaceutical, cosmetic, nutritional science, and agriculture. Pharmaceuticals are a significant opportunity for metabolomics, which can provide early information on drug toxicity[7] and pharmacokinetics to increase the chances of a drug proceeding through clinical trials and gaining regulatory approval through the FDA and EMA. Cosmetics also stand to benefit from metabolomics studies, which are being used to drive research into personalized skin care and, like drug toxicity studies, can characterize cosmetic product toxicity and activity[8]—a particularly important factor for companies that do not engage in animal testing.

Cosmetics also stand to benefit from metabolomics studies, which are being used to drive research into personalized skin care

Metabolomics can also assist food breeders in optimizing for traits such as flavor[9] and selecting the cultivars most likely to succeed commercially—as well as prevent large-scale losses by detecting food adulteration and contamination.[10] Other research groups and companies are leveraging microbial metabolomics to improve fertilizers, while still more are using metabolomics to better understand nutrition and address malnutrition on a global scale. It is also being used to drive innovation in animal nutrition, which can impact not only the health of our beloved pets but also the health of animals intended for food (and, therefore, our own health).

References

1. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020;30(6):492-506. doi:10.1038/s41422-020-0332-7

2. Vernocchi P, Del Chierico F, Putignani L. Gut Microbiota Metabolism and Interaction with Food Components. Int J Mol Sci. 2020;21(10):3688. Published 2020 May 23. doi:10.3390/ijms21103688

3. Jin Q, Black A, Kales SN, Vattem D, Ruiz-Canela M, Sotos-Prieto M. Metabolomics and Microbiomes as Potential Tools to Evaluate the Effects of the Mediterranean Diet. Nutrients. 2019;11(1):207. Published 2019 Jan 21. doi:10.3390/nu11010207

4. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet. 2012;13(5):343-357. Published 2012 Apr 3. doi:10.1038/nrg3173

5. Simithy J, Sidoli S, Garcia BA. Integrating Proteomics and Targeted Metabolomics to Understand Global Changes in Histone Modifications. Proteomics. 2018;18(18):e1700309. doi:10.1002/pmic.201700309

6. Liberti MV, Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? [published correction appears in Trends Biochem Sci. 2016 Mar;41(3):287] [published correction appears in Trends Biochem Sci. 2016 Mar;41(3):287]. Trends Biochem Sci. 2016;41(3):211-218. doi:10.1016/j.tibs.2015.12.001

7. Ramirez T, Daneshian M, Kamp H, et al. Metabolomics in toxicology and preclinical research. ALTEX. 2013;30(2):209-225. doi:10.14573/altex.2013.2.209

8. Jacques C, Jamin EL, Jouanin I, et al. Safety assessment of cosmetics by read across applied to metabolomics data of in vitro skin and liver models. Arch Toxicol. 2021;95(10):3303-3322. doi:10.1007/s00204-021-03136-7

9. Klevorn CM, Dean LL, Johanningsmeier SD. Metabolite Profiles of Raw Peanut Seeds Reveal Differences between Market-Types. J Food Sci. 2019;84(3):397-405. doi:10.1111/1750-3841.14450

10. Selamat J, Rozani NAA, Murugesu S. Application of the Metabolomics Approach in Food Authentication. Molecules. 2021;26(24):7565. Published 2021 Dec 14. doi:10.3390/molecules26247565

Chapter 5

Clinical Applications of Metabolomics

In the previous chapter of this guide, we provided a brief overview of the academic, clinical, and commercial applications of metabolomics. In this chapter, we dive deeper into past, current, and future clinical applications of metabolomics, including diagnostics, screening, and personalized medicine.

Clinical Screening and Diagnostic Applications of Metabolomics

Although metabolite tests, such as the heel-prick test performed on babies after their birth, and cholesterol screening tests have been used for decades to detect inborn errors in metabolism or disease risk/presence, the diagnostic potential of metabolomics is far more powerful than just these examples. Recent advances in automation and high-throughput techniques have enabled mass spectrometry to make significant contributions to disease diagnostics and screening landscape.

Metabolomics can and has identified several novel causes of various chronic diseases with previously uncharacterized etiologies, because it can probe complex biochemistry at both the cellular and organism levels as well as from both the host and environment (including the microbiome). This indicates that metabolites play a critical role in disease development, cellular signaling, and physiological control.

Perhaps the biggest contribution made by metabolomics in disease diagnostics is the growing understanding of cancer as a metabolic disease. Although the Warburg effect[1] has long been recognized as a hallmark of cancer, more recent large-scale metabolomics studies have solidified the presence of aerobic glycolysis and glutaminolysis in essentially all tumors and have additionally linked these metabolic processes[2] to many known oncogenes and tumor suppressors. Metabolomics has also facilitated the discovery of several oncometabolites, which are endogenous metabolites (such as 2-hydroxyglutarate) that sustain tumor growth and metastasis.

Perhaps the biggest contribution made by metabolomics in disease diagnostics is the growing understanding of cancer as a metabolic disease.

Metabolomics has also been used to identify an association[3] between increased serum levels of branched-chain amino acids (which function as insulin analogs) and type 2 diabetes risk. In fact, amino acid levels have been reported to be more predictive of disease onset[3] than GWAS studies or other genetic data. Other diseases for which a metabolic link to disease etiology has been reported include Alzheimer's disease, autism, asthma, and inflammatory bowel disease, among many others. A comprehensive list of diseases and their metabolic associations can be found by searching the Human Metabolome Database.

Metabolomics and Precision Medicine

Although metabolomics has several important applications in clinical diagnostics and screening, the biggest opportunity remains with precision medicine. Metabolomics is the measurement of metabolites or small molecules present within an organism, cell, or tissue. Current high-throughput technologies allow for the identification of hundreds to thousands of metabolites at a time within multiple samples at once. This makes it the perfect tool for enabling precision medicine,[4] which takes into account the individual variability that impacts disease etiology and responses to therapeutics to then create customized treatment plans.

Because metabolomics provides a deep look into the metabolites present in any given sample at any given time, it is particularly powerful for characterizing metabolic anomalies associated with disease, discovering and validating new therapeutic targets and biomarkers, and informing the design of personalized therapeutic approaches for patients based on their own unique metabolic profiles. Another aim of precision-based medicine is to facilitate a prevention-based health system. Because of metabolomics' ability to deeply characterize gene-environment interactions, scientists expect it[5] to play a critical role in enabling personalized medicine at large.

Although precision medicine is a relatively recent application area for metabolomics, metabolomics has already enabled several important advances[6] in precision/personalized medicine, including individualized drug-response monitoring,[7] the identification of novel biomarkers (ie, novel drug targets), and metabolic phenotyping of tumors,[8] which can help guide the development of more efficient, targeted cancer therapeutics. Other advances include the combination of DESI-MS (ie, desorption electrospray ionization mass spectrometry) with electro-incision techniques (such as the iKnife[9]) to facilitate precision surgery. As the surgeon's knife cuts through tissue, the vaporized tissue is immediately pushed through the DESI-MS, providing real-time information about which tissue is diseased and which is healthy, facilitating more precise surgical procedures and avoiding the unnecessary removal of healthy tissue.

Although precision medicine is a relatively recent application area for metabolomics, metabolomics has already enabled several important advances in precision/personalized medicine.

Metabolomics also has the potential to transform the drug development landscape. The current development paradigm, which relies on genetic screening and tests in animal models, sees concerningly high failure rates because only about 10% of diseases have a strong genetic basis[6] and are instead the result of interactions between genes and the environment. Because it is inherently phenotypic, metabolomics is purpose-built to inform better clinical trials with a higher chance of success. For example, one company used Metabolon's Global Discovery Panel to develop a more efficacious dosing strategy and redesign their clinical trial to bring a microbiome-based therapeutic that had failed Phase II clinical trials through a successful Phase III trial.[10]

Although clinical metabolomics research is growing, many laboratories don't have the necessary infrastructure and certifications to perform widespread clinical metabolomics. Metabolon offers two different targeted metabolomics panels for clinical research (the Quantose® Insulin Resistance Targeted Panel and the Quantose® Impaired Glucose Tolerance Targeted Panel) that are offered in Metabolon's CLIA-certified laboratory. Additionally, all global and targeted panels (including custom panels) offered by Metabolon adhere to ISO 9001 standards to facilitate biomarker discovery, drug development, and other pre-clinical and clinical research across a variety of disease landscapes.

References

1. *Liberti MV, Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? [published correction appears in Trends Biochem Sci. 2016 Mar;41(3):287] [published correction appears in Trends Biochem Sci. 2016 Mar;41(3):287]. Trends Biochem Sci. 2016;41(3):211-218. doi:10.1016/j.tibs.2015.12.001*

2. *Levine AJ, Puzio-Kuter AM. The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes [published correction appears in Science. 2012 May 11;336(6082):670]. Science. 2010;330(6009):1340-1344. doi:10.1126/science.1193494*

3. *Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. Nat Med. 2011;17(4):448-453. doi:10.1038/nm.2307*

4. *Clish CB. Metabolomics: an emerging but powerful tool for precision medicine. Cold Spring Harb Mol Case Stud. 2015;1(1):a000588. doi:10.1101/mcs.a000588*

5. *Di Minno A, Gelzo M, Caterino M, Costanzo M, Ruoppolo M, Castaldo G. Challenges in Metabolomics-Based Tests, Biomarkers Revealed by Metabolomic Analysis, and the Promise of the Application of Metabolomics in Precision Medicine. Int J Mol Sci. 2022;23(9):5213. Published 2022 May 6. doi:10.3390/ijms23095213*

6. *Wishart DS. Emerging applications of metabolomics in drug discovery and precision medicine. Nat Rev Drug Discov. 2016;15(7):473-484. doi:10.1038/nrd.2016.32*

7. *Balashova EE, Maslov DL, Lokhov PG. A Metabolomics Approach to Pharmacotherapy Personalization. J Pers Med. 2018;8(3):28. Published 2018 Sep 5. doi:10.3390/jpm8030028*

8. *Dos Santos GC, Saldanha-Gama R, de Brito NM, Renovato-Martins M, Barja-Fidalgo C. Metabolomics in cancer and cancer-associated inflammatory cells. Journal of Cancer Metastasis and Treatment. 2021; 7:1. http://dx.doi.org/10.20517/2394-4722.2020.86*

9. *Tzafetas M, Mitra A, Paraskevaidi M, et al. The intelligent knife (iKnife) and its intraoperative diagnostic advantage for the treatment of cervical disease [published correction appears in Proc Natl Acad Sci U S A. 2020 Aug 4;117(31):18892]. Proc Natl Acad Sci U S A. 2020;117(13):7338-7346. doi:10.1073/pnas.1916960117*

10. *McGovern BH, Ford CB, Henn MR, et al. SER-109, an Investigational Microbiome Drug to Reduce Recurrence After Clostridioides difficile Infection: Lessons Learned From a Phase 2 Trial. Clin Infect Dis. Jun 15 2021;72(12):2132-2140. doi:10.1093/cid/ciaa387*

Chapter 6

Academic Applications of Metabolomics

In the previous chapter of this guide, we took a deeper dive into the clinical applications of metabolomics, including routine screening, diagnosis, and precision medicine. But all clinical applications begin in the scientific research laboratory: academic research is vital to gaining new insights and advancing our understanding of biological systems to improve the diagnosis, prevention, and treatment of disease. In this chapter, we will explore the academic applications of metabolomics, including routine research and method development, and how these applications eventually lead to clinical translation.

The Human Metabolome

As with the human genome and the human microbiome, research efforts to fully understand the human metabolome require a comprehensive map, complete with compound concentrations, biofluid/tissue locations, subcellular locations, physical properties, known disease associations, nomenclature, descriptions, enzyme data, mutation data, and characteristic mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectra, to guide hypothesis generation and testing.

That map, the Human Metabolome Database (HMDB), was first introduced by Canadian researchers in 2007 and comprised nearly 2,200 endogenous metabolites, 1,200 drugs, and 3,500 food components.[1] The inclusion of drugs and food components in addition to host metabolites was critical because, as we've already discussed in previous chapters, metabolomics is the phenotypic expression of host-environment interactions.

Additionally, as recognition of the role the human microbiome plays in human health and disease increases, human metabolomics research increasingly includes metabolomic profiles of the human microbiome.[2] In 2019, the Virtual Metabolic Human database (VMH), comprising 5,180 unique metabolites, 17,730 unique reactions, 3,695 human genes, 255 Mendelian diseases, 818 microbes, 632,685 microbial genes, and 8,790 food items, was released to the scientific community as "a novel, interdisciplinary database for data interpretation and hypothesis generation."[3]

Databases like the HMDB and VMH, in addition to other human and bacterial metabolic resources and collections, have facilitated an amazing breadth of human metabolomics research, advancing our understanding of health and disease and the role metabolites play. Metabolomics is now standard scientific practice in many academic research labs, both for routine research applications and for technique development.

Metabolomics in Routine Research

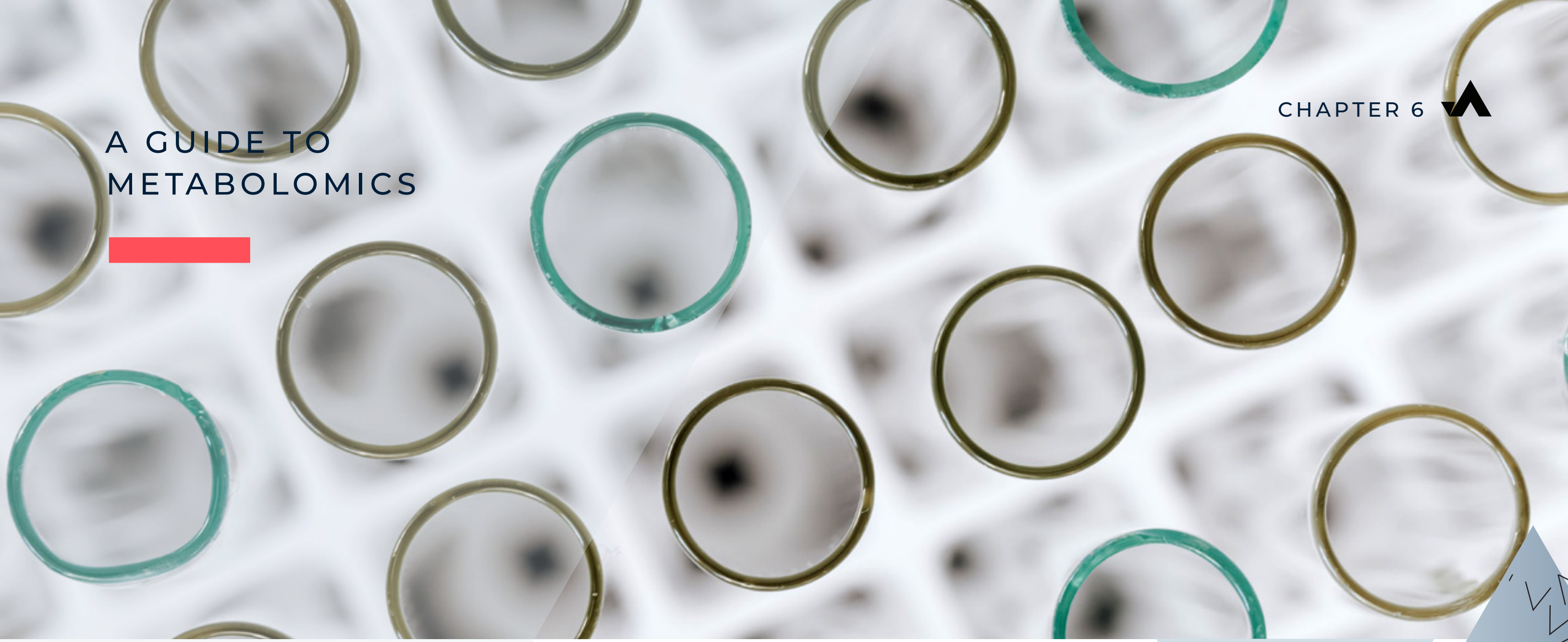
Studies of the relationship between genes and disease have improved our understanding of several diseases, including cancer. However, DNA sequences alone can't explain the intricate biological mechanisms that contribute to many "genetic" diseases. RNA sequencing (RNA-Seq) efforts to identify the impact of different types of RNA on gene and protein expression have shed some light on the molecular mechanisms behind disease, but a knowledge gap remains.

Combining metabolomics and genetic and/or transcriptomics datasets can complete the puzzle. For example, in prostate cancer, combining transcriptomics and metabolomics data has revealed that impaired sphingosine-1-phosphate receptor 2 signaling is the mechanism behind the specificity and sensitivity of sphingosine for distinguishing between prostate cancer and benign hyperplasia.[4] Research such as this lays the foundation for biomarker discovery and application in the clinic for a range of diseases, not just cancer.

Researchers have found biomarkers that can inform certain cancers, and, going deeper, other biomarkers that can diagnose and prognosticate other cancers.

Histone modification is also a rather large area academic research, because it plays such an integral role in life, from basic biological processes like DNA repair to complex physiologies like disease etiology.[5] Several metabolomics studies have reported crucial relationships between metabolites and histone modifications, and how fluctuations in these relationships can impact diseases such as cancer.[6] Basic research like this that provides mechanistic understanding is often the driver for drug discovery work, which can eventually make it to the clinic in the form of effective therapeutics.

No discussion of academic metabolomics studies would be complete without mentioning the human microbiome and its role in human health and disease. While the potential for leveraging the human microbiome to optimize human health has been recognized for several years, its therapeutic potential has been limited by knowledge gaps in precisely how the human microbiome interacts with its host. As we briefly touched on in Chapter 4 of this guide, by adding metabolomics components to their (traditionally DNA-based) studies, researchers are beginning to understand how the microbiome impacts our immune systems,[7] digestive systems,[8] metabolism,[9] skin,[10] and brain[11] function.



Metabolomics studies on the microbiome are laying the basic research foundation necessary for pre-clinical and, eventually, clinical studies that could make testing and addressing the microbiome in the clinic as common as measuring fasting plasma glucose. For example, a collaboration of academic investigators used Metabolon's services to link microbiome sequencing and metabolomics data in human twins to characterize microbiome-mediated differences in the incidence of food allergy.[12] Their results suggest a critical protective role against food allergy provided by the microbiome which lasts beyond the infant stage. Follow-up studies will determine the molecular mechanisms behind this relationship, which could identify biomarkers for diagnosis and novel targets for therapeutics.

As an increasing number of researchers view multi-omics research, particularly the combination of transcriptomics and metabolomics, as a new standard for human disease research, the demand for multi-omics tools has also increased. These tools address the challenges faced by researchers integrating omics datasets.

Technique Development with Metabolomics

Metabolomics research also plays a significant role in technique development, both by necessitating new research tools and techniques and by helping to support their development. A major area of development in the near term will be multi-omics tools capable of collecting, analyzing, and visualizing data. One of the biggest challenges facing the routine use of metabolomics datasets in many academic laboratories is the difficulty in combining metabolomics datasets with others, such as genetic and metatranscriptomic datasets.

New data analysis tools will address these challenges and fully realize the power of including metabolomics datasets in any research effort. As one group of researchers demonstrated, this could have significant implications for direct clinical translation.[13] Using the Metabolon Discover

Global Platform, the researchers identified several inflammatory pathways associated with childhood tuberculosis, and by combining this data with transcriptomics data, they accurately identified treatment responses and improved the interpretation of metabolic biomarkers in children with confirmed TB.

Metabolomics analyses can also support the development and use of novel animal models for furthering our understanding of disease and how to prevent or treat it. For example, researchers compared the plasma metabolomes of humans with a murine model of cardiac arrest to prove that their animal model metabolically replicates human disease and is therefore suitable for translational research on cardiac arrest.[14] This, of course, is just one example.

References

1. Wishart DS, Tzur D, Knox C, et al. HMDB: the Human Metabolome Database. Nucleic Acids Res. 2007;35(Database issue):D521-D526. doi:10.1093/nar/gkl923

2. Visconti A, Le Roy CI, Rosa F, et al. Interplay between the human gut microbiome and host metabolism. Nat Commun. 2019;10(1):4505. Published 2019 Oct 3. doi:10.1038/s41467-019-12476-z

3. Noronha A, Modamio J, Jarosz Y, et al. The Virtual Metabolic Human database: integrating human and gut microbiome metabolism with nutrition and disease. Nucleic Acids Res. 2019;47(D1):D614-D624. doi:10.1093/nar/gky992

4. Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics Data Integration, Interpretation, and Its Application. Bioinform Biol Insights. 2020;14:1177932219899051. Published 2020 Jan 31. doi:10.1177/1177932219899051

5. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet. 2012;13(5):343-357. Published 2012 Apr 3. doi:10.1038/nrg3173

6. Simithy J, Sidoli S, Garcia BA. Integrating Proteomics and Targeted Metabolomics to Understand Global Changes in Histone Modifications. Proteomics. 2018;18(18):e1700309. doi:10.1002/pmic.201700309

7. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020;30(6):492-506. doi:10.1038/s41422-020-0332-7

8. Vernocchi P, Del Chierico F, Putignani L. Gut Microbiota Metabolism and Interaction with Food Components. Int J Mol Sci. 2020;21(10):3688. Published 2020 May 23. doi:10.3390/ijms21103688

9. Jin Q, Black A, Kales SN, Vattem D, Ruiz-Canela M, Sotos-Prieto M. Metabolomics and Microbiomes as Potential Tools to Evaluate the Effects of the Mediterranean Diet. Nutrients. 2019;11(1):207. Published 2019 Jan 21. doi:10.3390/nu11010207

10. Roux PF, Oddos T, Stamatas G. Deciphering the Role of Skin Surface Microbiome in Skin Health: An Integrative Multiomics Approach Reveals Three Distinct Metabolite–Microbe Clusters. J Invest Dermatol. 2022;142(2):469-479.e5. doi:10.1016/j.jid.2021.07.159

11. Lai Y, Liu CW, Yang Y, Hsiao YC, Ru H, Lu K. High-coverage metabolomics uncovers microbiota-driven biochemical landscape of interorgan transport and gut-brain communication in mice. Nat Commun. 2021;12(1):6000. Published 2021 Oct 19. doi:10.1038/s41467-021-26209-8

12. Bao R, Hesser LA, He Z, Zhou X, Nadeau KC, Nagler CR. Fecal microbiome and metabolome differ in healthy and food-allergic twins. J Clin Invest. 2021;131(2):e141935. doi:10.1172/JCI141935

13. Dutta NK, Tornheim JA, Fukutani KF, et al. Integration of metabolomics and transcriptomics reveals novel biomarkers in the blood for tuberculosis diagnosis in children. Sci Rep. 2020;10(1):19527. Published 2020 Nov 11. doi:10.1038/s41598-020-75513-8

14. Shoaib M, Choudhary RC, Choi J, et al. Plasma metabolomics supports the use of long-duration cardiac arrest rodent model to study human disease by demonstrating similar metabolic alterations. Sci Rep. 2020;10(1):19707. Published 2020 Nov 12. doi:10.1038/s41598-020-76401-x

Through Metabolon's end-to-end service, we ensure the maximum value, utility, and applicability of your data to advance your research objectives.

A GUIDE TO
METABOLOMICS

Chapter 7

Commercial Applications of Metabolomics

In previous chapters of this guide, we explored the scientific discovery and breakthroughs enabled by metabolomics, from basic science research in the academic laboratory to clinical application and the growing future of precision medicine. In this chapter, we'll see how metabolomics supports a range of commercial applications, from nutrition to beauty and everything in between.

Drug Development (Pre-clinical and Clinical)

While drug development relies on academic and clinical research, it is not solely a research venture. The goal, of course, is to obtain regulatory approval (eg, FDA, EMA) and manufacture the drug at scale to serve the medical community while, of course, making a profit. Metabolomics can support several arms of early drug discovery and development:[1] identifying metabolites/biomarkers, identifying bioactive compounds, studying the metabolism of the compound, and characterizing the efficacy and toxicity of the compound. All of which are critical for establishing safety and efficacy of a drug and ultimately gaining regulatory approval.

Metabolomics can support multiple arms of early drug discovery and development.

Metabolomics can also support the design of efficacious clinical trials and/or troubleshoot trials that are failing for unknown reasons. For example, Metabolon's Global Discovery Panel was used to help develop a more efficacious dosing strategy and redesign of a clinical trial to bring a microbiome-based therapeutic that had failed Phase II clinical trials through a successful Phase III trial.[2] The impact is considerable because, by the time drugs make it to later stages of clinical trials, hundreds of millions of dollars have already been spent on their development. Ensuring successful clinical trials can improve the success rate[3] experienced by the pharmaceuticals industry today, ultimately lowering costs for everyone, including the patients who will be taking the drug.

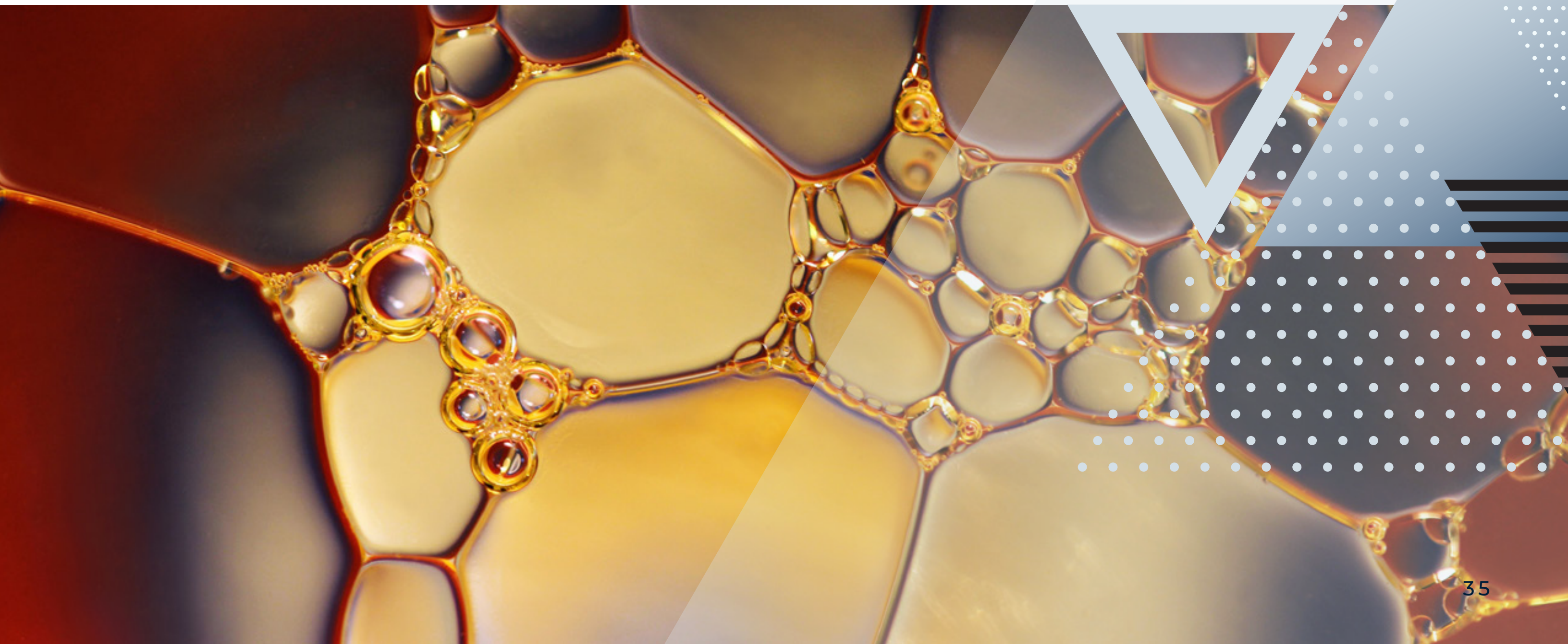
Metabolomics can also support the design of efficacious clinical trials and/or troubleshoot trials that are failing for unknown reasons.

Nutritional Sciences

Food and Drink Safety and Manufacturing

Most of us do not give a second thought about where our food comes from or the processes it went through to get to us. We don't wonder whether the piece of bread we're about to eat has nutritional value or worry whether it's safe to eat or contaminated with substances that could make us sick. We just eat. Metabolomics plays a crucial role in providing us the peace of mind to simply enjoy our food without having to think about it.

Industrial food and beverage production is a multifaceted process. Not only must flavor and nutritional profiles be upheld, but quality and health standards must also be met to avoid sickening the population. Metabolomics is a valuable tool used by the food and beverage industry[4] to ensure flavor, quality, and safety:



- **Flavor:** Food taste is usually linked to primary and secondary non-volatile metabolites. Profiling these metabolites can provide valuable information about the flavor of food. For example, profiling the impact of marination[5] on these metabolites in meat can reveal important impacts on flavor (and thus, consumer buying habits). Additionally, ensuring realistic taste is a major goal of the alternative meat/protein industry[6] (including plant-based products); doing so can make major inroads to releasing humanely produced and environmentally friendly protein sources on a global scale.

- **Quality:** Metabolomics can quickly and easily profile markers of nutritional quality,[4] including carotenoids, flavonoids, volatiles, and other metabolites. Tracking these markers over time can also inform strategies to preserve freshness and maximize shelf-life, as well as to identify issues in the supply chain that negatively impact food quality. Appearance can also be an indicator of quality to consumers; for example, the color of fresh meat[7] (changes which can be characterized with metabolomics) is a major factor impacting consumers' perception of meat quality.

- **Food safety:** Metabolomics is often used to identify whether microbial toxins and other contaminants are present in food. Contaminants are not just limited to bacterial and fungal pathogens; researchers have also used metabolomics to profile the levels of veterinary drugs in raw milk,[8] demonstrating the role of metabolomics tools in food safety in dairy processing specifically.

All the categories described above can and are impacted by the processing of foods and beverages, which can include heating, mixing, and drying—so continued metabolic profiling of food throughout the entire production process[9] can ensure the highest levels of flavor, quality, and safety.

Metabolomics can have a crucial role in ensuring that food and beverage production companies adhere to regulatory standards,[10] often around labeling. Production companies have been known to include false information about the origin, production, processing, and nutritional profile on their labels, switching out components for inferior ingredients to save money on the production of high-grade foods. Such fraudulent activities can be easily detected by metabolomics.[11] Similarly, foods with "organic" labels can be tested for their adherence to guidelines required to market a food or food product as organic (which often comes with steeper prices at the grocery store).

Transcripts often reveal signatures that cannot be detected through genomics approaches alone, such as RNA regulators of driver genes in oncology.

Vitamins and Supplements Quality Control

As with food and beverages, the effects of vitamins and supplements—including potential toxicity—should be carefully characterized and monitored. Although interest in vitamins and supplements has increased substantially over the past 20 years, they remain relatively understudied[12] as a substance intended for human consumption, largely due to differences in classification, regulation, and even definition around the world. Metabolomics (as well as other omics techniques) have played an important role in characterizing the metabolic and physiological impact and toxicity of the most common supplements, vitamins D, E, and A, and plant extracts such as resveratrol, green tea, ginseng, and curcumin.

Metabolomics has played an important role in characterizing the metabolic and physiological impact and toxicity of the most common supplements, vitamins D, E, and A, and plant extracts such as resveratrol, green tea, ginseng, and curcumin.

Plant-derived supplements are the fastest-growing segment[12] of the supplements industry and face the biggest issues with quality control and standardization. To address this, researchers have proposed frameworks[13] for the development of plant-derived/botanical supplements that mirror those of the typical pharmaceutical, both to ensure that the science around them is sound and reproducible and to ensure that safe and efficacious supplements are produced and released to the public. In the same way that metabolomics supports quality drug development, it can also support quality supplement development.

Like the food and beverage industry, metabolomics can also keep vitamin and supplement manufacturers accountable and honest by detecting inferior ingredients, contaminants, or fillers and by accurately tracking the source(s) of different substances to ensure that the products reaching the consumer are true-to-label and of high quality and purity.

Metabolomics studies of vitamins and supplements can also provide critical information for informing dosing, co-delivery with other vitamins, supplements, and drugs, and individualized responses to supplementation[14] that could inform personalized approaches to supplementation. Vitamin D is the most well-studied vitamin[12] in all these aspects, but these studies lay the foundation for others investigating any vitamin or supplement.

Personal Care and Cosmetics

Cosmetics and other products developed for topical application can be medicinal (e.g., skin-care products to treat acne), cosmetic (eg, makeup to cover up acne), or simply support good hygiene (eg, toothpaste). But regardless of their intended purpose, these products interact with and impact our skin, hair, mouth, and eyes, including the microbial communities that live in and on those body sites, and impact our health and well-being. Although studies are few compared to the food and supplement industries,

researchers are beginning to unravel how cosmetic ingredients impact the human metabolome,[15] for good or bad.

Metabolomics approaches are playing an increasingly important role in the responsible development of safe and effective personal care and cosmetics products. A leading multibillion-dollar cosmetics company leveraged Metabolon's services to understand the natural circadian rhythm of skin, characterize deficiencies in this rhythm as skin ages, and develop skincare products targeting those deficiencies to improve skin health. Others are using metabolomics to research alternative ingredients (such as cannabinoids[16]) as potential replacements for existing products containing substances that may do more harm than good, including to our endocrine systems and the environment.

Safety assessment is also a significant component of cosmetics production. With animal testing banned or frowned upon (depending on each country's regulatory standards), new methods leveraging metabolomics and in vitro assays[17] can comprehensively analyze a substance's impact on distribution, metabolism, and intracellular impacts.

With animal testing banned or frowned upon (depending on each country's regulatory standards), new methods leveraging metabolomics and in vitro assays can comprehensively analyze a substance's impact on distribution, metabolism, and intracellular impacts.

Many phenotypic traits are determined by a single gene, but most are the result of the interplay of multiple genes and the environment.

References

1. Alarcon-Barrera JC, Kostidis S, Ondo-Mendez A et al. Recent advances in metabolomics analysis for early drug development. Drug Discov Today. 2022;27(6):1763–1773. doi:10.1016/j.drudis.2022.02.018

2. McGovern BH, Ford CB, Henn MR, et al. SER-109, an Investigational Microbiome Drug to Reduce Recurrence After Clostridioides difficile Infection: Lessons Learned From a Phase 2 Trial. Clin Infect Dis. Jun 15 2021;72(12):2132-2140. doi:10.1093/cid/ciaa387

3. Wong CH and Siah KW. Estimation of clinical trial success rates and related parameters. Biostatistics. 2019;20(2):273–286. doi: 10.1093/biostatistics/kxx069

4. Pedrosa MC, Lima L, Heleno S et al. Food Metabolites as Tools for Authentication, Processing, and Nutritive Value Assessment. Foods. 2021;10(9):2213. doi: 10.3390/foods10092213

5. Yang Y, Ye Y, Pan D et al. Metabonomics profiling of marinated meat in soy sauce during processing. J Sci Food Agric. 2018;98(4):1325–1331. doi: 10.1002/jsfa.8596

6. Jacobs DM, van den Berg MA, and Hall RD. Towards superior plant-based foods using metabolomics. Curr Opin Biotechnol. 2021;70:23–28. doi: 0.1016/j.copbio.2020.08.010

7. Wideman N, O'Bryan CA, and Crandall PG. Factors affecting poultry meat colour and consumer preferences – A review. World's Poultry Sci Journal. 2015;353–366. doi: https://doi.org/10.1017/S0043933916000015

8. Zhan J, Yu XJ, Zhong YY et al. Generic and rapid determination of veterinary drug residues and other contaminants in raw milk by ultra performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2012;906:48–57. doi: 10.1016/j.jchromb.2012.08.018

9. Kim S, Kim J, Yun EJ et al. Food metabolomics: from farm to human. Curr Opin Biotechnol. 2016;37:16–23. doi: https://doi.org/10.1016/j.copbio.2015.09.004

10. Emwas AHM, Al-Rifai N, Szczepski K et al. You Are What You Eat: Application of Metabolomics Approaches to Advance Nutrition Research. Foods. 2021;10(6):1249. doi: 10.3390/foods10061249

11. Wei F and Wu B. Chapter 3.05: Use of Lipidomics for Food Quality Assurance and Authentication. In: Cifuentes, A. Comprehensive Foodomics. Elsevier Inc. 2021;44–61. doi: 10.1016/B978-0-08-100596-5.22829-1

12. Steg A, Oczkowicz M, and Smołucha G. Omics as a Tool to Help Determine the Effectiveness of Supplements. Nutrients. 2022;14(24):5305. doi: 10.3390/nu14245305

13. Van Breemen B. Development of Safe and Effective Botanical Dietary Supplements. J Med Chem. 2015;58(21):8360–8372. doi: 10.1021/acs.jmedchem.5b00417

14. McClorry S, Slupsky CM, Lind T et al. Effectiveness of vitamin D supplementation in Swedish children may be negatively impacted by BMI and serum fructose. J Nutr Biochem. 2020;75:108251. doi: 10.1016/j.jnutbio.2019.108251

15. Bouslimani A, da Silva R, Kosciolek T et al. The impact of skin care products on skin chemistry and microbiome dynamics. BMC Biology. 2019;17(1):47. doi: 10.1186/s12915-019-0660-6

16. Aliferis K and Bernard-Perron D. Cannabinomics: Applications of Metabolomics in Cannabis (Cannabis sativa L.) Research and Development. Front Plant Sci. 2020;11:554. doi: 10.3389/fpls.2020.00554

17. Jacques C, Jamin EL, Jouanin I et al. Safety assessment of cosmetics by read across applied to metabolomics data of in vitro skin and liver models. Arch Toxicol. 2021;85(10):3303–3322. doi: 10.1007/s00204-021-03136-7

Chapter 8

Regulatory Applications of Metabolomics

Until now, this guide has been focused on the scientific applications of metabolomics across various disciplines, from basic science research to clinical trials to cosmetics. In this chapter, we'll explore the ways in which metabolomics can help define and uphold governmental and agency regulations and guidelines.

FDA and Agency Standards

The United States Food and Drug Administration (FDA) oversees numerous activities,[1] from the approval of new drugs to guidelines around safe food production. Utilizing metabolomics can help the FDA do its job more quickly and accurately, and there are several use cases for metabolomics:

- Assuring safe, wholesome, sanitary, and properly labeled foods.[1] Metabolomics techniques have been used to define the nutritional profile and quality of foods,[2] detect microbial[3] and environmental[4] toxins in foods, and ensure that food manufacturers don't cheat food labels[5] by switching out high-end ingredients for cheaper ingredients and fillers to save money.

- Ensuring safe and effective drugs and medical devices.[1] Safe, efficacious drugs must elicit the desired physiological effect without exceeding acceptable toxicity levels. Metabolomics can identify and characterize drug and other host metabolites to profile the mechanism of action[6] and define the toxicity profile[7] of new drugs. Metabolomics can also play a key role in the quality control of manufactured drugs and has already been used in proof-of-concept quality control of natural product-derived medicines.[8]

- Assuring safe, properly labeled cosmetics and dietary supplements.[1] Similar to foods, metabolomics can ensure that cosmetics[9] and dietary supplements[10] are safe for human use and consumption and that labels are accurate.

It is important to note that systematic, standardized application of metabolomics in the regulatory setting hasn't yet been achieved, despite the use cases presented above. Different stakeholders are working for regulatory buy-in by developing validated protocols and establishing the relevance of outcomes data from metabolomics studies.[11] One of the most well-recognized efforts is the Metabolomics standards Initiative in Toxicology (MERIT). Although MERIT is a European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC)-supported project "providing guidance on best practice, quality standards, and the reporting of analytical and computational metabolomics methods used in regulatory toxicology," it is an international effort that "relies on worldwide collaboration."[12] A follow-up to this, called the Metabolics Reporting Framework (MRF), was published by the Organisation for Economic Co-operation and Development (OECD) in 2021.[13]

Different stakeholders are working for regulatory buy-in by developing validated protocols and establishing the relevance of outcomes data from metabolomics studies.

Other organizations and associations are also contributing to a growing effort to support the widespread use of metabolomics (including regulatory applications). For example, the Metabolomics Society, which has over 1,000 members from across the globe, was founded to promote the growth of metabolomics internationally and help establish collaborations and partnerships across academia, government, and industry.[14] The American Heart Association also released a scientific statement outlining the need for "strategies for determining the true clinical relevance of metabolites observed in association with cardiovascular disease outcome,"[15] an achievement that would certainly impact the development and approval of new therapeutics.

Safety and Dangerous Substance Monitoring

Chemical risk assessment is how the environmental and human health impacts of chemicals in the environment are assessed. Unfortunately, there are an incredibly large number of chemicals present in the environment. Traditional tests for detecting them are both expensive and time-consuming, and the use of animals for toxicity tests is also waning due to ethical concerns. Metabolomics is a sensitive, high-throughput alternative with significant potential for using this approach in environmental pollution and chemical safety applications. Scientists even suggest that metabolomics can make significant contributions to guide decision-making around chemical regulation and management.[16]

The OECD, which also developed the MRF discussed above, has coordinated an international Adverse Outcome Pathways (AOP) development effort, which acts as a framework for evaluating the biomolecular effects of chemicals on various levels, from macromolecular interactions to population-level.[16] The ultimate goal is to use these AOPs to predict the future impacts of various chemicals and devise effective remediation strategies. Metabolomics is expected to play a key role in characterizing these AOPs; for example, researchers have used metabolomics to describe the AOPs of silver[17] and other metal-bearing[18] nanoparticles, selenium as it relates to brain toxicity,[19] and the pharmaceutical spironolactone (i.e., a water pill),[20] to name just a few examples.

Metabolomics protocols also contribute to improved registrations to the European Chemicals Agency's (ECHA) REACH (registration, evaluation, and authorization of chemicals) Regulation, which governs the manufacture and import of chemical substances into the European Union. Metabolomics can be used to find similarities between biological responses to different chemicals to facilitate chemical grouping for read across of adverse events,[16] one of the most common methods used by the ECHA for data gap filling in

REACH registrations.[21] Metabolomics has facilitated chemical groupings for several chemical classes, including herbicides[22] and bisphenols.[23]

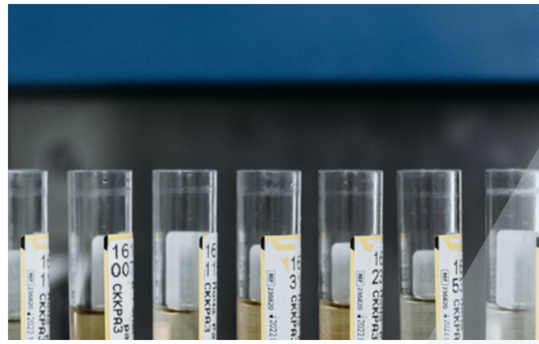
Causation-Correlation Analysis

The precedent has been set for using metabolomics to establish causal relationships between single molecular traits and complex phenotypes because metabolomics is the only omics technique that measures phenotype.[24] Although critical for information changes to regulatory standards and oversight, establishing causation, rather than simply correlation, isn't as straightforward as defining causality between genome and phenotype. One reason for this is that different environmental exposures may be heavily correlated with each other or may act together, making the assignment of causality difficult, if not impossible, when studying them one at a time.[25] Therefore, it is essential to find tools that can measure the cumulative impact of multiple exposures alongside their interactions with the genetic background of individuals. To identify causal links between environmental exposures and disease, the "meet-in-the-middle" (MITM) approach, which searches for intermediate biomarkers elevated in disease and then retrospectively searches for links between those biomarkers and environmental exposures, is one method that has been used for over 10 years.[16]

Metabolomics-based protocols have been reported for several MITM studies:

- Air toxic exposure linked to oxidative stress associated with several common complex diseases and allergic respiratory diseases[26]
- Identification of 10 biomarkers associating increased exposure by children and adolescents to industrial carcinogens with early carcinogenic biological events[27]
- Linkage of six tryptophan and vitamin B3 pathway metabolites to air pollution exposure and decreased probability of live birth[28]
- Arsenic exposure connected to male infertility[29]

This method, however, identifies what could more accurately be called associations, rather than causative relationships. To identify these associations, scientists suggest using approaches that can measure the cumulative impact of multiple exposures alongside their interactions with genetic elements.[30] Regardless of which protocols are eventually developed and utilized, metabolomics will play a part.



It is essential to find tools that can measure the cumulative impact of multiple exposures alongside their interactions with the genetic. background of individuals.

References

1. *(2021, June 28). What does FDA do? https://www.fda.gov/about-fda/fda-basics/what-does-fda-do Accessed December 28, 2022.*

2. *Pedrosa MC, Lima L, Heleno S et al. Food Metabolites as Tools for Authentication, Processing, and Nutritive Value Assessment. Foods. 2021;10(9):2213. doi:10.3390/foods10092213*

3. *Jadhav SR, Shah R, Karpe AV et al. Detection of Foodborne Pathogens Using Proteomics and Metabolomics-Based Approaches. Front Microbiol. 2018;9:3132. doi:10.3389/fmicb.2018.03132*

4. *Zhan J, Yu XJ, Zhong YY et al. Generic and rapid determination of veterinary drug residues and other contaminants in raw milk by ultra performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2012;906:48–57. doi:10.1016/j.jchromb.2012.08.018*

5. *Emwas AHM, Al-Rifai N, Szczepski K et al. You Are What You Eat: Application of Metabolomics Approaches to Advance Nutrition Research. Foods. 2021;10(6):1249. doi:10.3390/foods10061249*

6. *Alarcon-Barrera JC, Kostidis S, Ondo-Mendez A et al. Recent advances in metabolomics analysis for early drug development. Drug Discov Today. 2022;27(6):1763–1773. doi:10.1016/j.drudis.2022.02.018*

7. *Ramirez T, Daneshian M, Kamp H et al. Metabolomics in Toxicology and Preclinical Research. ALTEX. 2013;30(2): 209–225. doi:10.14573/altex.2013.2.209*

8. *Lee KM, Jeon JY, Lee BJ et al. Application of Metabolomics to Quality Control of Natural Product Derived Medicines. Biomol Ther. 2017;25(6):559–568. doi:10.4062/biomolther.2016.249*

9. *Bouslimani A, da Silva R, Kosciolek T et al. The impact of skin care products on skin chemistry and microbiome dynamics. BMC Biology. 2019;17(1):47. doi:10.1186/s12915-019-0660-6*

10. *Steg A, Oczkowicz M, and Smołucha G. Omics as a Tool to Help Determine the Effectiveness of Supplements. Nutrients. 2022;14(24):5305. doi:10.3390/nu14245305*

11. *Viant MR, Ebbels TMD, Beger RD et al. Use cases, best practice and reporting standards for metabolomics in regulatory toxicology. Nat Commun. 2019;10(1):3041. doi:10.1038/s41467-019-10900-y*

12. *Beger RM, Viant T, Ebbels J et al. MEtabolomics standaRds Initiative in Toxicology (MERIT). Metabolomics 2018, Seattle, WA, June 24 – 28, 2018.*

13. *(2021 May). The Organisation for Economic Co-operation and Development. Metabolomics Reporting Framework (MRF). https://www.oecd.org/chemicalsafety/testing/metabolomics-reporting-framework.pdf Accessed December 27, 2022.*

14. *https://metabolomicssociety.org*

15. 15.Cheng S, Shah SH, Corwin EJ et al. Potential Impact and Study Considerations of Metabolomics in Cardiovascular Health and Disease: A Scientific Statement From the American Heart Association. Circ Cardiovasc Genet. 2017;10(2):e000032. doi:10.1161/HCG.000000000000003

16. Bedia C. Metabolomics in environmental toxicology: Applications and challenges. Trends in Env Anal Chem. 2022;34:e00161. doi:10.1016/j.teac.2022.e00161

17. Maria VL, Licha D, Scott-Fordsmand JJ et al. Multiomics assessment in Enchytraeus crypticus exposed to Ag nanomaterials (Ag NM300K) and ions (AgNO3) – metabolomics, proteomics (& transcriptomics). Environ Pollut. 2021;286:117571. doi:10.1016/j.envpol.2021.117571

18. Dekkers S, Williams TD, Zhang J et al. Multi-omics approaches confirm metal ions mediate the main toxicological pathways of metal-bearing nanoparticles in lung epithelial A549 cells. Environ Scie: Nano. 2018;5:1506–1517. doi:10.1039/C8EN00071A

19. Li X, Liu H, Li D et al. Dietary seleon-L-methionine causes alterations in neurotransmitters, ultrastructure of the brain, and behaviors in zebrafish (Danio rerio). Environ Sci Technol. 2021;55:11894–11905. doi:10.1021/acs.est.1c03457

20. Davis JM, Ekman DR, Skelton CA et al. Metabolomics for informing adverse outcome pathways: androgen receptor activation and the pharmaceutical spironolactone. Aquat Toxicol. 2017;184:103–115. doi:10.1016/j.aquatox.2017.01.001

21. Sperber S, Wahl M, Berger F et al. Metabolomics as read-across tool: An example with 3-aminopropanol and 2-aminoethanol. Regul Toxicol Pharmacol. 2019;108:104442. doi:10.1016/j.yrtph.2019.104442

22. van Ravenzwaay B, Sperber S, Lemke O et al. Metabolomics as a read-across tool: a case study with phenoxy herbicides. Regul Toxicol Pharm. 2016;81:288–304. doi:10.1016/j.yrtph.2016.09.013

23. Oliveira Pereira EA, Labine LM, Kleywegt S et al. Metabolomics reveals that bisphenol pollutants impair protein synthesis-related pathways in Daphnia magna. Metabolites. 2021;11(10):666. doi:10.3390/metabo11100666

24. Auwerx C, Sadler MC, Reymond A et al. Exploiting the mediating role of the metabolome to unravel transcript-to-phenotype associations. Preprint. bioRxiv. 2022. doi:10.1101/2022.06.08.495285

25. Patel CJ and Ioannidis JPA. Studying the elusive environment in large scale. JAMA. 2014;311(21):2173–4. doi:10.1001/jama.2014.4129

26. Chen CHS, Yuan TH, Shie RH et al. Linking sources to early effects by profiling urine metabolome of residents living near oil refineries and coal-fired power plants. Environ Int. 2017;102:87–96. doi:10.1016/j.envint.2017.02.003

27. Chen CHS, Kuo TC, Kuo HC et al. Metabolomics of children and adolescents exposed to industrial carcinogenic pollutants. Environ Sci Technol. 2019;53:5454–5465. doi:10.1021/acs.est.9b00392

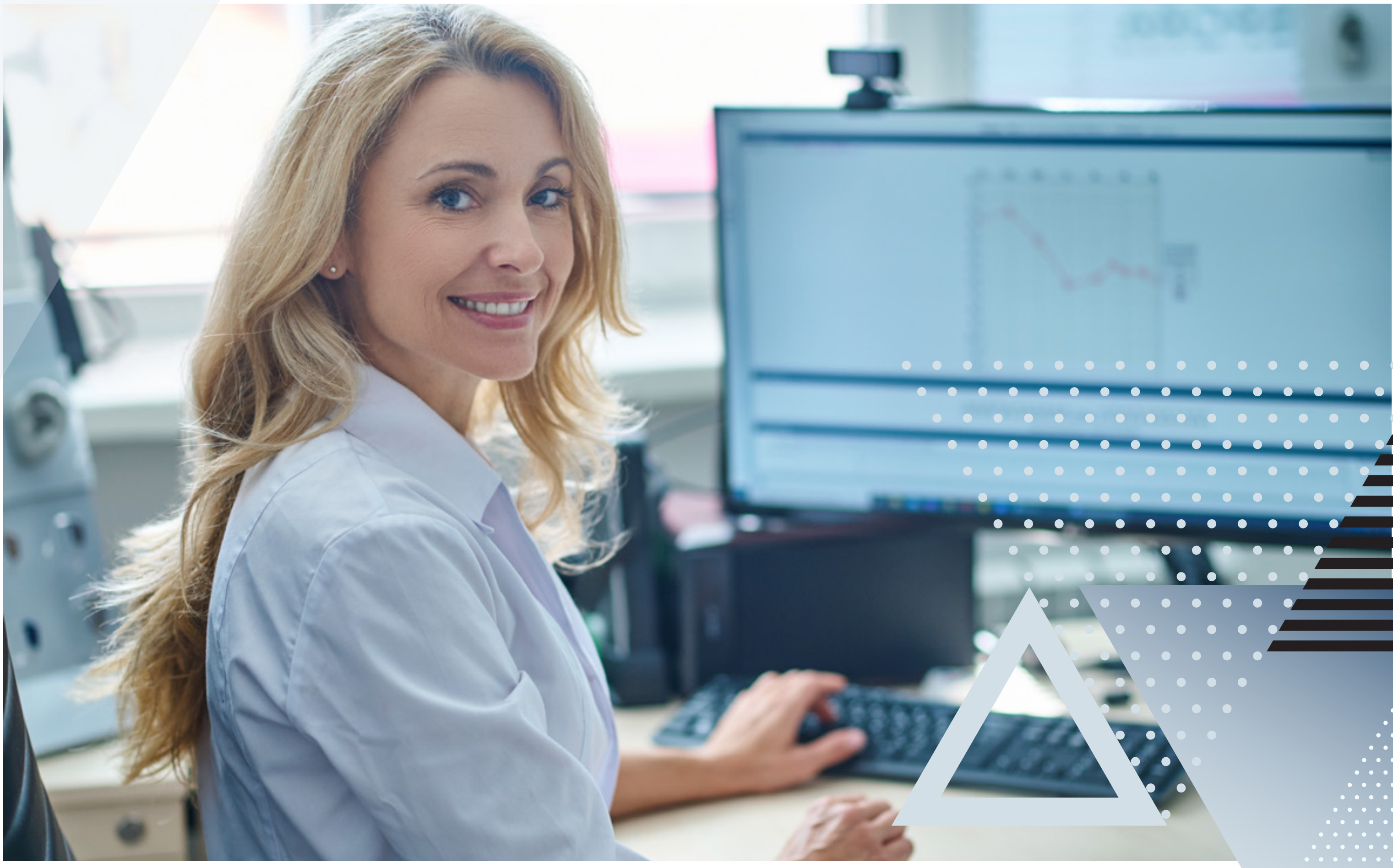
28. Gaskins AJ, Tang Z, Hood RB et al. Periconception air pollution, metabolomic biomarkers, and fertility among women undergoing assisted reproduction. Environ Int. 2021;155:106666. doi:10.1016/j.envint.2021.106666

29. Wu Y, Ding R, Zhang X et al. Meet-in-metabolite analysis: a novel strategy to identify connections between arsenic exposure and male infertility. Environ Int. 2021;147:106360. doi:10.1016/j.envint.2020.106360

30. Rattray NJ, Deziel NC, Wallach JD et al. Beyond genomics: understanding exposotypes through metabolomics. Hum Genomics. 2018;12(1):4. doi:10.1186/s40246-018-0134-x

Chapter 9

Designing a Metabolomics Study



As you've learned throughout this guide, metabolomics analyses are powerful additions to any scientific study, from basic research to toxicology studies. Agencies around the world are also working to make metabolomics an important component to uphold existing regulatory standards and inform the development of new ones. But achieving a controlled and insightful research outcome is highly dependent on the quality of your study preparation. In this final installment of our metabolomics guide, we'll walk you through the fundamentals of how to design and execute a robust metabolomics study to answer your research question(s).

What's Your Study Goal?

The most important part of any metabolomics study is defining the scope of your study. Two approaches may be leveraged: global or targeted. Global metabolomics approaches are semi-quantitative and consider all output metabolite data, while targeted studies utilize panels of known metabolites to quantify the amounts of those specific metabolites in the sample(s) of interest. Which approach you choose, or whether you use a combination, depends on your research question.

For those interested in identifying novel biomarkers or seeking to understand the presence of a broad set of metabolites, global metabolomics is the better choice. If, however, you know what you are looking for and want to know how much of a specific metabolite or groups of metabolites are present in your sample, you'll need a targeted approach. Targeted approaches can facilitate pretty much any scientific question; at Metabolon, we provide targeted panels for amino acids, bile acids, central carbons, complex lipids, fatty acids, impaired glucose tolerance, insulin resistance, short chain fatty acids, and more. And, of course, we can help you develop custom target panels specific to your research goals.

At Metabolon, we provide targeted panels for amino acids, bile acids, central carbons, complex lipids, fatty acids, impaired glucose tolerance, insulin resistance, short chain fatty acids, and more.

Due to their nature, targeted panels necessarily have limited analytical coverage compared to global panels, but they are more sensitive and reproducible than untargeted approaches. It can also be difficult to identify unknown compounds using global panels, mostly due to the limited availability of analytical standards and spectral information in public databases.[1] Often, researchers opt for a two-pronged approach to get the best of both worlds: starting with a global approach and then using targeted metabolomics to further understand key analytes of interest.

Regardless of the approach or combination of approaches you choose, it's important to define this study parameter at the outset, as this will inform the sample types and quantities required to make data collection as straightforward as possible.

Which Technology is Best for Your Study?

As briefly discussed in previous chapters of this guide, there are two main technologies used to obtain metabolite data: nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS).

NMR: NMR exposes samples to a magnetic field and radio frequency pulse to identify compounds based on the resonance energy of electromagnetic radiation absorbed and re-emitted by cellular nuclei. Specifically for metabolomics, proton atoms from small molecules are typically analyzed (1H-NMR). It's a highly reproducible method capable of measuring tens to one hundred metabolites from various sample types.

LC-MS: Mass spectrometers measure small molecules by ionizing (adding charge to) them and then detecting them as they move through a spectrometer. By using liquid chromatography (LC) to physically separate the molecules present in a sample prior to ionization in the mass spectrometer, researchers can work with the very small sample sizes (down to 0.1 µL) supported by LC to detect thousands of metabolites from diverse sample types.[2-5]

Each method has its advantages and disadvantages. The main advantage of NMR is that it is non-invasive and doesn't destroy the sample,[6] meaning you can re-use your samples for additional experimental investigation (including complementary LC-MS, if you wish).

Samples aren't destroyed during NMR because the technology doesn't require any sample pre-processing or separation prior to identification, unlike LC-MS, making sample preparation for NMR much simpler.[6] Additionally, NMR is highly reproducible and quantitative, because electromagnetic peak intensities are directly proportional to the number of nuclei in the sample.[6] It is also a powerful method for identifying unknown metabolites,[6] making it a great tool for novel biomarker discovery.[7]

LC-MS is a much more sensitive method than NMR, however, and it is better suited for targeted metabolomics analyses.[7] Unlike NMR, LC-MS can measure thousands of metabolites at a time and is often used to analyze different classes of metabolites from the same sample, achieving a wider coverage of the metabolome than NMR.[6] Currently available MS techniques are incredibly sensitive and accurate and offer a high mass resolution, meaning that more than 80% of the pathways present in the KEGG (Kyoto Encyclopedia of Genes and Genomes) database can be detected using this method.[1]

LC-MS can measure thousands of metabolites at a time and is often used to analyze different classes of metabolites from the same sample, achieving a wider coverage of the metabolome than NMR.

Do It Yourself or Outsource?

The next thing to determine before you even begin collecting samples is whether you will execute your project in-house or outsource. Asking this question up front may save you a lot of time and money, and it will help you focus your research objectives appropriately considering the limitations, risks, and costs associated with each option.

In-house

- **Pros:** no risk of samples becoming lost or damaged in transit, can begin processing samples immediately, you know exactly when your data are coming back.

- **Cons:** need to purchase or rent instrumentation and access to analysis software, need your own research standards and reference libraries, requires know-how for confident data interpretation.

While some kits can be purchased to facilitate in-house metabolomics projects, these are typically semi-quantitative and therefore do not support targeted studies. They also may not have the best coverage, meaning you might miss important scientific discoveries. Unless you are going to be performing metabolomics analyses on a regular basis, the financial investment into instruments, software, and personnel trained in handling your samples, running the instruments, and data analysis might not be worth it.

Outsourced

- **Pros:** access to metabolomics experts who run and interpret metabolomics data daily, access to the best available technology for the highest accuracy and confidence, fully validated methods for reproducibility and reliability across cohorts.

- **Cons:** may not be feasible for urgent analyses due to shipping and data turnaround times this, fee-for-service may not be in scope for every project or budget.

Outsourcing your metabolomics studies can be nerve-wracking, especially if you're working with limited samples that are hard to obtain, which is why it is essential that you choose a trustworthy metabolomics partner with expertise you can count on. Metabolon offers several different models for working with you to meet your research needs, from sample processing and data only to complete analysis and interpretation. Our 20 years of experience optimizing protocols and working with hundreds of sample types combined with our reference library containing over 5,400 metabolites means that we are fully equipped to help you design your study, collect the right samples the right way, and obtain the highest quality data possible with meaningful, definitive answers to your research questions.

Sample Collection, Preparation, Storage, and Shipment

As we briefly discussed in Chapter 4 of this guide, several different sample types can be analyzed using metabolomics protocols, including cells in culture, tissue, feces, blood, urine, and sweat. The sample source you used depends on your research goal; for example, you'll select stool if you want to study the impact of the gut microbiome on host nutrient acquisition, but you will work with tissue samples if you want to identify biomarkers that differentiate primary tumors and metastases. Planning ahead will help you map out your budget early and avoid the unexpected need to add more samples later on or request funding extensions.

Each sample type requires different handling and preparation, which is also impacted by whether you've selected a targeted or untargeted approach and whether you're using NMR or LC-MS. If you are doing your project in-house, you'll need to make sure you know how to handle your samples appropriately; and for particularly difficult samples, outsourcing is often the easier and cheaper option–especially if you're working with limited sample material. With every project, Metabolon offers a free Study Success Sample Handling Kit, customized specifically for your study. With the kit, we include barcoded tubes specific to your matrix (ie, biofluids, solid samples, or cell pellets) with instructions for mailing your samples to us to ensure they arrive safely. If you store large quantities of samples in a biorepository or biobank, we'll even work directly with personnel there to prepare and ship your samples.

Metabolon offers a free Study Success Sample Handling Kit, customized specifically for your study.

Data Interpretation

Data interpretation is often the most difficult part of any metabolomics project. Instruments don't produce easy-to-read summaries with beautiful graphics; instead, you get tables with endless columns and values that you must transform into understandable insights using any one of several available software tools (some of which are free, others of which are not). If no one on your team is trained in analyzing NMR or LC-MS data, you may lack confidence in your interpretation and the likelihood of missing important, actionable observations is high. Outsourcing data analysis and interpretation can save you a lot of time and money and prevent you from having to re-do experiments.

Working with Metabolon, you'll never be left in the dark when interpreting your data. Global panels come with continuous access to the MyMetabolon Portal, where you can access customized interpretation by our PhD-level study directors, statistical tests they performed, and fully customizable data visualizations. And while targeted panels don't have the same statistical analysis and visualization as global panels, we do provide a formatted data table that is ready for import and analysis with third-party tools such as R and Python.

For those research groups that want to outsource metabolite identification and quantification but not data interpretation, we also offer data-only. This can be a good option for labs with the necessary expertise for data analysis but who don't necessarily have access to or funds for appropriate instrumentation or who are working with sample types outside of their expertise.

What's Next?

After going through this guide, you are ready to start executing your own metabolomics studies and experience the scientific power they have to offer. We are here to help you on your journey, whether you simply need us to process your samples or work with you one-on-one from start to finish to design and execute an insightful metabolomics study. Contact us today to get started!

References

1. *Bedia C. Metabolomics in environmental toxicology: Applications and challenges. Trends in Env Anal Chem. 2022;34:e00161. doi: 10.1016/j.teac.2022.e00161*

2. *Silva Siqueira Sandrin V, Moraes Oliveira G, Weckwerth GM et al. Analysis of Different Methods of Extracting NSAIDs in Biological Fluid Samples for LC-MS/MS Assays: Scoping Review. Metabolites. 2022;12(8):751. doi: 10.3390/metabo12080751*

3. *Thevia M, Krug O, Geyer H et al. Expanding analytical options in sports drug testing: Mass spectrometric detection of prohibited substances in exhaled breath. Rapid Commun Mass Spectrom. 2017;31(15):1290–1296. doi: 10.1002/rcm.7903*

4. *Frank N, Bessaire T, Tarres A et al. Development of a quantitative multi-compound method for the detection of 14 nitrogen-rich adulterants by LC-MS/MS in food materials. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2017;34(11):1842–1852. doi: 10.1080/19440049.2017.1372640*

5. *Zwiener C and Frimmel FH. LC-MS Analysis in the aquatic environment and in water treatment – a critical review. Part I: Instrumentation and general aspects of analysis and detection. Anal Bioanal Chem. 2004;378(4):851–861. doi: 10.1007/s00216-003-2404-1*

6. *Nagana Gowda GA and Raftery D. NMR Based Metabolomics. Adv Exp Med Biol. 2021;1280:19–37. doi: 10.1007/978-3-030-51652-9_2*

7. *Emwas AH, Roy R, McKay RT et al. Recommendations and Standardization of Biomarker Quantification Using NMR-Based Metabolomics with Particular Focus on Urinary Analysis. J Proteome Res. 2016;15(2):360–373. doi: 10.1021/acs.jproteome.5b00885*

Chapter 10

The End of the Guide... the Beginning of Your Metabolomics Studies

Throughout this guide, you've learned everything you need to know to start performing your own metabolomics studies: from the basics about metabolites and why studying them is important to how metabolomics analyses can support basic science research, clinical practice, and even everyday products such as food, supplements, and cosmetics, and, of course, important considerations for designing your own metabolomics study. We encourage you to refer back to previous chapters of this guide at any time—treat it like your basic metabolomics

handbook. Here's a quick review of the main points of discussion in each chapter that you can use to help you find what you need quickly:

Chapter 1—Introduction

In Chapter 1, we explored the relationship between genes, environment, and functional capacity, defined metabolites and the metabolome, and provided a basic introduction to the importance of including metabolomic information in scientific studies.

Chapter 2—Other Omics Sciences and Metabolomics

In Chapter 2, we defined and provided basic introductions to the other core omics approaches: genomics, transcriptomics, and proteomics. Additionally, using examples from the primary literature, we demonstrated how metabolomics can add additional layers of information to each of these other omics techniques, and why metabolomics is a key enabling technology for multi-omics studies.

Chapter 3—Metabolite Identification and Detection

In Chapter 3, we more deeply explored the types of metabolites (endogenous and exogenous) that can be identified and the sample types from which they can be identified. We provided examples of the metabolic reactions that can be identified via metabolomics studies and introduced the two main approaches for measuring metabolites: nuclear magnetic resonance (NMR) and mass spectrometry (MS). Finally, we introduced global and targeted approaches and the circumstances under which one or the other might be used.

Chapter 4—The Importance of Metabolic Insights

In Chapter 4, we expanded upon the ideas introduced in earlier chapters of this guide. Beginning with a discussion of the many benefits associated with metabolomics studies, this chapter then focused on some of the academic, clinical, and commercial applications of metabolomics analyses, including unraveling how the microbiome impacts human health, improving clinical trial design, and facilitating toxicity studies of supplements and cosmetics.

Chapter 5—Clinical Applications of Metabolomics

In Chapter 5, we built upon the ideas introduced in Chapter 4, deeply exploring the many clinical applications of metabolomics. From decades-old tests such as the heel-prick test on babies to the present and future of precision medicine, we provided several examples of metabolomics' place in the clinic. This chapter also highlighted the work done by one of our customers, who used Metabolon's services to redesign their clinical trial and move their microbiome-based therapeutic successfully through phase III clinical trials.

Chapter 6—Academic Applications of Metabolomics

Chapter 6 expanded ideas introduced in Chapter 4 through an in-depth discussion of metabolomics applications in the academic laboratory. We introduced some of the databases available to academic researchers for exploring metabolites from humans and the human microbiome before presenting several examples from the primary literature demonstrating how academic metabolomics research is laying the foundation for biomarker discovery and drug discovery and leveraging the human microbiome clinically. We also showed how the explosion of metabolomics research in academic laboratories is leading to the development of novel animal models for studying human diseases as well as sophisticated tools for effectively analyzing and visualizing metabolomics and multi-omics datasets.

Chapter 7—Commercial Applications of Metabolomics

Chapter 7 was the final of three chapters expanding on Chapter 4, providing a detailed introduction to several commercial applications benefitting from metabolomics analyses: drug development, food and drink safety and manufacturing, vitamin and supplement development and manufacturing, and cosmetics development. Through several examples from the primary literature, we demonstrated how metabolomics plays and will continue to play a critical role in each of these industry areas as they grow and evolve.

Chapter 8—Regulatory Applications of Metabolomics

In this chapter, we discussed how metabolomics supports not just the research and development aspects of drugs, foods and beverages, supplements, and cosmetics, but how it also enables manufacturers to meet and uphold regulatory standards. From ensuring that foods, supplements, and cosmetics are properly labelled to profiling drug toxicity, metabolomics plays an essential role in our ability to consume and use everyday products without worry. This chapter also introduced the various global organizations working toward establishing standards for using metabolomics in regulatory toxicity, safety, and dangerous substance monitoring, and other applications.

Chapter 9—Designing a Metabolomics Study

In this chapter, we explained why achieving a controlled and insightful research outcome is highly dependent on the quality of your study preparation. We then layed out the most important questions to answer prior to embarking on any metabolomics study so you can make sure you're able to execute a successful metabolomics study with your specific budget: what is your study goal?; which technology is best for your study?; should you do it yourself or outsource?; considerations for sample collection, preparation, storage, and shipping; and data analysis and interpretation. We also explained how Metabolon can support you at each and every one of these steps to help you get the most out of your metabolomics studies.

What's Next?

We hope this guide has fully equipped you to get started on your own metabolomics journey with confidence. We're here to support your projects in any way you need—from experimental design to data interpretation. Don't hesitate to reach out to us today to learn more about how we can work with you to gain as much scientific insight as possible from your metabolomics research. It isn't difficult to imagine how metabolomics and genomics datasets can add important contextual information to one another to elucidate why specific genetic anomalies are associated with specific phenotypes. The integration of genomic and metabolomic data has been extensively used to elucidate metabolite chemical structures, functions, and biosynthetic origins, and several tools have been developed to aid such efforts. Genome-metabolome integrations have also proven particularly useful in oncology research, as cancer has critical genetic and metabolomic characteristics. Recent work has even used genomics-metabolomics integration to help elucidate why genetic aberrations can be observed in both healthy and cancerous cells and why specific environmental and nutrient cues act as “selectors” during oncogenesis.

Metabolon

SMALL MOLECULES, BIG INSIGHTS.™

Metabolomic insights: Don't get left behind

Both Seres and Siolta have harnessed the power of metabolomics to overcome drug development obstacles and inform critical decision-making to advance their programs. Metabolon has long been at the forefront of metabolomics utilization, having executed more than 10,000 projects over the last 20 years. Our unique ability to provide key data and interpret them for our clients makes us a reliable collaborator as you navigate the complex journey of drug development.

To learn more about how Metabolon can help your study, please contact us today.

+1 (919) 572-1711
www.metabolon.com/contact-us

617 Davis Drive, Suite 100, Morrisville, NC, 27560

AR.DDCT.091721