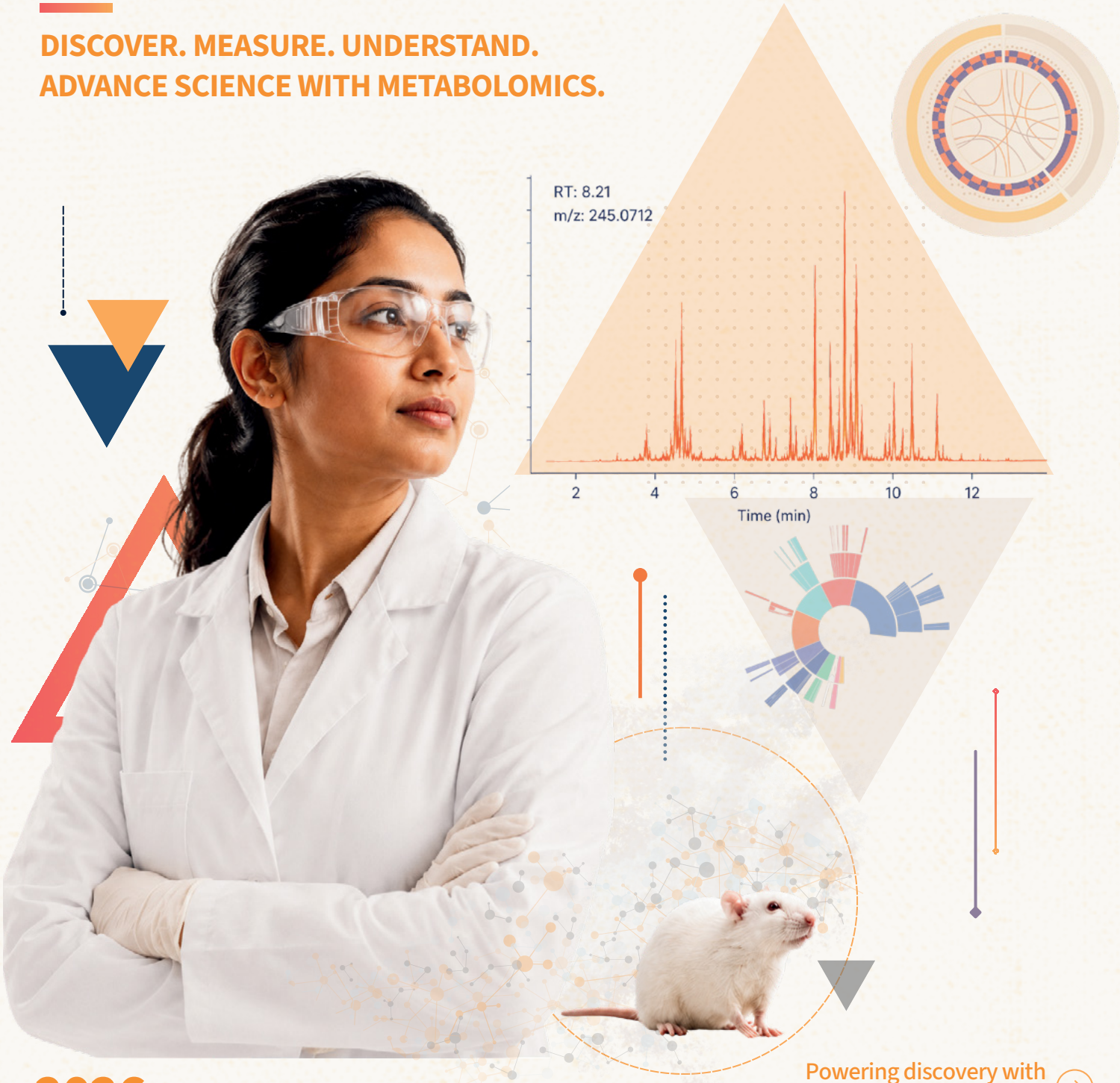
Metabolon

RESEARCH GUIDE

The Guide to Metabolomics

DISCOVER. MEASURE. UNDERSTAND. ADVANCE SCIENCE WITH METABOLOMICS.



2026

Powering discovery with comprehensive metabolomics.

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Foreward

When I began my tenure at Metabolon in 2004, metabolomics was a niche field characterized by limited standardization and poor reproducibility. Early efforts in the discipline were therefore heavily focused on establishing standardized methodologies and rigorously validating biochemical compound identities to improve data quality and reliability.

Since then, metabolomics has advanced significantly. These improvements have enabled the field to move beyond simple data generation toward more efficient and accurate data interpretation, while also expanding into a broader range of applications. Today, validated protocols exist for a wider variety of sample matrices than ever before, and metabolomics can be readily integrated with other omics disciplines to deepen biological insights.

From a business perspective, these advancements have been translated into tangible value. Metabolomics accelerates research and development timelines by enabling earlier and more confident decision-making, reducing costly late-stage failures. It enhances product differentiation by uncovering unique biochemical signatures that can support claims in areas such as pharmaceuticals, diagnostics, agriculture, and consumer products. Additionally, the ability to generate high-resolution, actionable data supports the development of personalized solutions, opening new revenue streams and strengthening competitive positioning. Improved reproducibility and standardization also increase confidence among regulators and partners, facilitating smoother pathways to commercialization.

As a result, metabolomics is now widely recognized by scientists and industry leaders as essential to the success of studies spanning clinical research, commercial applications, and both basic and translational science.

In this Third Release of our Guide to Metabolomics, we provide an overview of the field's current state, highlight key studies demonstrating its impact on modern applications, and illustrate how incorporating metabolomics can uncover insights not accessible through other approaches.

As the field continues to evolve, Metabolon remains committed to staying at the forefront, leveraging our metabolomics platform to drive biological discovery and advance breakthroughs in science and medicine, with the ultimate goal of enabling a healthier world.

Sincerely,

Annie Evans, Ph.D., Senior Director, Head of Core Research

Chapter 1

Introduction to Metabolomics

AT A GLANCE

In this chapter we will address:

- What metabolomics is and why is it important to scientific discovery.
- How metabolomics is performed.
- What two main profiling methods are used in metabolomics and when one should be used over the other.
- How sample matrix selection impacts results and interpretation of metabolomics data.

Defining Metabolomics

Metabolomics is the study of metabolites, the small molecule reactants, intermediates, and products of metabolism as well as molecules from diet, exposure, and pharmaceutical origin. Metabolites, along with genes, transcripts, and proteins, represent different but interrelated levels of cellular processes that are organized according to the central dogma of biology. Since metabolites typically represent the terminal step in cellular processes, these molecules are uniquely positioned to receive inputs from the genome, transcriptome, and proteome. In addition, they are positioned to receive direct input from other factors including the microbiome and the environment (**Figure 1**). Thus, metabolites not only play a crucial role in mechanisms that directly impact the phenotype, but they also reflect an organism's real-time biological status more closely than other molecule types. For these reasons, **metabolomics is recognized for its ability to reveal deep phenotypic insights that cannot be deduced from genomics, transcriptomics, or proteomics alone**. The ability of metabolomics to 'complete the picture' in omics studies has played a major role in advancing scientific discovery, accelerating drug development, validating product quality, and delivering other actionable insights that drive measurable business outcomes and propel research forward.

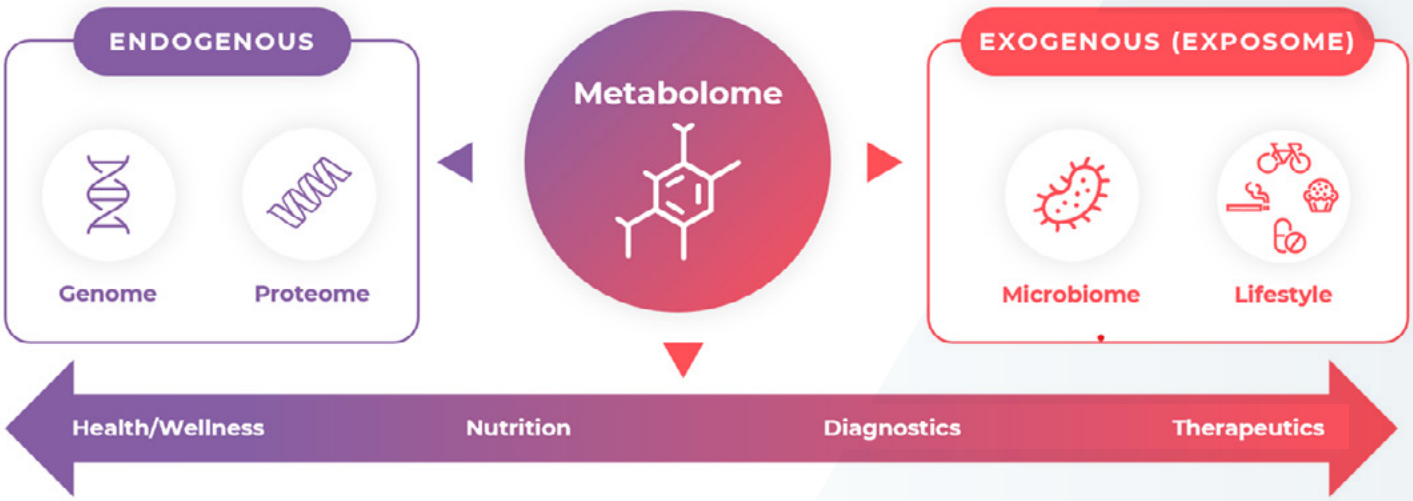

Figure 1. Inputs from cellular molecules and external factors converge on metabolites. The central dogma of biology dictates that cellular processes are carried out by each set of molecules propagating a signal according to inputs from the one(s) that come before it. Metabolites are uniquely placed to receive inputs from each set of cellular molecules as well as from the microbiome and other external factors, making them the closest reflection of the phenotype.

The Purpose of this Guide

In this guide we will discuss the basic principles of metabolomics and explore peer reviewed scientific studies that showcase the utility of metabolomics in basic science, translational science, and various applied markets. This guide is written specifically for principal investigators, research scientists, R&D leaders, and innovation strategists, and is intended to show you how metabolomics can amplify study insights beyond traditional omics sciences. In this guide you will learn:

1. The basics of a metabolomics workflow, profiling methods, and sample selection (**Chapter 1**)
2. Metabolon's solutions to challenges associated with metabolomics studies, and how our chemistry-centric (chemo-centric) approach elevates Metabolon-generated data above others (**Chapter 2**)
3. Metabolomics for Commercial Applications (**Chapter 3**)
    a. Drug Development
    b. Human Nutrition
    c. Animal Husbandry and Companion Animal Health
4. Metabolomics for Translational Studies (**Chapter 4**)
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    c. Population Health
    d. Alternative Sample Matrices
6. How to Design a Metabolomics Study (**Chapter 6**)

Metabolomics Workflow

Metabolomics is typically performed using either nuclear magnetic resonance (NMR) or liquid chromatography-mass spectrometry (LC-MS). NMR identifies and measures metabolites based on energy emitted from cell nuclei after they are exposed to electromagnetic radiation. This approach has the advantage of keeping the sample intact, which allows alternative follow-up analyses to be performed. However, sample preservation forces a trade-off with sensitivity, which is relatively low with this technique. By contrast, LC-MS offers vastly superior sensitivity and resolution, and thereby greater scientific insight.

In an LC-MS workflow metabolites are isolated from their biological milieu, separated from each other using LC, and analyzed by MS. Mass spectrometers operate by converting the analyte molecules to a charged (ionized) state and then analyzing those ions and any ion fragments that are produced during the ionization process. Metabolites can then be identified by matching the signatures they produce in the MS coupled with their chromatographic (LC) characteristics to a biochemical reference library.

Fast MS scanning speeds enable a high degree of multiplexing and the identification and measurement of hundreds to thousands of compounds in a single analytical run. LC-MS accurately detects metabolites in concentrations that range from picomolar to the molar levels, which enables wide-coverage biochemical profiling.

Profiling Methods

Metabolomics is generally performed using either a targeted or untargeted (global) approach, depending on the study objective or hypothesis being tested. Targeted metabolomics is a quantitative technique that detects and measures a predefined set of metabolites, whereas global metabolomics is semi-quantitative and is used to detect a wide range of metabolites in a biological sample without prior selection (**Figure 2**).

The broad coverage offered by global metabolomics makes it particularly useful for generating hypotheses and conducting discovery studies, while targeted metabolomics is ideal for validating prior findings or testing a hypothesis associated with a known mechanism or set of biochemical pathways. The features of both profiling methods and their unique applications are demonstrated in the case studies discussed in **Chapters 3-5**.

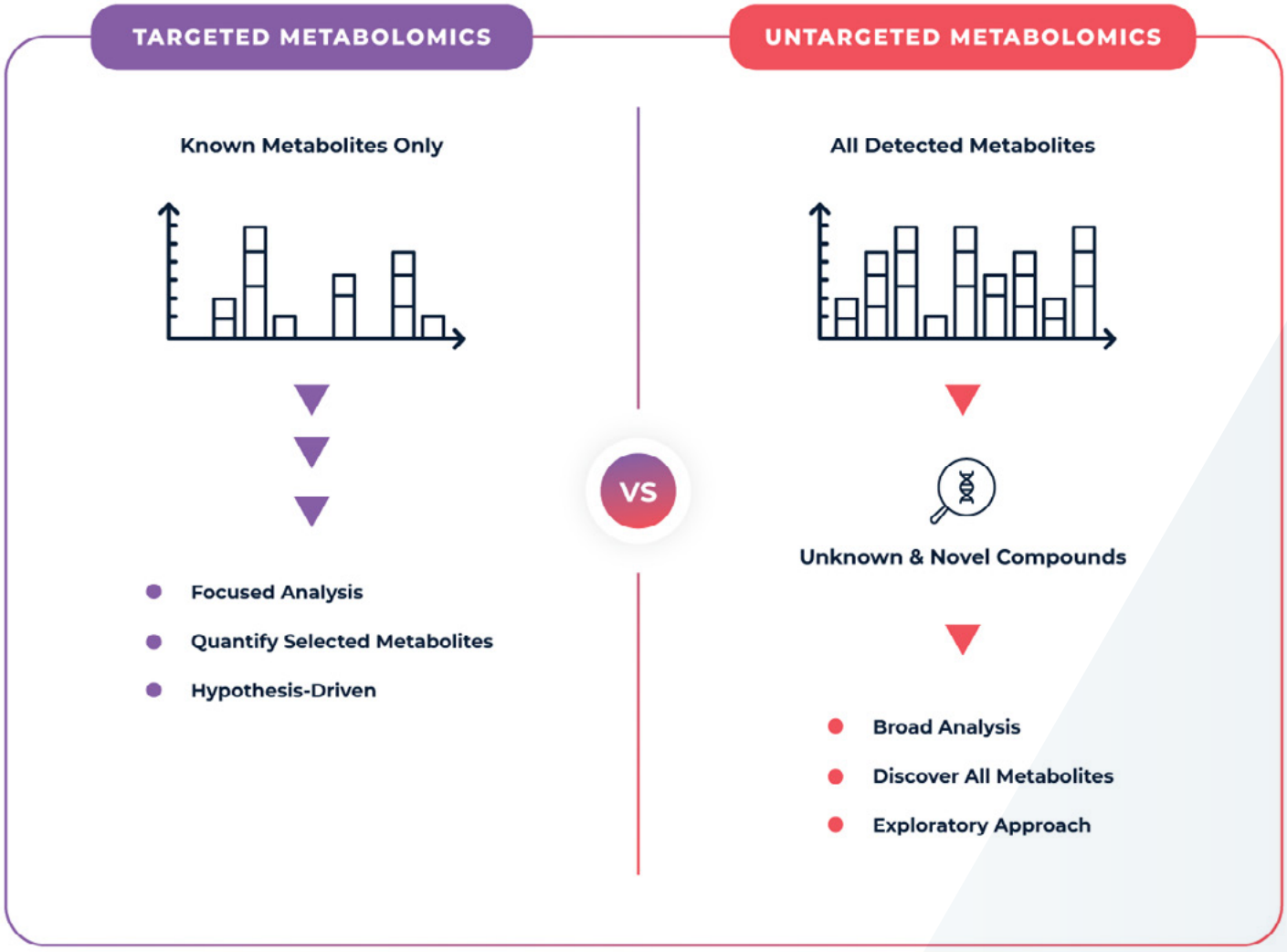


Figure 2. Summary of targeted and untargeted approaches.

Sample Selection

Numerous sample matrices have been validated in metabolomics workflows including but not limited to various types of tissues, cells, plants, and biological fluids. In some cases, two sample types may be analyzed together to comprehensively evaluate a topic of interest. For example, an investigator looking to characterize mechanisms of irritable bowel disease may profile both serum and feces to determine how alterations in the gut microbiome affect circulating biomarkers of inflammation. Likewise, an investigator studying NAD(H) metabolism may profile serum and muscle tissue because certain metabolites in NAD(H) pathways are more abundant in one matrix over the other and thus profiling both provides a more comprehensive picture of the biological flux. Metabolon's extensive experience with commonly used and alternative sample matrices are discussed in **Chapter 5**.

REVIEW

Chapter Takeaways

- Due to their biological positioning, metabolomics can reveal insight into biological processes that cannot be deduced from other omics sciences alone.
- Metabolomics is performed using either targeted or untargeted profiling and each approach is uniquely suited to specific types of scientific inquiry.
- Metabolomics is compatible with numerous sample types, making it broadly applicable to life science disciplines and applied markets.

With a basic understanding of metabolomics in mind, we will now discuss challenges associated with metabolomics studies, and innovations that Metabolon has developed to address them. We will also discuss Metabolon's chemical-centric (chemo-centric) approach to data analysis and explain how it elevates the quality of Metabolon-generated data above the rest of the industry.

Chapter 2

Metabolon's Solutions and Chemo-Centric Approach

AT A GLANCE

In this chapter we will address:

- Some common challenges associated with metabolomics studies.
- The technical solutions Metabolon has developed and how they improve metabolomics data.
- What a chemical-centric (chemo-centric) approach is and how it elevates data quality.

Metabolomics Challenges and Solutions

LC-MS is a favored method for conducting metabolomics studies due to its high sensitivity and wide biochemical coverage, and metabolomics is recognized for its ability to provide deep phenotypic insight beyond traditional omics sciences. However, there are challenges associated with metabolomics and traditional LC-MS approaches that can limit the insight gained from the data. These challenges include insufficient pathway coverage, a lack of reliable metabolite identification, and a shortage of context-specific data interpretability.

Metabolon's LC-MS platform incorporates strategic innovations into the conventional analytical approach, which overcomes key limitations to deliver broader, deeper, and more precise coverage than traditional LC-MS technologies. These innovations are:

- **Four chromatography methods.** Samples are subjected to four methods of chromatography, each optimized to separate hydrophilic, hydrophobic, basic, and acidic compounds. Using four chromatography methods results in superior compound separation and coverage and builds analytical fail-safes into the method, so compounds with overlapping chemical characteristics are identified with the highest confidence.

- **Biochemical reference library.** Metabolon's library is the largest commercial biochemically relevant reference library in the world. It contains more than 5,400 metabolites and metabolite intermediates, most of which have achieved Level 1 identification status, which denotes the highest level of confidence in a metabolite's identity, as it confirms correct chromatographic, mass, and fragmentation properties (**Figure 3**). Although open-source reference libraries tend to be larger, the methods used to identify their entries are undisclosed, differ across users, and contain less data for compound annotation, which leaves room for uncertainty regarding biochemical identity. By containing the largest number of accurately annotated reference points, Metabolon's library enables superior analytic accuracy over other LC-MS platforms.

- **Data acquisition software.** Metabolon's proprietary data acquisition software allows thousands of biochemicals from 70+ pathways to be rapidly and accurately annotated and aligned with data from scientific literature. These tools also enable metabolomics data from various studies to be merged and for metabolomics datasets to be integrated with other omics datasets to maximize biological insight and interpretability.

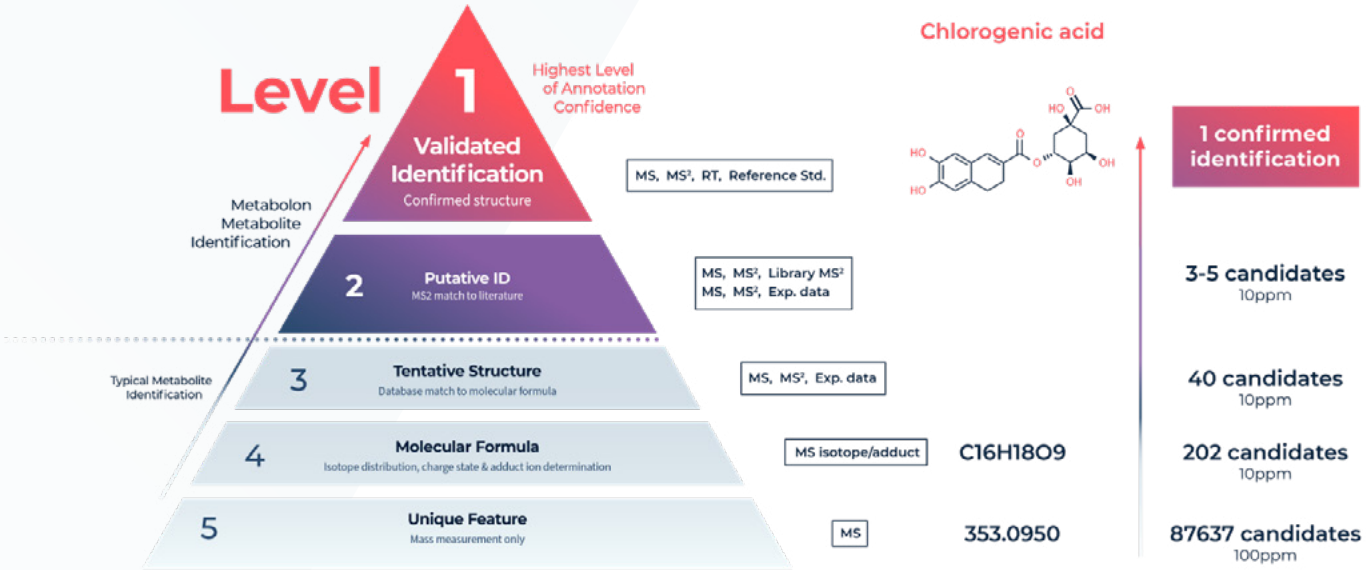


Figure 3. A summary of each level of metabolite identification. The confidence in a metabolite's identity is defined by 5 levels that are categorized according to the analytical evidence of that identity. Level 1 denotes the highest confidence in identity, and the majority of biochemical entries in Metabolon's library have achieved this level of identification.

Metabolon's Chemo-Centric Approach

In global metabolomics studies that use mass spectrometry, 10s to 100s of thousands of mass spectrometry signals, often referred to as ion features, are detected in a single sample. In the traditional ion-centric or feature centric approach to data analysis, only the statistically significant ion features are determined from the full dataset of 100,000+ features (**Figure 4, bottom panel**). This can introduce errors into data analysis because in a dataset this size many of the detected ion features are redundant and irrelevant to the study hypothesis. Keeping redundant features in the dataset can skew the statistical analysis, and irrelevant features only muddy the interpretation of the data, limiting insight. Moreover, distinguishing meaningful ion features from the rest in such a large dataset is tedious and time-consuming, and often done incorrectly. This forces the unchanging ion features (those compounds that are not affected by the study design) to be overlooked due to lack of time and leads to incorrect classification of important ion features, limiting insight further and introducing confounding data.

To address this challenge, Metabolon pioneered the chemo-centric approach, which brings the compound or biochemical to the forefront by using Metabolon's robust biochemical reference library to appropriately account for extraneous and redundant ion features from the dataset before statistical analyses are performed (**Figure 4, top panel**). The chemo-centric workflow deconvolutes the ion feature data, which allows accurate

identification of the statistically significant compounds (not features) as well as identification of compounds that did not change over the course of the study, extracting maximum insight from the dataset. Additionally, all entries in Metabolon's biochemical reference library are identified by unique ChemID numbers, which enable Metabolon data to be compared across studies. The use of open-source biochemical reference libraries leads to rampant non-concordance of reported metabolite calls, which constrains the comparison of data generated by independent parties.

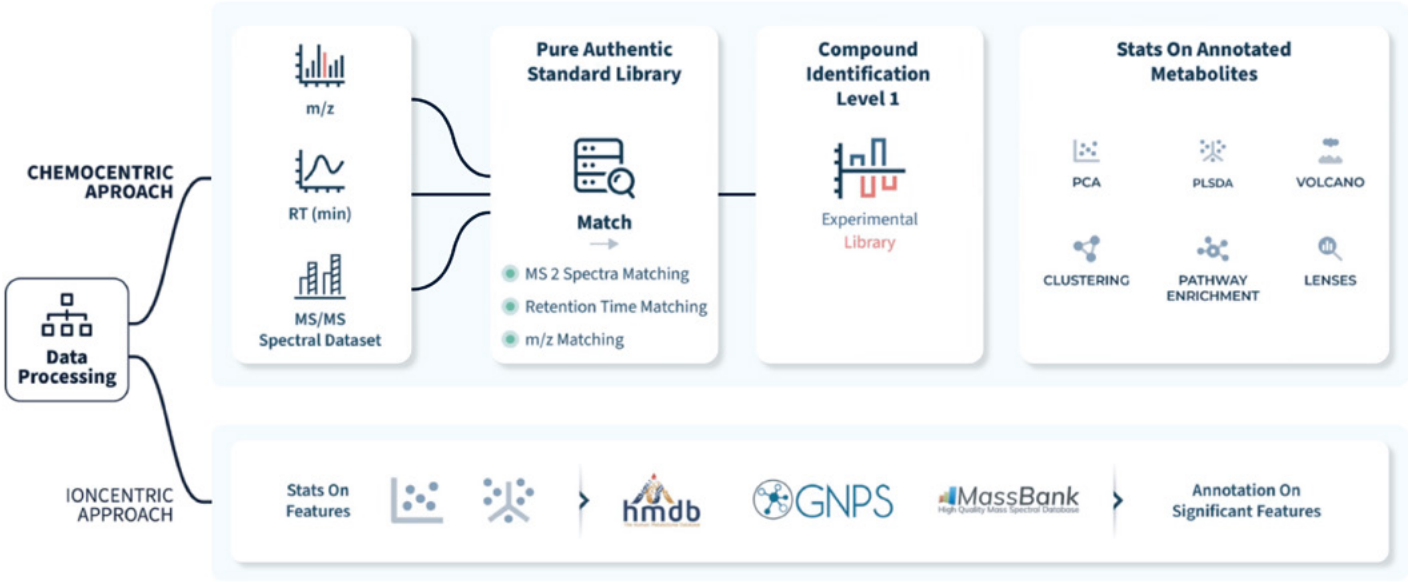


Figure 4. Summary of Metabolon's chemo-centric approach. Global metabolomics typically identifies 10s to 100s of thousands of ion features per sample. Metabolon pioneered the approach of identifying compounds and accounting for redundant features using an in-house authentic standard reference library (**Top**). Unlike the traditional ion centric approach (**Bottom**) the library enables accurate compound identification based on multiple criteria including retention time, mass, and fragmentation spectra. Metabolon's vast library contains information on the ionization products of numerous compounds, which facilitates their efficient removal from the data stream.

The innovations discussed above, coupled with Metabolon's chemo-centric approach set Metabolon data apart from that of other metabolomics providers.

REVIEW

Chapter Takeaways

- Metabolon has developed industry-leading technologies, methods, and data analysis tools that address many limitations of traditional metabolomics workflows.
- These tools impart Metabolon data with industry-leading quality, reproducibility, and insightfulness.

Armed with Metabolon's tools, numerous investigators, clinicians, and industry professionals have made discoveries that were vital to advancing their projects to the next step. Over the next three chapters we will discuss quintessential studies that show how this was done. We hope that seeing metabolomics in action across diverse scientific disciplines and sectors inspires your own research endeavors.

Chapter 3

Metabolomics for Commercial Applications

AT A GLANCE

In this chapter we will address:

- How metabolomics can reveal functional insight into disease phenotypes that traditional biomarkers fail to provide.
- How metabolomics can identify high performing diagnostic biomarkers that are invisible to other omics sciences.
- How metabolomics can inform human nutrition to mitigate disease and improve overall health.
- How metabolomics can inform food animal health to reduce culling and improve the nutritional value of meat.

Overview

Metabolomics has become an invaluable tool in various applied markets including drug development, human nutrition, animal husbandry, and pet care. Metabolic changes can elucidate novel therapeutic targets and mechanisms of drug action, identify connections between diet and disease phenotypes, and reveal biomarkers that inform animal health. Here, we discuss a few studies that show how metabolomics helped solve a problem and/or provided key scientific insight into topics related to selected applied markets.

DRUG DEVELOPMENT

Gaining Insight into Drug-Induced Toxicity that Traditional Biomarkers Failed to Provide

Dyslipidemia, an abnormally high level of low- and very low-density lipoproteins (LDL/VLDL) in the blood, is a major risk factor for cardiovascular disease and stroke. Nicotinic acid receptor (NAR) agonists treat dyslipidemia by activating receptors on fat cells to inhibit triglyceride metabolism and reduce circulating levels of LDL and VLDL. In one study, investigators at Merck Research Laboratories were developing a NAR agonist (SCH 900424) to treat dyslipidemia, but in their investigations identified potential drug-induced toxicity which they wanted to better understand and monitor [1].

In preclinical studies SCH 900424 unexpectedly caused rapid morbidity and acute kidney injury (AKI) in mice, implying that the drug was affecting both the renal and central nervous systems (CNS). Traditional markers of kidney function including creatinine and BUN were not sensitive enough to either predict or characterize the drug-induced toxicity, and the underlying mechanism(s) were unknown. The goals of this study were to identify sensitive and early biomarkers of SCH 900424-related toxicities in mice and elucidate the mechanism(s) that led to toxicity.

Adult male CD-1 mice were dosed with low, medium, and high concentrations of SCH 900424 or a control agonist. Plasma, urine, and brain samples were collected 1, 4, 8, and 24 hours post dose. Samples were processed then analyzed using global metabolomics to identify perturbations in relevant biochemical pathways. Targeted metabolomics was then used to measure biochemicals of interest identified in the global discovery analyses. Three sample matrices were analyzed to ensure a comprehensive mechanistic study of both AKI- and CNS-related pathologies.

Results. In plasma, 3-indoxyl sulfate (3IS), a gut-derived uremic toxin produced from tryptophan metabolism, emerged as a top biomarker of SCH 900424-induced AKI. This marker increased significantly in treated mice relative to controls and also rose earlier and more drastically than creatinine or urea (**Figure 5, High Dose**), showing that 3IS is a more sensitive marker of AKI than traditional markers of kidney function.

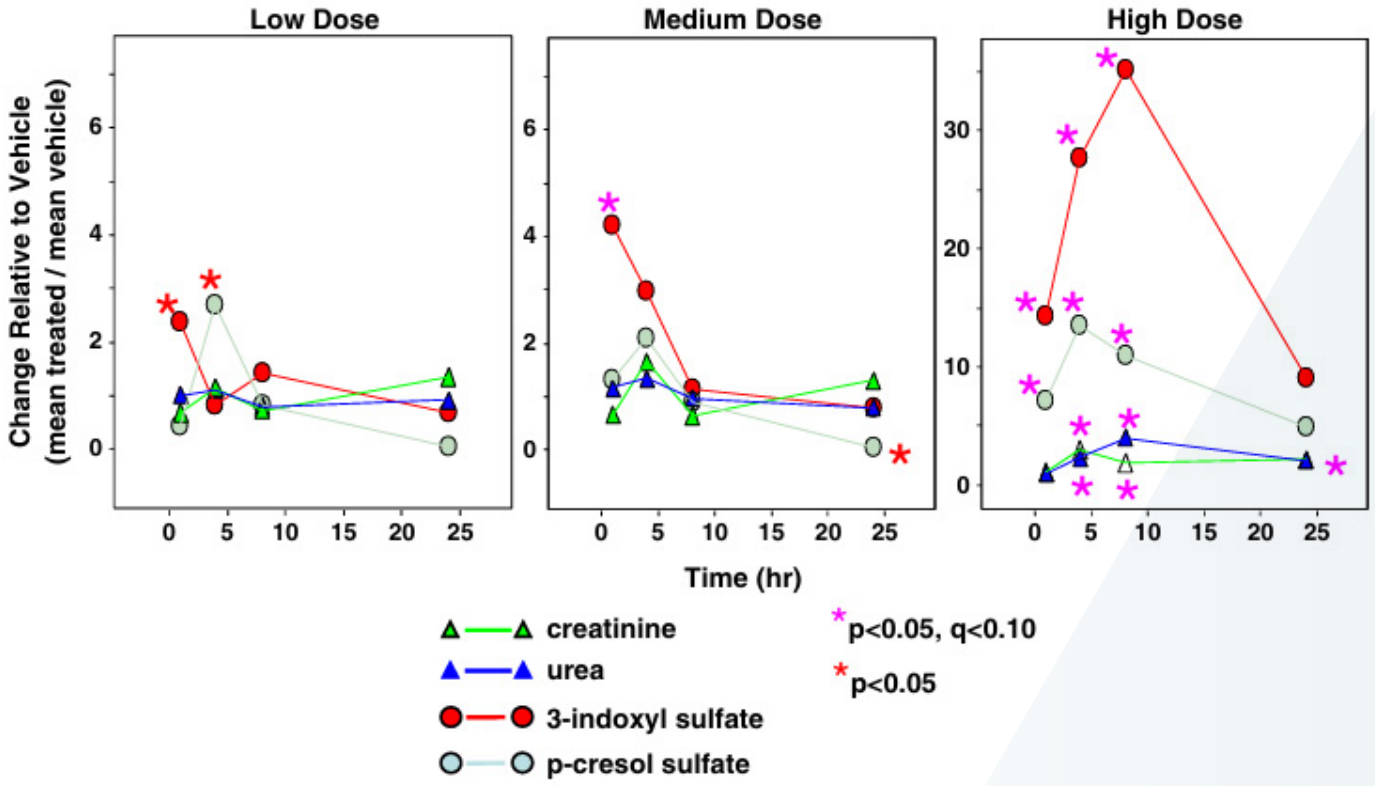


Figure 5. Fold-changes relative to vehicle in plasma for 3IS, PCS, urea, and creatinine following administration of SCH 900424. Based on the comprehensive global metabolomic analysis, 3IS and p-cresol sulfate were identified as the most statistically significant plasma markers of renal toxicity in this study and were more sensitive than urea and creatinine. Image reproduced from Zgoda-Pols et al., *Toxicol Appl Pharmacol*, 2011, licensed under CC BY 4.0.

Plasma levels of P-cresol sulfate, a uremic toxin produced from metabolism of tyrosine and phenylalanine, was also significantly elevated (**Figure 5, High Dose**). Several amino acids, including tryptophan, significantly decreased in plasma while many tryptophan catabolites including kynurenine, kynurenate, and indole-3-lactate were significantly increased (**Figure 6**). In urine, significant increases in glucose and amino acids were observed. Under normal conditions, nearly all filtered amino acids and sugars are reabsorbed in the proximal tubules. However, a kidney injury would impair reabsorption and cause them to be excreted into the urine. The metabolic signature observed across plasma and urine supported true renal dysfunction rather than isolated metabolic changes.

Another important study finding was the significant and dose-dependent elevation of 3IS in brain tissue after administration of SCH 900424. This increase occurred in tandem with significant reduction in urinary levels 3IS, indicating impaired clearance leading to its accumulation in the brain. Overall, these findings suggested that accumulated 3IS in plasma and brain may not only contribute to AKI, but also to CNS toxicity and rapid morbidity in mice.

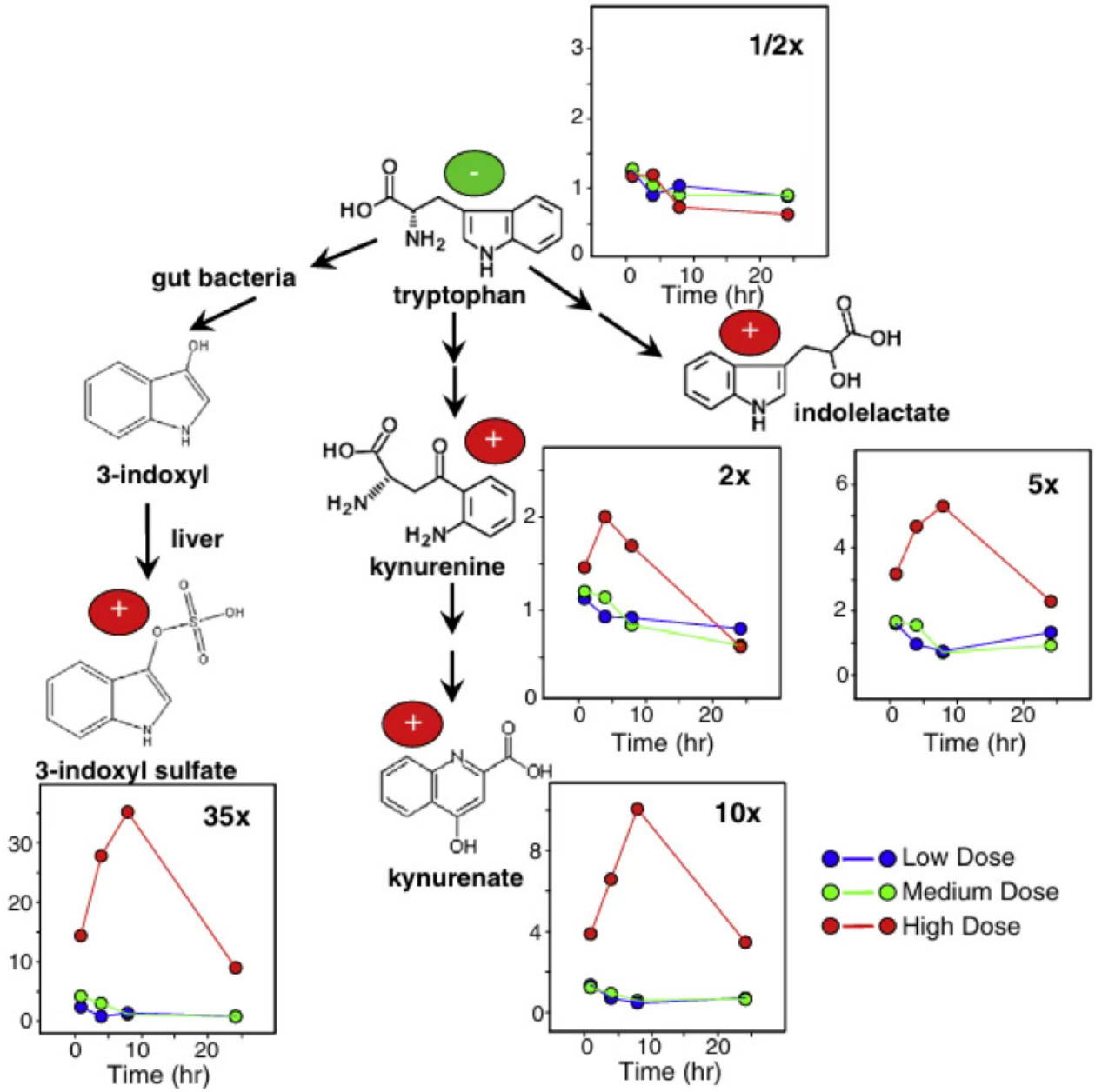


Figure 6. Catabolic pathways of tryptophan with fold-changes relative to vehicle for individual catabolites. While tryptophan decreased over time with the higher doses of SCH 900424, its catabolites, and especially 3IS, increased dramatically. Image reproduced from Zgoda-Pols et al., *Toxicol Appl Pharmacol*, 2011, licensed under CC BY 4.0.

Study Conclusions

- This study's findings suggest that 3IS is an early and more sensitive marker of SCH 900424-induced renal toxicity than traditional chemistry markers. In practice, this could improve early detection of nephrotoxicity in drug development.
- The observed accumulation of gut-derived uremic toxins, disrupted tryptophan metabolism, and patterns of reduced urinary excretion provided mechanistic evidence of uremic toxin buildup due to renal dysfunction.
- Accumulation of 3IS in brain tissue, rather than only plasma, suggests a mechanistic link between kidney failure and rapid morbidity in mice; a novel hypothesis that can be tested in future studies.
- This study shows how metabolomics can help turn an uncharacterized phenotypic observation into a mechanistically informed biomarker story to inform on drug candidate selection with greater speed and accuracy.

Rescuing a Drug Development Program

Background. *C. difficile* infection (CDI) causes inflammatory colitis and subsequent diarrhea that is quite debilitating to patients. Even with antibiotic treatment, 20-30% of patients have recurrent CDI due to continued disruption of their microbiome. In a healthy gut, gut bacteria suppress the growth and germination of *C. difficile* by converting conjugated primary bile acids to secondary bile acids and by producing short- and medium-chain fatty acids. Fecal microbiota spores produced by members of the Firmicutes phylum have been shown to recolonize the gut with bacteria that guard against recurrent CDI. In one study, investigators at Seres Therapeutics developed VOS (formerly SER-109), an orally administered microbiome-based live biotherapeutic product intended for treatment and prevention of recurrent CDI [2].

Preliminary Data and Study Goals. VOS engraftment was consistent among patients and only 13.3% of patients experienced CDI recurrence across a range of doses administered in an open-label phase 1 study. However, VOS did not meet its primary efficacy endpoint in a follow-up randomized, placebo-controlled phase 2 study, where it was administered at a fixed dose that had shown efficacy in phase 1. In response, this group performed several follow-up metabolomics studies. The goal of their first follow-up study was to reconcile these disparate results by evaluating VOS-induced changes to the microbiome and microbe-associated metabolites [3]. Their second goal is described in the next section titled 'Success in a Phase 3 Trial Despite Failure in Phase 2'.

Methods. In the phase 1 and 2 clinical trials, VOS engraftment was assessed by comparing the number of VOS-associated bacterial species in stool samples 1, 4, and 8 weeks after dosing and CDI recurrence was determined based on clinical symptoms. Whole-metagenomics shotgun sequencing and global metabolomics analyses were conducted post-hoc on phase 1 and phase 2 datasets to evaluate the impact of VOS on clinical outcomes.

Results. In the phase 2 clinical trial, treated participants who did not develop recurrent CDI had significantly more VOS species one week after dosing than those who experienced a recurrence. A post-hoc analysis of the phase 1 and phase 2 trial results were performed to assess the magnitude of engraftment one week after participants received a high (phase 1), low (phase 1), or fixed (phase 2) dose. Phase 1 subjects who received the high dose had a significantly greater number of VOS species by week 1 than the other 2 dosing cohorts (**Figure 7**). Furthermore, week 1 engraftment was shown to be highly variable in phase 2 participants, suggesting that successful engraftment was dependent on dose and that dosing was suboptimal in the phase 2 trial.

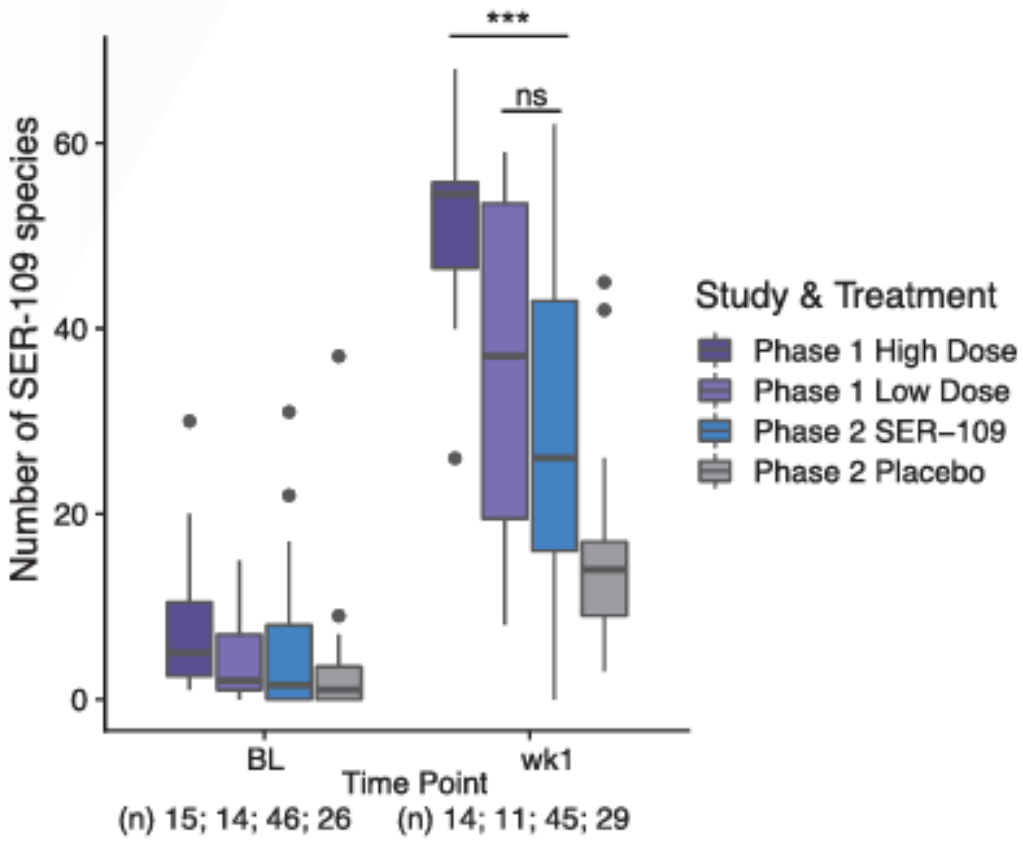


Figure 7. Relationship of engraftment of VOS species to doses administered in the phase 1 and 2 studies. Participants who received the high dose in the phase 1 study had significantly more VOS species than participants in the treatment arm of the phase 2 study who received a low fixed dose ($p < 0.001$). Image reproduced from McGovern et al., *Clin Infect Dis*, 2021, licensed under CC BY 4.0.

Interestingly, there was also a significant positive correlation between the number of VOS species and the abundance of the secondary bile acids, lithocholic acid (LCA) and deoxycholic acid (DCA), suggesting increased species engraftment leads to increased conversion of conjugated primary bile acids to secondary bile acids, a key step in suppressing growth of *C. difficile* and returning to normal gut health (**Figure 8**).

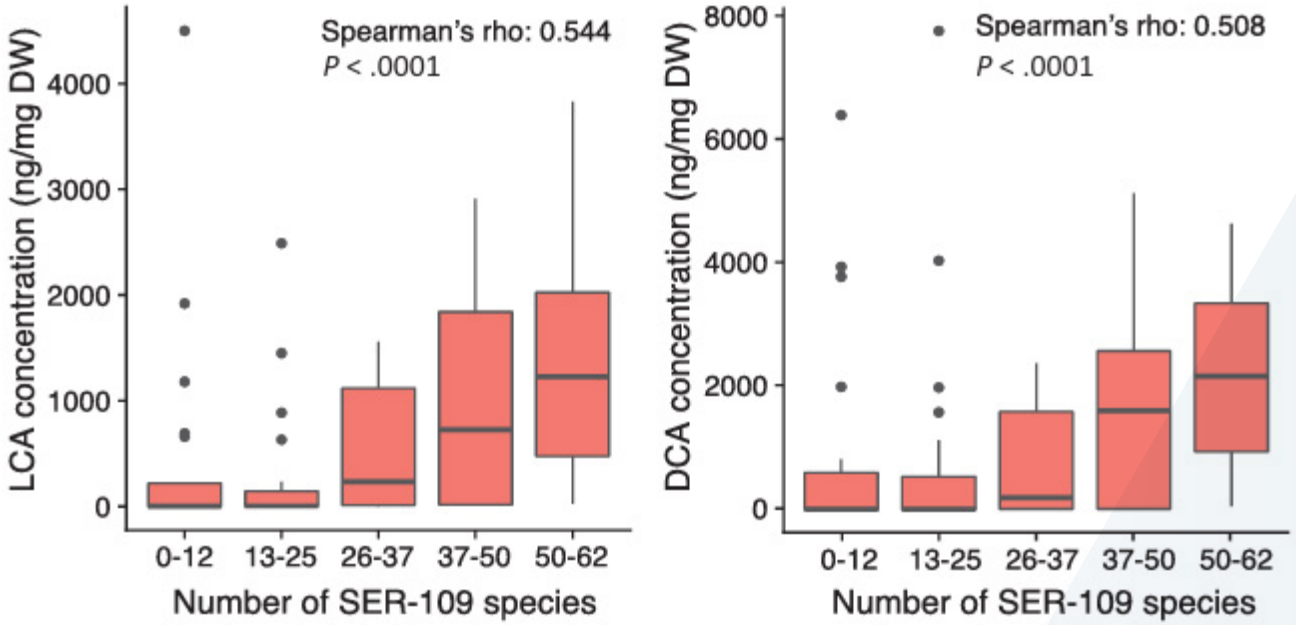


Figure 8. The relationship between the engraftment of VOS (formerly SER-109) species and concentration of secondary bile acids in the phase 2 study. The number of VOS species one week after dosing was significantly associated with the concentration of LCA and DCA. Abbreviations: DCA- deoxycholic acid, DW- dry weight, LCA- lithocholic acid. Image reproduced from McGovern et al., *Clin Infect Dis*, 2021, licensed under CC BY 4.0.

Study Conclusions

- This study showed that the phase 2 clinical trial was probably unsuccessful because of suboptimal dosing which led to inconsistent engraftment, rather than because of poor therapeutic efficacy.
- These findings also generated the hypothesis that VOS engraftment leads to important functional changes through key microbe-associated metabolites, which break the cycle of recurrent CDI.
- In this first follow-up study, metabolomics was instrumental in showing why results from the phase 1 and phase 2 studies disagreed with each other, which allowed the team to proceed with a redesigned phase 3 trial despite failure in phase 2, thus saving a promising therapeutic from being shelved and avoiding the loss of a commercial investment. The next case study discusses the outcome of this group's phase 3 trial.

A Successful Phase 3 Trial Despite Failure in Phase 2

Preliminary Data and Study Goals. In light of the results described in the above case study, investigators redesigned the phase 3 study to have a more efficacious dosing strategy [4]. Treatment arm participants were given a fixed dose of VOS that was 10-fold higher than the dose administered in phase 2 with the goal of testing the therapeutic efficacy of VOS under the proper conditions for engraftment.

Methods. Efficacy was evaluated based on the reduction of relative risk and sustained clinical response up to 8 weeks after dosing. Specifically, relative risk was defined as the percentage of patients with recurrence in the VOS group divided by the percentage in the placebo group. Sustained clinical response was defined as no recurrence through the 8 weeks that patients were followed. Global metabolomics analyses were conducted on stool samples collected 1, 2, and 8 weeks after dosing to evaluate bile acid profiles in the gut.

Results. VOS (formerly SER-109) was found to be superior to placebo in reducing the risk of CDI recurrence, and in generating a sustained clinical response in patients (**Figure 9**).

The VOS treatment group demonstrated significantly larger increases in secondary bile acids from baseline compared to placebo (**Figure 10**), which concurred with reduced amounts of proinflammatory Enterobacteriaceae bacteria and increases in Firmicutes bacteria, species that are thought to be key regulatory suppressors of CDI.

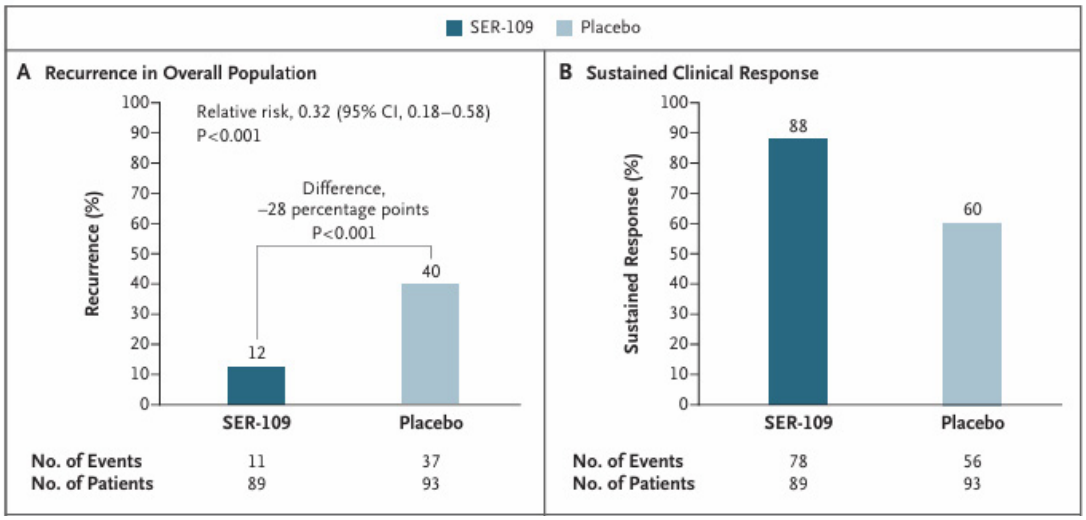


Figure 9. Recurrence of CDI up to 8 weeks. (**A**) primary efficacy analysis showed superiority of VOS (SER-109) compared to placebo in reduction of the risk of CDI recurrence. (**B**) VOS was also superior to placebo in generating a sustained clinical response. Image reproduced from Feuerstadt et al., *N Engl J Med*, 2022, licensed under CC BY 4.0.

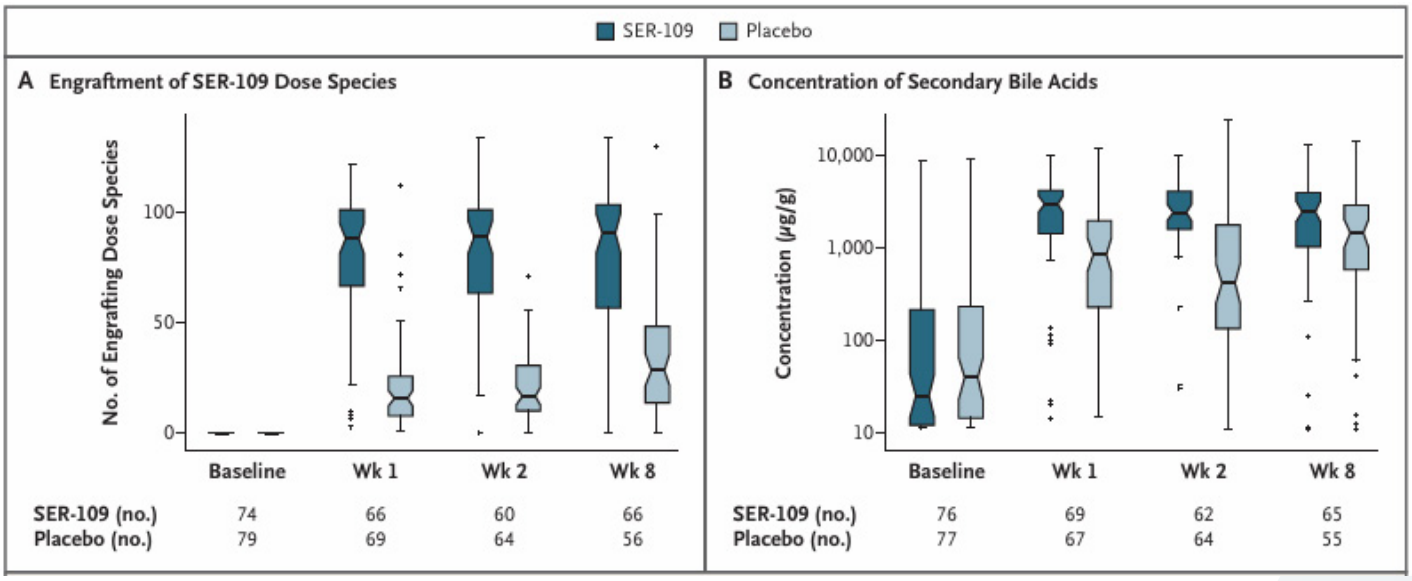


Figure 10. Compositional and metabolomic changes in the microbiome after receiving VOS (SER-109) or placebo. (**A**) The number of engrafting dose species and (**B**) the concentration of pooled secondary bile acids detected in patient stool specimens 1, 2, and 8 weeks after receipt of VOS or placebo. Image reproduced from Feuerstadt et al., *N Engl J Med*, 2022, licensed under CC BY 4.0.

Study Conclusions

- A phase 3 trial redesigned for optimal dosing based on metabolomics data enabled VOS to properly engraft and thereby demonstrate its superiority over placebo in preventing the recurrence of CDI.
- The metabolomics data from this and the previous clinical studies suggest that VOS engraftment is dose-dependent, and its mechanism of action may be linked to production of secondary bile acids.
- These findings led to a highly reasonable hypothesis about the mechanism of action of VOS. Since understanding the MOA of a therapeutic agent is essential for optimizing treatment efficiency, predicting side effects, and determining correct dosages, this study may facilitate cost-effective decision making for follow-up clinical studies.

Introduction. Survival rates of colorectal cancer (CRC) could be improved by early detection and non-invasive biomarkers to facilitate this are a significant need. Changes in energy metabolism are a hallmark of tumorigenesis because cancer cells rely on aerobic glycolysis to generate energy and glycolytic intermediates, which serve as precursors for lipids and animo acids. Differences in plasma and serum amino acid and lipid profiles have demonstrated high sensitivity and specificity in distinguishing CRC patients from healthy controls.

Preliminary Data and Study Goals. A growing body of evidence suggests that platelets contribute to cancer progression. Co-culture experiments have shown that platelet rich plasma (PRP) fosters a proliferative phenotype in cancer cells, and platelet adhesion is thought to facilitate tumor metastasis. In this study investigators theorized that PRP with high biological activity may reveal metabolic changes that could aid in early detection of CRC. The goal of this study was to analyze the metabolic profiles of platelet rich plasma (PRP) in patients with CRC and healthy controls to identify metabolic changes that could help detect early-stage CRC [5].

Methods. Global metabolomics analysis was performed on PRP collected and grouped into individuals with Stage I and II CRC, Stage III and IV CRC, and healthy controls. Cluster analysis and class enrichment were used to evaluate the differences in profiles between the three cohorts.

Results. Lactose, glycerol-3-phosphate, 2-hydroxyglutaric acid, isocitric acid, and citric acid were significantly elevated in PRP collected from CRC patients compared to controls (**Figure 11**). Although the levels of these metabolites were not significantly different between early- (Stage I and II) and late- (Stage III and IV) stage malignancies, their consistently higher concentrations relative to controls did not preclude them as biomarker candidates for early detection of CRC.

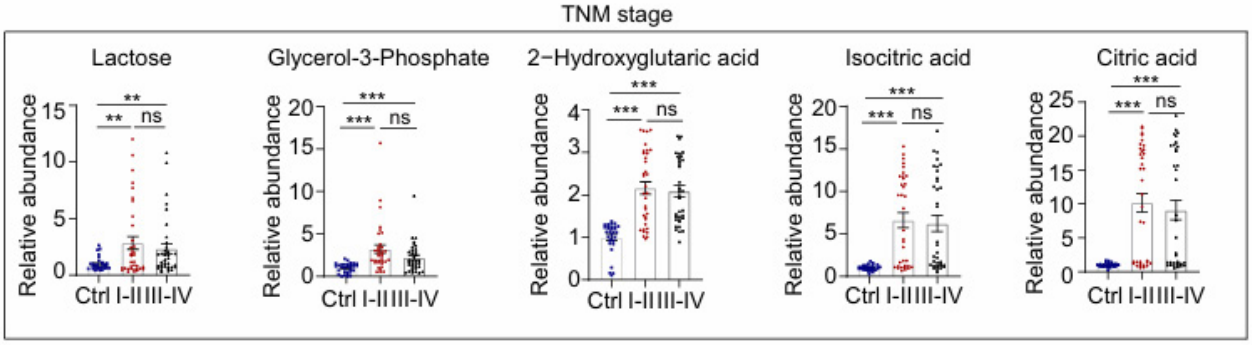
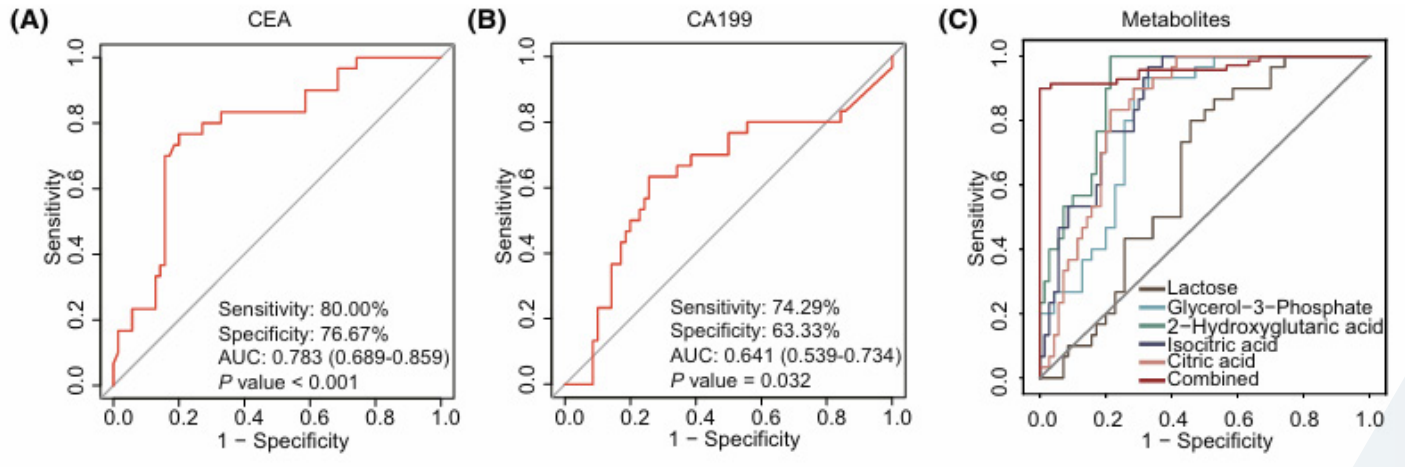


Figure 11. The relative abundance of five key carbohydrate metabolites in different stages of CRC progression. These metabolites were significantly different between CRC patients and controls. No difference between early- (Stage I and II) and late-stage (Stage III and IV) malignancies was observed. Image reproduced from Hu et al., *Mol Oncol*, 2025, licensed under CC BY 4.0.

The diagnostic performance of this 5-metabolite signature was compared to that of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), two classic blood-based biomarkers of CRC. CEA demonstrated 80% sensitivity, 76.67% specificity, and an area under the receiver operating characteristic curve (AUC) value of 0.783; CA 19-9 achieved 74% sensitivity, 63% specificity, and an AUC of 0.641. By contrast, the metabolomics signature performed much better, distinguishing CRC from healthy controls with 90% sensitivity, 96.67% specificity, and an AUC of 0.961. (**Figure 12**).



(D)

Metabolites	Sensitivity (%)	Specificity (%)	AUC (95%CI)	*P* value
Lactose	54.29	80.00	0.638 (0.536-0.732)	0.012
Glycerol-3-Phosphate	71.43	90.00	0.811 (0.720-0.882)	<0.001
2−Hydroxyglutaric acid	78.57	100.00	0.902 (0.827-0.953)	<0.001
Isocitric acid	67.14	96.67	0.861 (0.777-0.922)	<0.001
Citric acid	78.57	83.33	0.843 (0.756-0.908)	<0.001
Combined	90.00	96.67	0.961 (0.922-0.998)	<0.001

Figure 12. Diagnostic performance of the metabolic signature versus traditional biomarkers of CRC. (**A**) Diagnostic efficacy of CEA (**B**) CA 19-9, and (**C**) each signature metabolite alone and in combination. (**D**) The sensitivity, specificity, and AUC of each signature metabolite in distinguishing CRC patients from healthy controls. Image reproduced from Hu et al., *Mol Oncol*, 2025, licensed under CC BY 4.0.

Study Conclusions

- In this study metabolomics identified a biomarker signature for CRC that outperformed traditional protein biomarkers.
- This study demonstrates how metabolite levels reflect the combined influence of pathogenic germline mutations, transcriptional changes, enzyme activity, nutrient availability, and the tumor microenvironment to capture the functional phenotype of cancer rather than simply upstream molecular changes.
- The biomarkers identified in this study would not have been detected by other omics sciences alone, showing how metabolomics can help set a drug development program apart from the competition.

HUMAN NUTRITION

Identifying Mechanistic Links Between Diet and Chronic Kidney Disease

Introduction. Chronic kidney disease (CKD) affects 800 million people worldwide and incurs an annual cost of approximately $407 billion. Diet significantly impacts kidney health, and based on recent findings, plant-based dietary patterns may offer a cost-effective way to prevent CKD. The Adventist Health Study-2 (AHS-2) is a multi-year study intended to measure connections between lifestyle, diet, and disease. Recent findings from this cohort showed an association between vegetarian dietary patterns and lower incidence of diabetes and hypertension, two key risk factors for CKD. The vegetarian diet was also strongly associated with reduced mortality from renal disease. Despite these strong associations, it was not clear how vegetarian dietary patterns seemed to protect kidney health.

Preliminary Data and Study Goals. Previous metabolomics analyses in the ASH-2 cohort showed that vegans had higher plasma levels of polyphenol-derived, microbial, and vitamin-related metabolites while nonvegetarians had higher levels of proinflammatory lipids and amino acids. With the goal of better understanding the potential cause and effect relationships between CKD risk, progression, and diet this study examined associations between vegetarian dietary patterns, kidney function, and diet-related metabolites using estimated glomerular filtration rate (eGFR) as a measure of kidney health [6].

Methods. 899 participants from the AHS-2 cohort were grouped according to dietary patterns which included vegan, pesco-vegetarian, and non-vegetarian. Global metabolomics was performed on serum samples collected from these study participants. Serum creatinine levels were used to calculate eGFR. For analyses of metabolite subclasses, individual component metabolite values within a subclass were averaged to obtain a composite mean for that subclass. T-scores were obtained by dividing the standard deviation of the composite means, accounting for the covariances between the metabolites. The number of significant or differential metabolites for each subclass was determined, along with the number of differential metabolites positively or inversely associated with the dietary pattern.

Results. Many metabolites were uniquely associated with diet and creatinine levels, which revealed specific connections between dietary patterns and metabolic phenotype. For example, relative to non-vegetarians, vegans had significantly lower levels of certain uremic toxins including p-cresol, 3-methyl catechol sulfate, and glucuronide, which are associated with lower eGFR and subsequently lower kidney function. Further analyses identified differential

metabolite subclasses (rather than individual metabolites) associated with different dietary patterns and serum creatinine. Comparisons between vegan with non-vegetarian dietary patterns showed that plasmalogens and lysoplasmalogens had the largest proportion of significant metabolites in one or both groups (**Figure 13**). Plasmalogens and lysoplasmalogens have been shown to mediate membrane repair during oxidative stress. Reduced circulating levels of these metabolite subclasses have been observed in patients with advanced CKD, likely due to high oxidative stress.

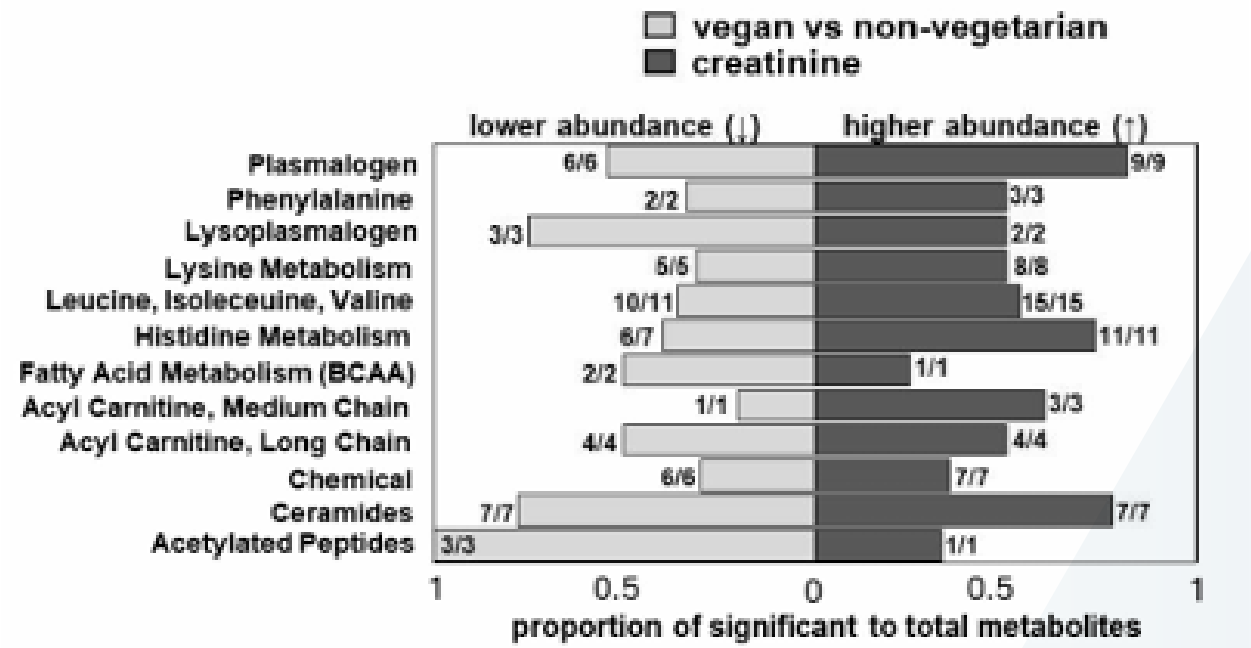


Figure 13. Differential subclasses showing opposite associations of dietary patterns and serum creatinine with plasma metabolites. Differentially abundant subclasses were determined after calculating the composite mean (averaging component metabolites) for each dietary group and subsequently t-scores. Bars reflect the proportion of differential metabolites to total metabolites on panel associated positively with creatinine (right of 0) or inversely with the indicated dietary pattern (left of 0). Ratios represent the number of significant metabolites associated in the same direction (inversely or positively) out of the total number of significant metabolites in the subclass. The predominant direction of change in abundance for each subclass, reflecting the overall direction of component metabolites, is plotted for contrasts of creatinine with vegan vs. non-vegetarian. Image reproduced from Butler et al., *J Ren Nutr*, 2025, licensed under CC BY 4.0.

Study Conclusions

- This study identified metabolic changes that were unique to different dietary patterns, to reveal mechanistic links between diets and eGFR.
- Metabolomics enabled investigators to look beyond the standard creatinine measurement to see underlying metabolic changes, providing more robust evidence that diet-driven metabolic pathways are directly linked to kidney health.

Addressing Limitations of Dietary Self-Reporting by Standardizing Biomarker Fingerprints of Food Intake

Introduction. Traditional methods of dietary assessment, which often rely on self-reporting, are prone to error and bias. Metabolomics can address this limitation by capturing food-derived compounds to provide an objective molecular fingerprint of food intake. Although many dietary biomarkers have been identified, most studies have focused on individual foods rather than whole dietary patterns, and few controlled feeding trials included a randomized cross-over approach to reduce heterogeneity when quantifying associations with specific diets.

Preliminary Data and Study Goals. To address the gaps in modern day dietary metabolomic reporting, this study conducted a randomized cross-over controlled feeding trial comparing a healthy Australian diet (HAD), which was based on current Australian Dietary Guidelines, to a typical Australian diet (TAD). The investigators' goals were to 1) describe metabolic changes in plasma and urine in response to these two dietary patterns, and 2) identify discriminatory metabolites specific to the healthy diet in order to develop a metabolome-derived diet quality score that could help evaluate the association of the healthy diet with markers of cardiometabolic health [7].

Methods. In an 8-week randomized cross-over feeding trial healthy adults completed two controlled 2-week diet interventions, healthy diet and typical diet, separated by a washout period. All meals were provided to ensure adherence and minimize diet variability. Fasting blood and urine samples were collected before and after each intervention then analyzed using untargeted metabolomics profiling. Elastic net regression was used to identify metabolites that best distinguished the healthy diet from the typical diet. These metabolites were used to build a composite metabolome-derived diet quality score, which was then tested for associations with cardiometabolic markers including blood pressure, lipids, and fasting glucose.

Results. After two weeks, 408 plasma and 319 urinary metabolites differed from baseline, many of which belonged to lipid, amino acid, and xenobiotic pathways. Direct comparison of the two diets identified hundreds of metabolites that differed significantly. 43 of them showed consistent changes across plasma and urine, suggesting that each dietary pattern produced a robust metabolic signature (**Figure 14**). Machine-learning analysis identified 31 plasma and 34 urine metabolites that best distinguished the healthy diet (HAD) from the typical diet (TAD). These metabolites were used to create the metabolome-derived diet quality score (**Figure 15 A, B**). Higher scores, which indicated close adherence to the HAD, were significantly associated with improved cardiometabolic markers including lower blood pressure, triglycerides, LDL-cholesterol, and fasting plasma glucose (**Figure 15 C-H**).

Figure 14. Magnitude and direction of change in 43 metabolites after two weeks of consuming a HAD compared to a TAD. Concentrations of these metabolites differed consistently between groups in both plasma and urine. Image reproduced from Stanford et al., *Mol Nutr Food Res*, 2025, licensed under CC BY 4.0.

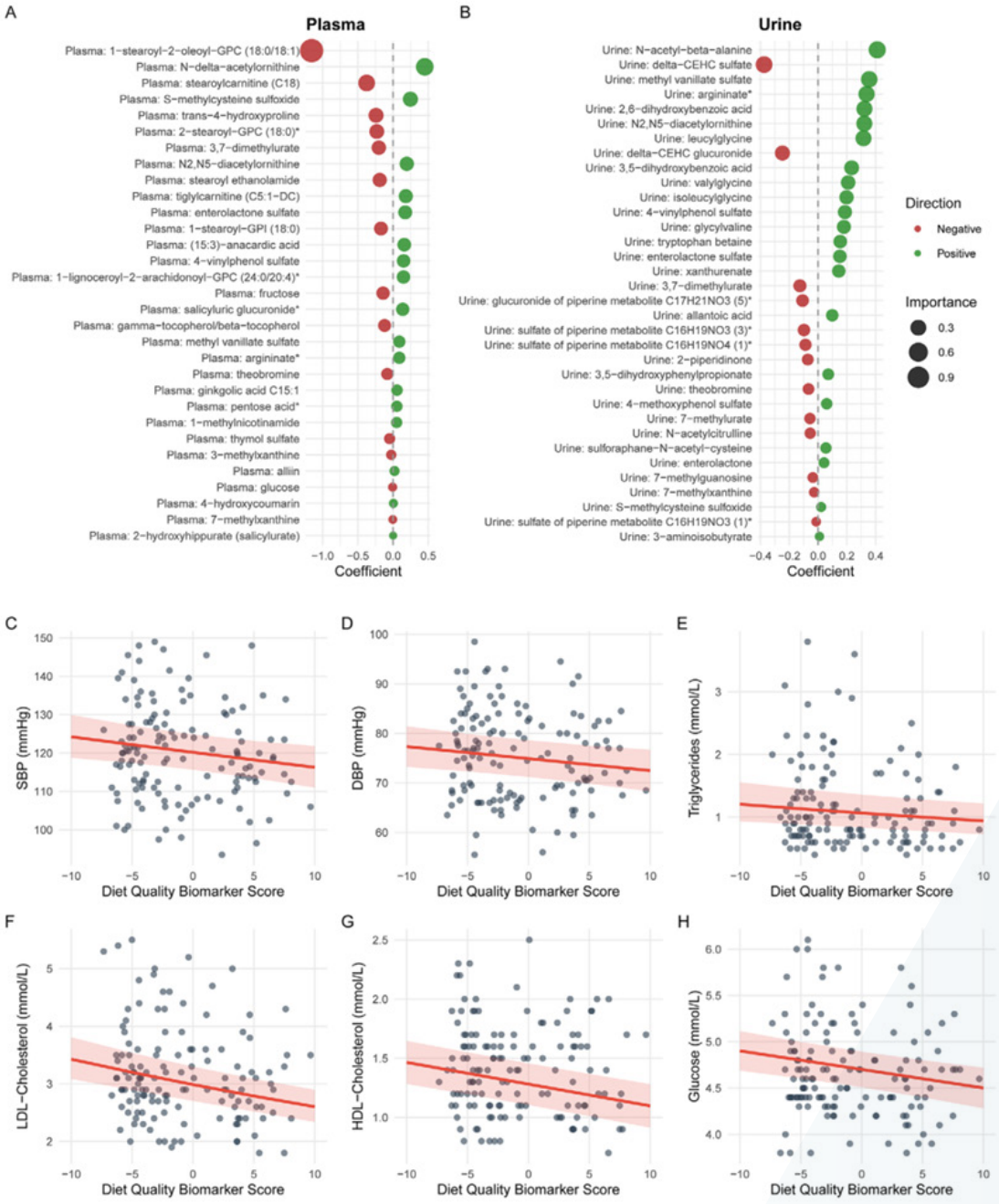


Figure 15. Development of a metabolome-derived diet quality biomarker score and its association with markers of cardiometabolic health. Discriminative metabolites and their coefficients from (**A**) plasma and (**B**) urine are shown. The coefficients were used to construct a composite diet quality score, where a higher score was associated with significantly improved cardiometabolic outcomes including (**C**) lower systolic blood pressure, (**D**) diastolic blood pressure, (**E**) triglycerides, (**F**) LDL-cholesterol, (**G**) HDL-cholesterol, and (**H**) fasting glucose plasma. Image reproduced from Stanford et al., *Mol Nutr Food Res*, 2025, licensed under CC BY 4.0.

Study Conclusions

- In this study, investigators used metabolomics to derive a score that quantitatively measures diet adherence, which addresses bias and error inherent to self-reported dietary intake. Going forward, this diet quality score could significantly improve interpretability and reproducibility of nutrition-based studies to subsequently improve efficiency and cost-effectiveness of future study designs.

Developing Tools to Improve Measurability of Ultra Processed Food Intake

Introduction. Ultra processed foods (UPFs) are characterized by industrial ingredients and additives not typically used in home cooking including artificial sweeteners and modified starches. Although food processing plays an important role in food safety and shelf life, observational studies have consistently associated high consumption of UPFs with increased risk of obesity, cardiovascular disease, cancer, and all-cause mortality. Currently, nutritional studies involving UPFs are limited by a lack of tools to accurately measure UPF intake. Common dietary assessment questionnaires and 24-hour recall lack the detailed ingredient and processing information needed to classify foods according to industry classification systems.

Preliminary Data and Study Goals. The goal of this study was to address the need for objective biomarkers that can reliably capture UPF intake and its metabolic effects, with the long-term goal of improving the study of diet-disease relationships [8].

Methods. Controlled feeding trials, in which all foods are provided and intake is closely monitored, are ideal for identifying relevant biomarkers of UPFs. This study was a secondary analysis of a randomized, crossover, controlled-feeding trial in which 20 adults consumed either a diet high in UPFs or a diet entirely devoid of UPFs. Diets were matched for calories and nutrients. Blood and urine samples were collected at multiple timepoints throughout the study then analyzed by global metabolomics profiling. Metabolite levels were compared between diets using linear mixed-effects models, and additional analyses evaluated pathway level changes.

Results. Relative to the control diet, the high UPF diet induced widespread and consistent metabolic changes. As expected, several metabolites directly linked to food additives and preservatives were significantly increased in the high UPF diet while metabolites associated with whole fruits and vegetables were reduced, reflecting lower intake of these foods. Changes were also observed in pathways related to lipid metabolism, bile acids, amino acids, and gut microbial metabolites, showing that UPF consumption alters both host and microbiome-related metabolism. Importantly, many of these metabolite changes were consistent across plasma and urine, supporting their potential as robust candidate biomarkers of UPF intake and short-term metabolic response (**Figure 16**).

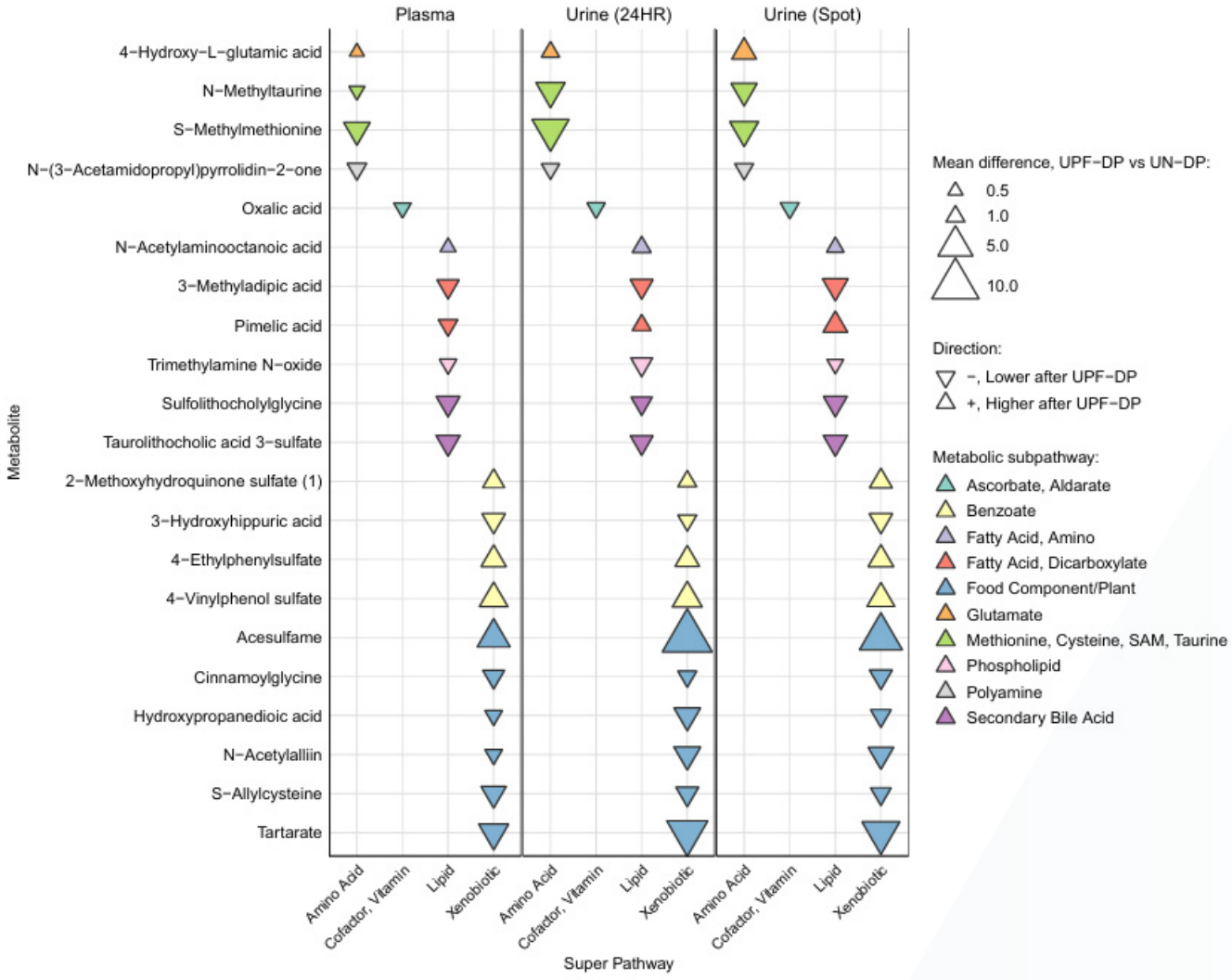


Figure 16. Magnitude and direction of mean differences at week 2 in metabolites after consumption of a high UPF diet compared with a control diet. Metabolites listed (n=21) here differed within individual participants (n=20) between UPF-DP and UN-DP at week 2 for plasma as well as week 1 and week 2 for 24-h urine. Mean differences in metabolites were estimated via a linear mixed model adjusted for diet, phase, sequence, and time point for urine only and calculated EI during the week prior to sample collection with subject-specific random intercepts. Image reproduced from O'Connor et al., *J Nutr*, 2023, licensed under CC BY 4.0.

Study Conclusions

- This study shows that consuming a diet high in UPFs results in clear and measurable changes in the plasma and urine metabolome. Metabolites directly linked to UPFs including benzoate preservatives and artificial sweeteners, as well as those associated with shifts in the microbiome, could serve as objective biomarkers of UPF intake.
- These biomarkers could help address some longstanding limitations of traditional dietary assessment methods and improve the accuracy and reproducibility of studies aimed at characterizing the relationship between diet and health.

PET HEALTH AND ANIMAL HUSBANDRY

Characterizing the Mechanism of Action of a Widely Used but Poorly Understood Therapy for Chronic Large-Bowel Diarrhea in Dogs

Introduction. Chronic large-bowel diarrhea in dogs is a common gastrointestinal disorder driven by complex interactions between the gut microbiome, diet, host metabolism, immune responses, and environmental factors. Recent evidence suggests that microbial dysbiosis contributes to this condition by altering metabolic outputs in the gut, including the production of inflammatory or harmful metabolites. Supplementing the diet with fiber may improve gastrointestinal health by supporting beneficial microbial fermentation and increasing the production of protective postbiotics including short-chain fatty acids (SCFAs), secondary bile acids, and indoles. Although nutritional intervention is considered the primary therapy for chronic large-bowel diarrhea in dogs, the mechanisms behind its therapeutic effect are poorly understood.

Preliminary Data and Study Goals. Investigators at Hill's Pet Nutrition, Inc., showed that a proprietary blend of soluble and insoluble fibers chosen for their pre- and postbiotic activity improved stool scores. The goal of this follow-up study was to evaluate the mechanistic impact of fiber-supplemented dietary intervention on GI health in dogs actively experiencing large bowel diarrhea [9].

Methods. Investigators conducted an 8-week clinical trial on adult dogs experiencing chronic large-bowel diarrhea. All dogs received a high-fiber therapeutic diet formulated with multiple soluble and insoluble plant fibers. Fecal and serum samples were collected at baseline and several timepoints during the intervention. Untargeted metabolomics was used to profile fecal and serum to evaluate changes in gastrointestinal health and metabolic activity.

Results. The fiber-supplemented dietary intervention rapidly improved clinical signs of diarrhea. Significant increases in stool firmness were observed within one day and diarrhea resolved in most dogs within several weeks. Metabolomic analyses revealed significant changes in fecal and circulating metabolites that indicated a transition in gut microbial metabolism from protein fermentation (putrefaction) toward carbohydrate fermentation (saccharolysis) (**Figure 17**). The intervention reduced metabolites associated with bacterial protein breakdown and increased plant-derived metabolites and beneficial microbial postbiotics including indole derivatives. Additional metabolomic changes suggested improvements in inflammatory pathways, lipid metabolism, intestinal barrier integrity, and host-microbe interactions.

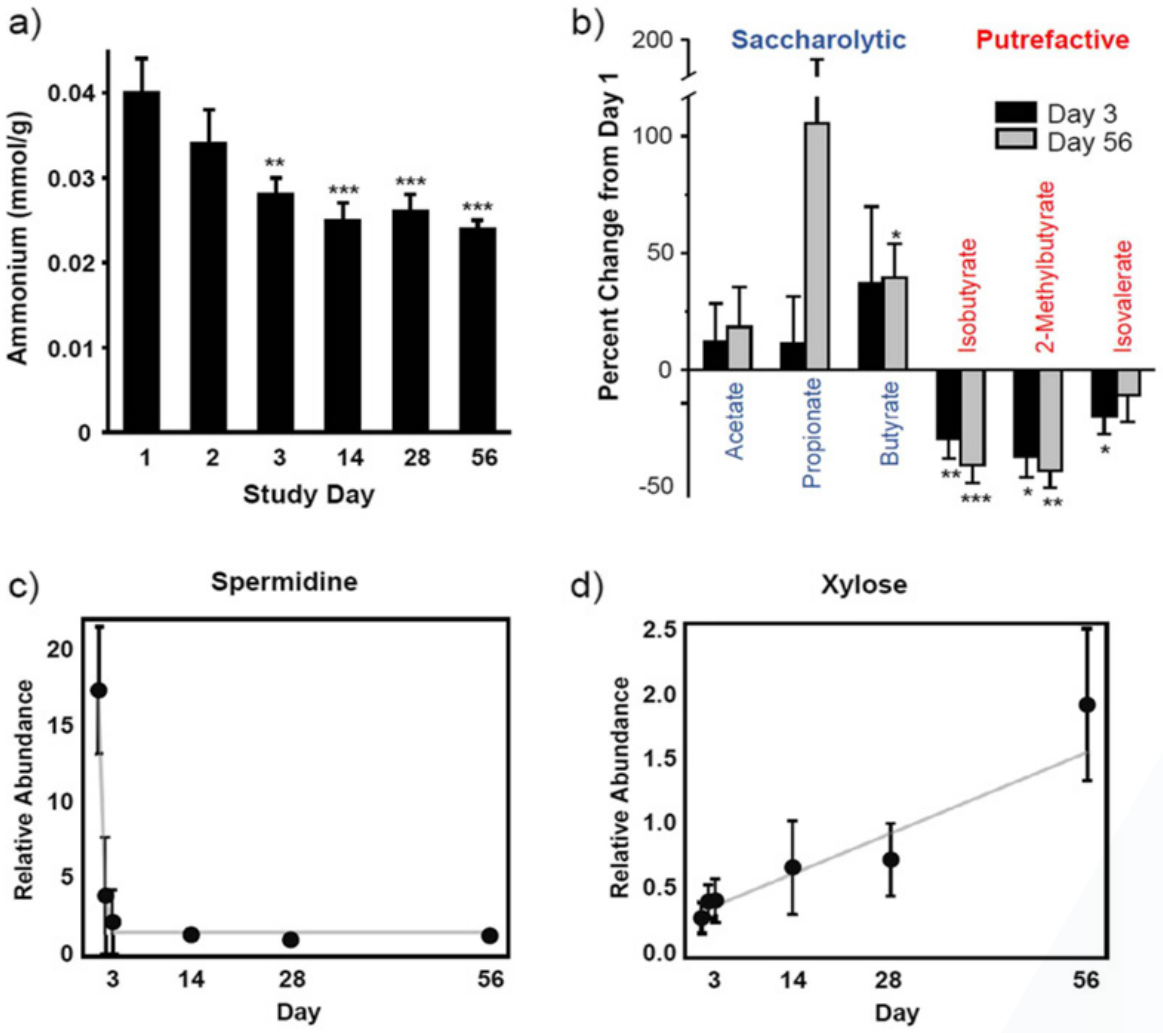


Figure 17. The impact of dietary intervention on fermentative metabolism. Dietary fiber intervention shifted metabolism away from protein fermentation towards carbohydrate metabolism as evidenced by reductions in (**A**) Fecal ammonium concentrations, (**B**) short chain fatty acids, and (**C**) spermidine and increased (**D**) monosaccharide xylose. Image reproduced from Fritsch et al., *BMC Vet Res*, 2022, licensed under CC BY 4.0.

Study Conclusions

- Overall, these study findings indicate that dietary fiber supplementation positively alters both microbial metabolism and host gastrointestinal physiology, demonstrating how dietary fiber confers its therapeutic effect.
- Metabolomics enabled investigators to directly measure the metabolic products produced in the gut, which provided functional insight into these mechanisms that could not have been deduced otherwise.
- The mechanistic evidence provided by the metabolomics data provided evidence of the efficacy of the nutritional intervention that could be used to support marketing claims.

Informing Early-Life Nutrition to Reduce Culling in Dairy Cows

Introduction. Early-life nutrition plays a crucial role in shaping long-term metabolic health. In dairy calves, insufficient nutrition before weaning is associated with reduced growth, impaired immune function, and poorer long-term productivity, while adequate nutrition tends to increase milk yield and support early reproductive maturity. The biological mechanisms linking early-life nutrition to productivity in adult cows are well documented but poorly understood.

Preliminary Data and Study Goals. Previous studies have shown that an adequate preweaning nutrient supply alters metabolic pathways related to energy, amino acid metabolism, and insulin sensitivity that remain in adulthood. In this study, investigators theorized that greater preweaning nutrient supply improves energy metabolism and long-term productivity in dairy cows [10]. The goal of this study was to test their hypothesis by determining how early-life milk intake influences metabolomic profiles, milk production, and survival through multiple lactations.

Methods. 78 Holstein cows were studied from the first to the fifth calving. Calves were randomly assigned to an elevated nutrient group which received 8 liters of milk replacer per day or to a restricted group which received 4 liters per day until weaning. Growth, reproductive performance, milk production, feed intake, and survival across lactations were monitored. Serum samples were collected on day 60 of lactation and analyzed using untargeted metabolomics to evaluate metabolic status during peak milk production.

Results. Calves receiving elevated levels of milk replacer showed greater early growth and improved insulin sensitivity later in development compared to restricted-fed calves. Elevated milk replacer cows also produced higher milk fat yields and fat content, and produced more milk when volumes were corrected for percentages of body fat and protein. Reproductive performance was similar between groups in the first lactation, but in the second lactation elevated milk replacer cows had a higher rate of conception after the first mating than restricted fed cows. Survival analysis showed that cows receiving higher early life nutrition had substantially lower risk of culling before later calvings (**Figure 18**). Metabolomic profiling at peak lactation revealed persistent metabolic differences between groups, including changes in amino acid, lipid, nucleotide, and carbohydrate metabolites (**Figure 19**). Pathways analyses identified alterations in metabolism of purines, pyrimidines, sphingosine, fatty acids, and the TCA cycle, suggesting that early-life nutrition influences long-term metabolic programming.

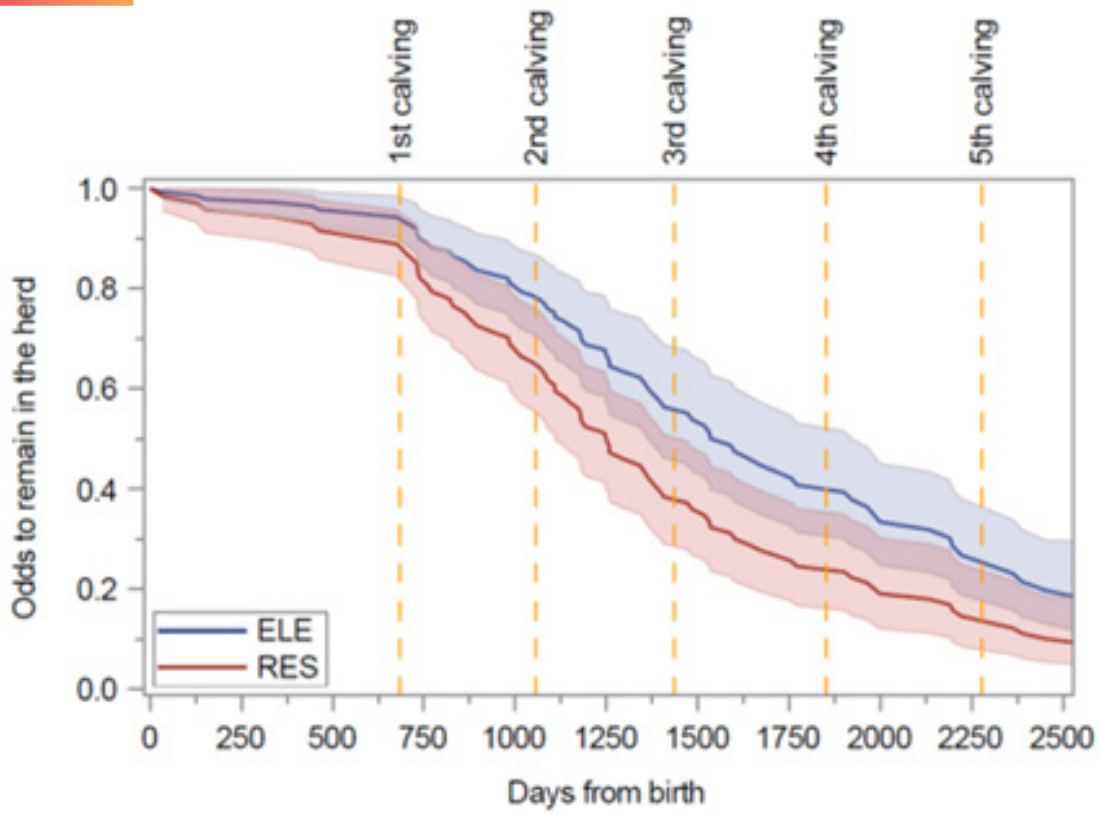


Figure 18. Probability that cows will remain in the herd, and thus avoid culling, in relation to days from birth for cows fed an enhanced (blue line) or restricted (red line) amount of nutrient intake before weaning. Image reproduced from Leal et al., *J Dairy Sci*, 2025, licensed under CC BY 4.0.

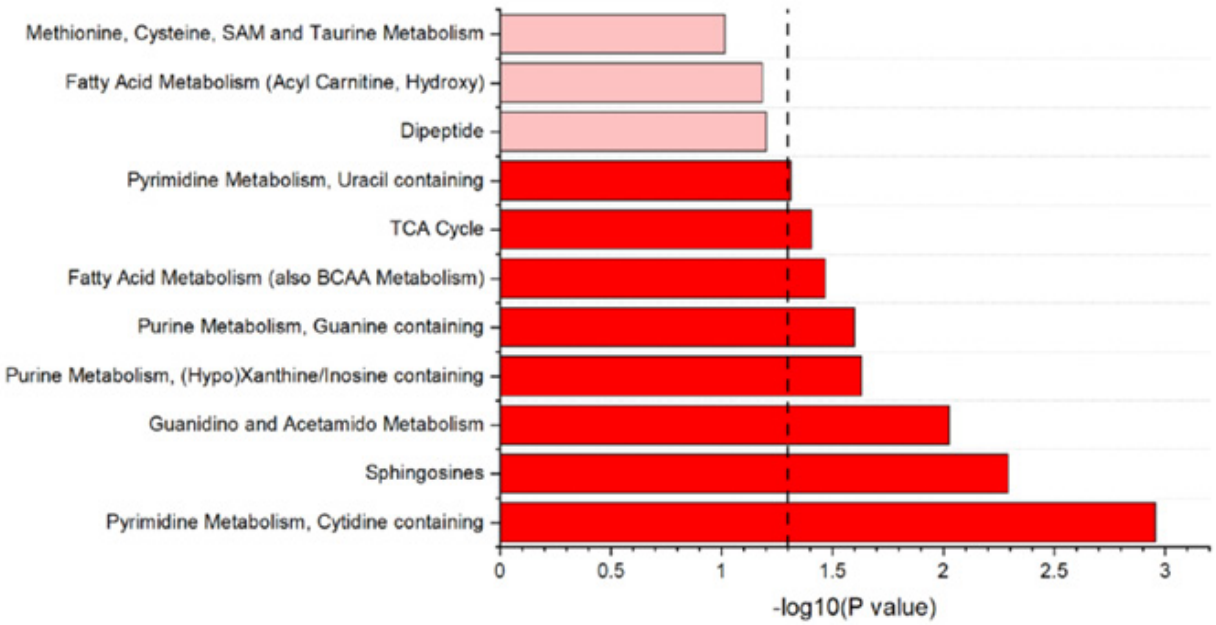


Figure 19. Visualization of meaningful metabolic pathways evidenced by enrichment analysis based on metabolites detected in serum of dairy cows at peak lactation. Metabolic pathways shown in darker red had $p < 0.05$; pathways shown in lighter red had $p < 0.10$. Image reproduced from Leal et al., *J Dairy Sci*, 2025, licensed under CC BY 4.0.

Study Conclusions

- This study's findings demonstrate a direct link between early-life nutrition and long-term metabolic function, which can inform feed decisions to ultimately improve lifetime productivity and sustainability in dairy cattle leading to cost savings and nutritional benefits to humans.

Improving Beef Quality by Characterizing the Relationship Between Soil Quality, Feed, and Cattle Health

Introduction. In the American beef industry, cattle are usually fed corn-based diets for the last several months of life to maximize their growth and carcass quality. Animal feed crop production relies on monocultures, synthetic fertilizers, and irrigation which deplete soil nutrients, degrade soil structure, and contaminate water sources. Finishing animals on pasture using adaptive and rotational grazing practices has been shown to improve plant diversity, soil carbon, and general ecosystem function. Animals that graze fresh forages consume higher and more diverse polyphenol pools, and enrichment of these compounds in live-stock diets can improve animal metabolic health and increase the broader nutrient density of meat and milk. To better inform animal grazing practices more extensive research is needed to establish potential linkages between metrics of soil, animal, and plant health.

Preliminary Data and Study Goals. The goals of this study were to 1) determine the potential differences in the metabolite profiles of grass-fed and grain-fed beef, 2) evaluate them for associations with soil health and abundance of nutrients, and 3) evaluate health biomarkers in animals to gain insight into the potential impacts of varying nutritional statuses on their health and meat nutritional composition [11].

Results. Notable differences in soil health, feed composition, and beef metabolite profiles between pasture-based and grain-fed production systems were observed. Pasture soils generally showed higher soil organic matter, nutrient availability, and mineral levels compared to cornfield soils used for feed production. Untargeted metabolomics revealed substantial biochemical variation between pasture forage and grain-based mixed rations (**Figure 20**). Pasture contained higher levels of phytochemicals, phenolics, and omega-3-realted compounds, which grain rations had higher levels of dipeptides and omega-6-related metabolites. These dietary differences translated to the meat itself. Grass-fed beef contained higher levels of phenolic compounds, omega-3-fatty acids, long-chain acyl carnitines, antioxidants, and vitamin E and vitamin A derivatives. By contrast, grain-fed beef contained higher levels of certain dipeptides, sugars, B-vitamins, and markers of oxidative stress (**Figure 21**).

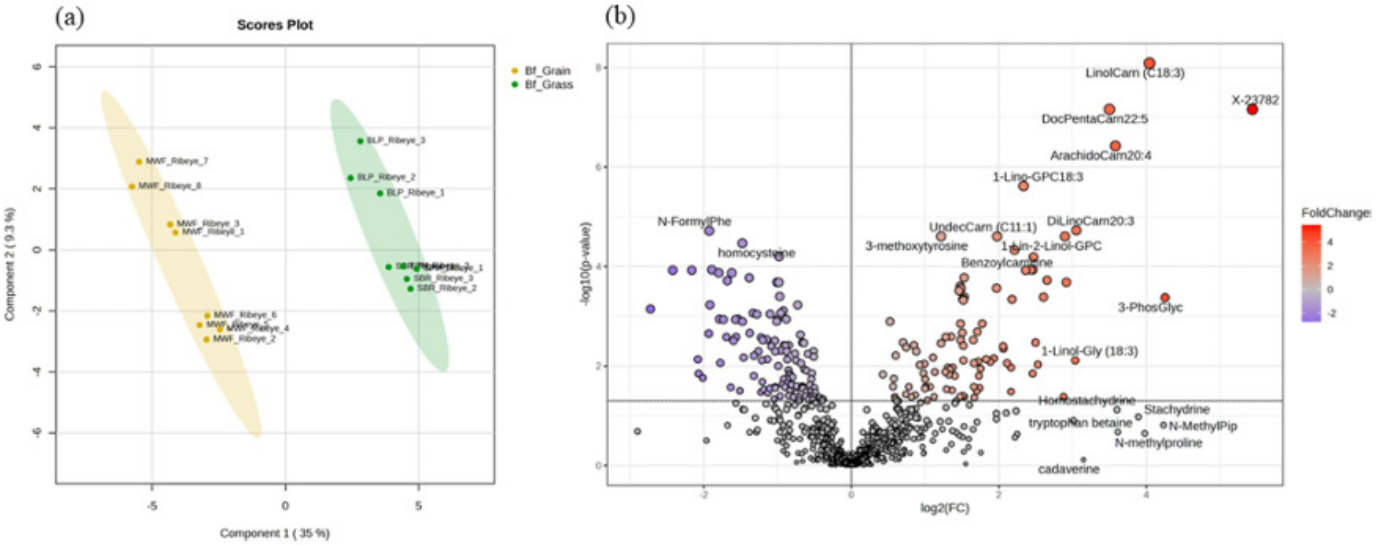


Figure 20. Multi- and univariate analysis of metabolite profiles in grass-fed and grain-fed beef samples. (**A**) Partial Least Squares Discriminant Analysis (PLS-DA) and (**B**) Volcano plot of differential metabolites in grass-fed (n=8) and grain-fed (n=8) beef samples. Image reproduced from Ahsin et al., *NPJ Sci Food*, 2025, licensed under CC BY 4.0.

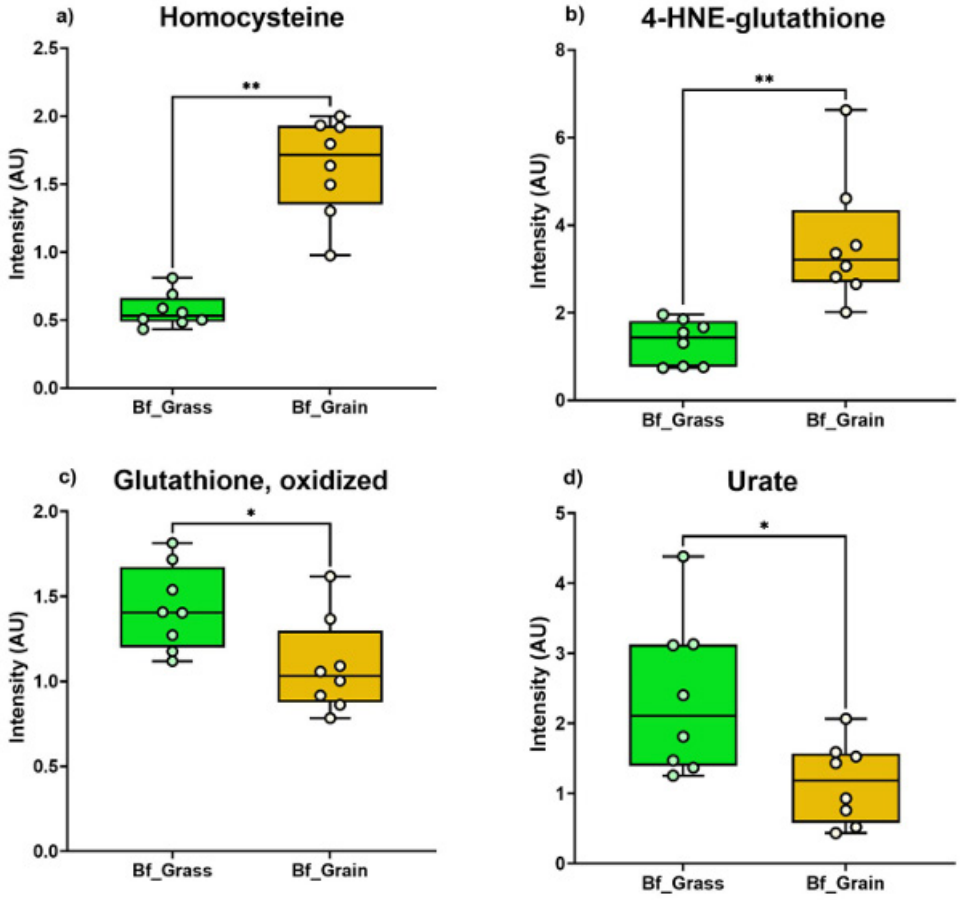


Figure 21. Oxidative stress and antioxidant markers in grass-fed (Bf_Grass) and grain-fed (Bf_Grain) beef. The bar graph shows the abundance of oxidative stress markers: (**A**) Homocystine, (**B**) 4-HNE-glutathione, and antioxidants: (**C**) Oxidized Glutathione, (**D**) Urate across both cattle groups. Image reproduced from Ahsin et al., *NPJ Sci Food*,2025, licensed under CC BY 4.0.

Study Conclusions

- These findings show that beneficial nutrient compounds from forage are transferred directly or metabolically into beef, suggesting that cattle diet influences the metabolic composition and potential nutritional properties of the final meat product.
- Metabolomics provided evidence that directly linked soil quality to cattle feed and cattle feed to meat quality. Such information will be important to future decision-making about grazing practices and feed selection to enhance human health.

REVIEW

Chapter Takeaways

- Metabolomics is a highly valuable tool for gaining biological insight beyond what other omics sciences can provide.
- The insight gleaned from metabolomics can be key to cost savings and gaining an edge on the commercial competition.

Chapter 4

Metabolomics for Translational Research

AT A GLANCE

In this chapter we will address:

- How metabolomics can help develop disease models that most accurately reflect human biology.
- How metabolomics can be used to characterize novel mechanisms of disease progression in preclinical models that translate remarkably well to human patients.
- How metabolomics can help explain why standard of care treatments for a disease are only partially effective, and which biological process should be targeted to address this gap in care.

Overview

As the closest omic to the phenotype, metabolomics has revealed important insights into the relationship between causative factors and disease. Unlike genes and proteins, biochemical pathways are conserved across species. Thus, metabolomics has been instrumental in characterizing disease biomarkers and pathways in cells or animal models that translate to humans. In this chapter, we will discuss key studies where metabolomics played an integral role in identifying an important biomarker signature or therapeutic target, characterizing a disease mechanism that was particularly relevant in human patients, or developing a novel disease model significantly closer to human pathophysiology than existing models. The case studies in this chapter cover these topics as follows:

Disease models – Case Studies 1, 2, and 4

Biomarkers – Case Studies 2, 3, and 4

Mechanisms – Case studies 1, 2, 3, and 4

DISEASE MODELS, BIOMARKERS, AND MECHANISMS

Case Study 1: Developing Animal Models of Dyslipidemia that Accurately Reflect Human Biology

Introduction. Cardiovascular disease (CVD) is a leading cause of death worldwide, and dyslipidemia is a major risk factor for this condition. Although statins can be highly effective at lowering LDL cholesterol and reducing CVD risk, many patients experience adverse events, which highlight the need for new therapeutic targets. However, efforts to develop novel treatments have yielded low clinical success, which are often attributed to inadequate preclinical animal models that do not accurately reflect human lipid metabolism.

Preliminary Data and Study Goals. To address the limitations of today's animal models of lipid metabolism, study investigators used metabolomics to analyze and compare plasma lipid profiles across multiple animal species and humans with the goal of determining which models most closely resemble human dyslipidemia, both at baseline and in response to statin treatment [12].

Methods. Multiple mouse strains, other non-primate species, and non-human primates were analyzed alongside dyslipidemic humans. Animals were fed either standard laboratory chow or a diet high in fat or high in cholesterol. Most species received simvastatin treatment or vehicle control for two weeks to allow comparison between baseline and drug-responding lipid profiles. Plasma samples were analyzed for lipids and lipoproteins using metabolomics. Lipids were also measured using traditional clinical measurements including total cholesterol, LDL, HDL, and triglycerides. Distance-based methods were used to quantify how closely each animal model resembled human dyslipidemia across multiple lipid fractions.

Results. Substantial differences in baseline plasma lipid profiles across animal models were observed, with only a subset closely resembling dyslipidemic humans. Nonhuman primates showed the greatest similarity to humans regarding total cholesterol, LDL/HDL balance, and overall lipoprotein distribution (**Figure 22**). Many commonly used models including mice, rabbits, and rats differed significantly from humans. For example, many rodent models carried most cholesterol in HDL (an atheroprotective profile), unlike humans, whereas nonhuman primates and some modified models such as CETP-expressing mice exhibited more human-like lipid patterns. Fatty acid composition across major lipid classes was broadly conserved in some species but diverged in others. In humans, most fatty acids came from nonessential and omega-6 pathways, and this pattern was best replicated in nonhuman primates, dogs, and a few rodent models. When comparing overall lipid composition across eight major lipid fractions nonhuman primate models ranked closest to humans (**Figure 23**). In response to simvastatin treatment, nonhuman primates and dogs showed lipid changes most consistent with humans, while most rodent models showed little or even opposite responses.

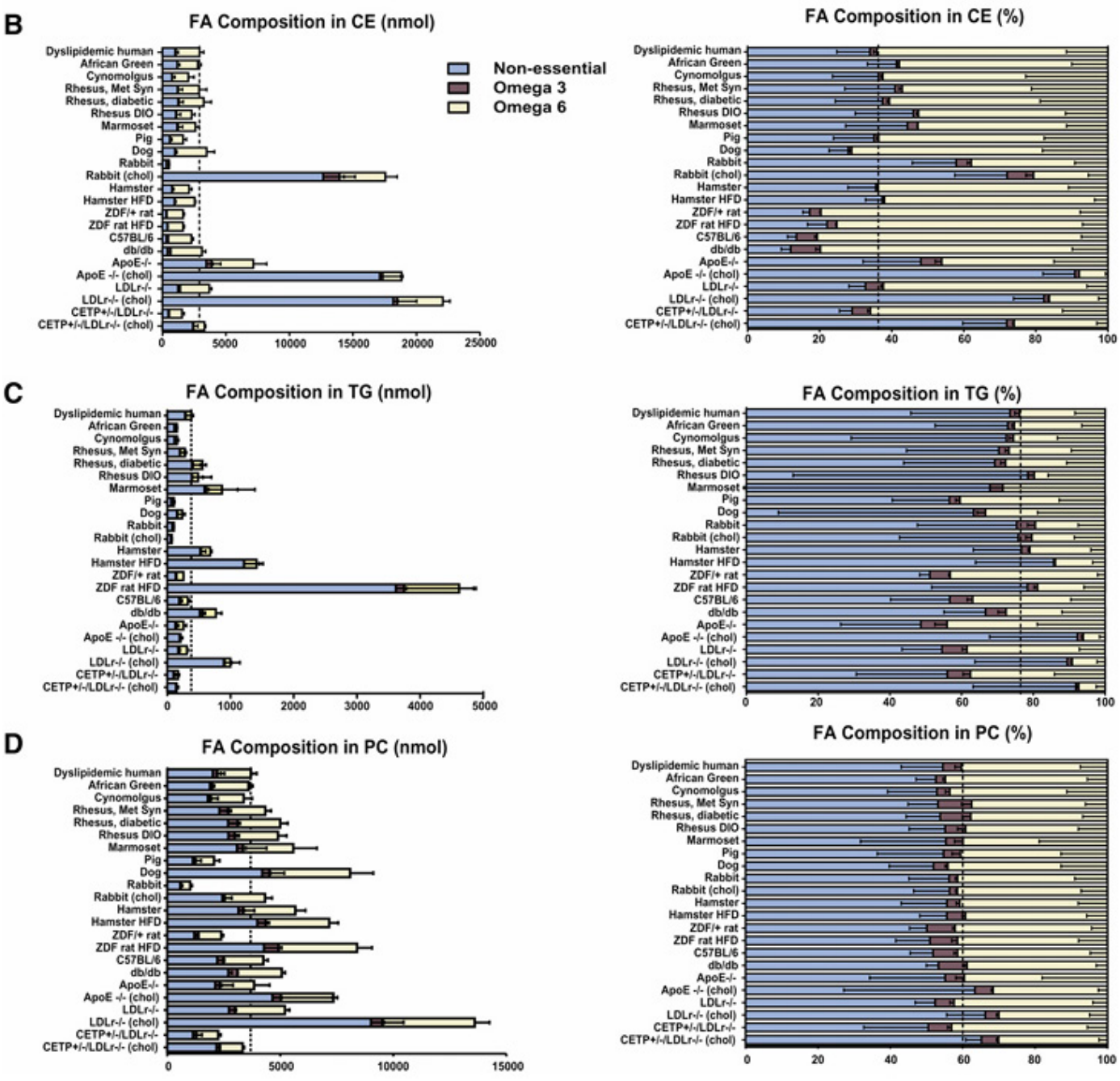


Figure 22. Fatty acid (FA) composition in plasma cholesterol esters (CEs), triglycerides (TGs), and phosphatidylcholine (PC) across animal species. FAs are shown within three categories: nonessential FAs (light blue), omega-3 (maroon) pathway FAs, and omega-6 (light yellow) pathway FAs. Measured FAs were summed for each pathway. Left panel shows the absolute amounts (nmol) of FAs in each pathway and right panel shows the relative amounts (%) for plasma CEs, (A) TGs (B), and (C) PCs. Image reproduced from Yin et al., *J Lipid Res*, 2012, licensed under CC BY 4.0.

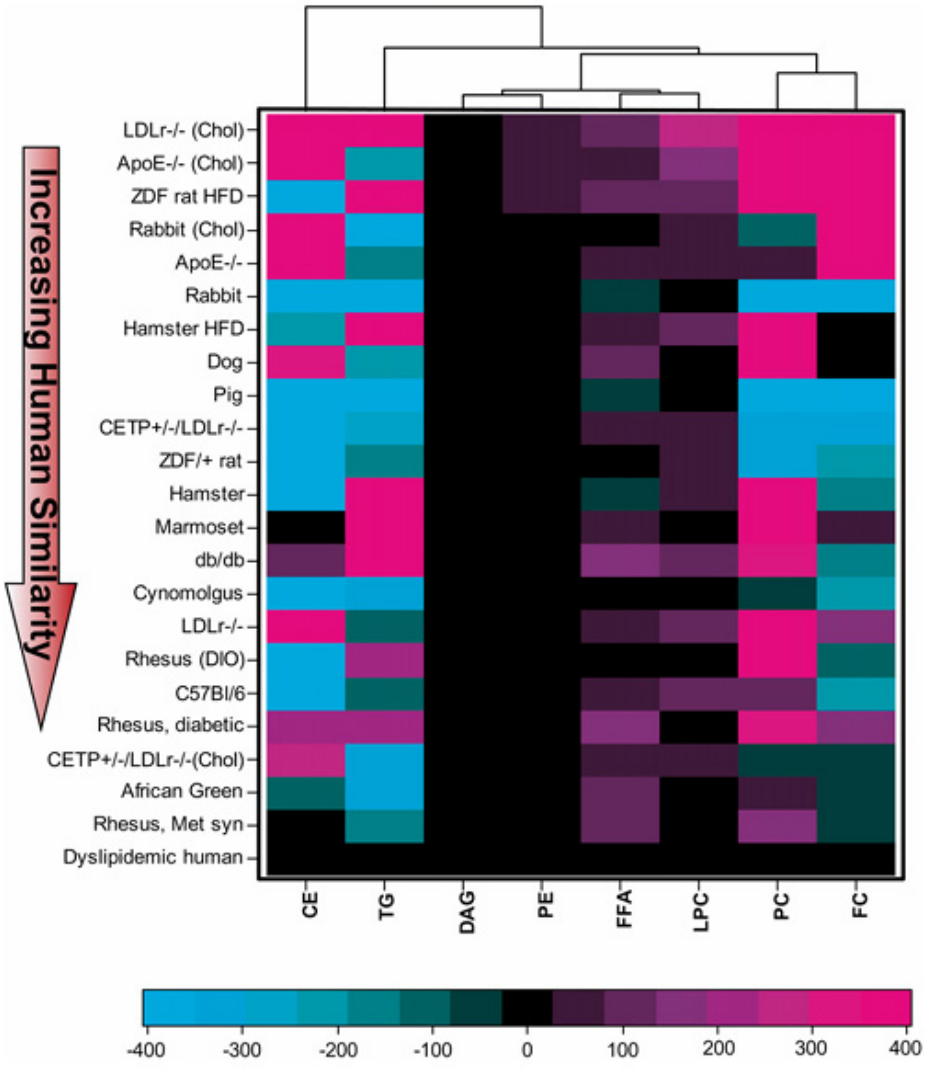
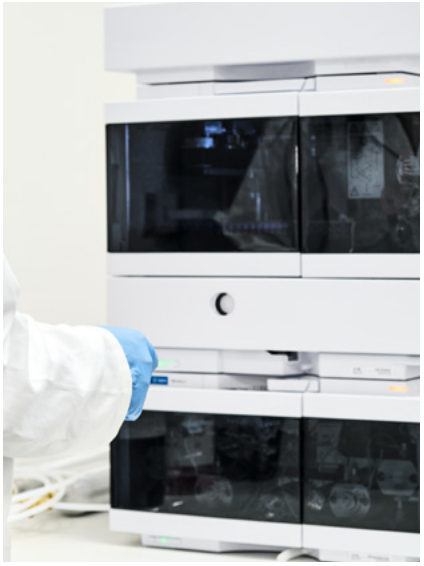


Figure 23. Dendrogram comparison of baseline plasma lipid similarity based on eight major circulating lipid fractions (CE, TG, DAG, PE, FFA, LPC, PC, and FC) across species. The difference of any given lipid fraction between the means of each animal model and dyslipidemic human was calculated and weighted according to the proportion the same lipid fraction over total lipid in humans. The overall weighted distance of each animal model from dyslipidemic humans was calculated, and the models were sorted by distance. Image reproduced from Yin et al., *J Lipid Res*, Licensed under CC BY 4.0.

Study Conclusions

- This study shows that baseline and treatment-responded lipid profiles are crucial for evaluating how well animal models reflect human disease.
- Nonhuman primates are the most representative models of human dyslipidemia, and dogs are similar in regards to drug response. By contrast, many widely used animal models including ApoE-/- and LDLr-/- mice were significantly different from humans.
- Not only did metabolomics enable a comprehensive comparison of lipid profiles between several animal models and humans, but it also showed that integrating comprehensive lipid profiling (lipidomics) with functional drug response data provides a more accurate framework for evaluating disease similarity and improving target validation.

Case Study 2: Characterizing Mechanisms of Niemann-Pick Disease that Mirror the Phenotype of Human Patients



Introduction. Niemann-Pick type C1 (NPC1) disease is a rare, progressive, neurodegenerative disorder caused primarily by mutations in the NPC1 gene that lead to impaired intracellular cholesterol trafficking and accumulation of lipids in lysosomes. The disease typically presents in childhood with ataxia and cognitive decline, and without timely intervention, patients usually succumb to it in adolescence. A major challenge in managing NPC1 disease is the lack of a simple, non-invasive diagnostic test, which can delay diagnosis often by several years.

Preliminary Data and Study Goals. NPC1 disease is strongly associated with oxidative stress, as demonstrated by increased reactive oxygen species (ROS) and lipid peroxidation in cellular and animal models, and as reduced antioxidant capacity in patients. The oxidative environment, combined with excess cellular cholesterol, promotes the formation of cholesterol oxidation products (oxysterols). Based on these previously reported findings, the investigators of this study theorized that circulating cholesterol oxidation products could serve as sensitive and specific blood-based biomarkers for NPC1 disease [13]. The goal of this study was to determine whether oxysterols in plasma could distinguish NPC1 disease patients from healthy individuals and whether oxysterol levels correlate with disease severity.

Methods. Npc1-/- mice and a feline model of NPC1 disease were used to evaluate oxysterols as biomarkers for NPC1 disease. Plasma, liver and brain tissue, and cerebrospinal fluid (CSF) were collected from animals across their lifespan. In parallel, human plasma, CSF, and fibroblast samples were collected from NPC1 disease patients, healthy controls, heterozygous carriers of a NPC1 mutation, and individuals with other lysosomal storage diseases to assess biomarker specificity. Oxysterols were measured using targeted metabolomics. Differences in oxysterol levels and correlation with disease severity were determined using ANOVA and correlation analyses.

Results. Multiple oxysterols were significantly elevated in plasma and tissues of Npc1-/- mice, with a moderate increase seen before the onset of neurological symptoms and increasing further as disease progressed. In human subjects, the non-enzymatic oxysterols 3β,5α,6β-triol and 7-ketocholesterol (7-KC) were significantly increased in NPC1 patients compared to controls and heterozygous carriers, which detected cases with high sensitivity and specificity. These biomarkers clearly separated NPC1 patients from healthy individuals and those with other lysosomal storage disorders (**Figure 24**). Higher concentrations of 3β,5α,6β-triol and 7-KC were associated with earlier disease onset and greater disease severity, and an

index combining these markers improved predictive power. In the NPC1 feline model, treatment with cyclodextrin significantly reduced oxysterol levels, showing that these biomarkers can also track therapeutic response (**Figure 25**).

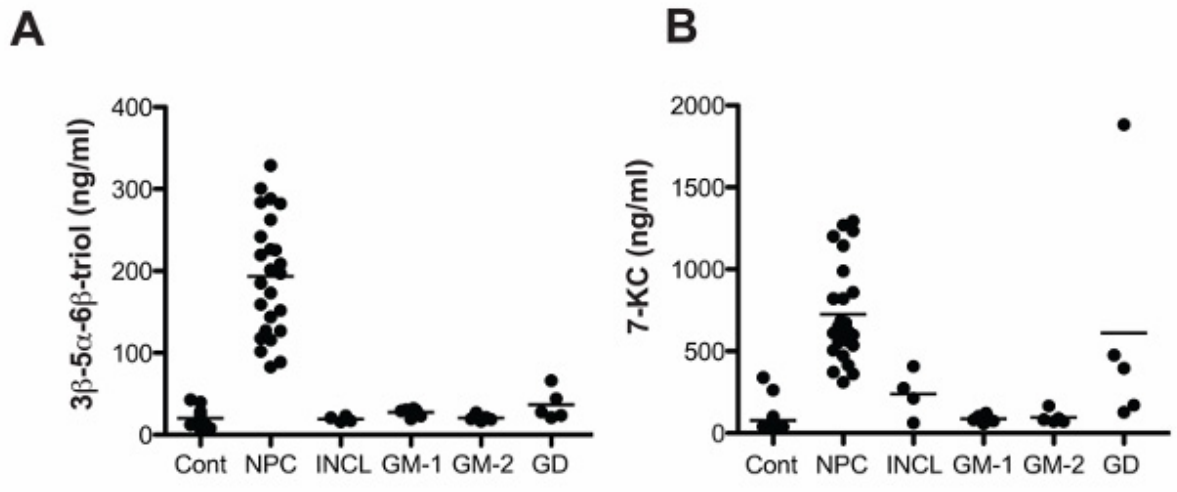


Figure 24. Comparison of plasma oxysterol concentrations in NPC1 disease and other lysosomal storage diseases. (**A**) 3β,5α,6β-triol and (**B**) 7-KC concentrations in fasting plasma samples from control, NPC1, infantile neuronal ceroid lipofuscinosis (INCL), GM-1 gangliosidosis (GM-1), GM-2 gangliosidosis (GM-2) and Gaucher disease (GD) subjects. Image reproduced from Proter et al., *Sci Transl Med*, 2010, licensed under CC BY 4.0.

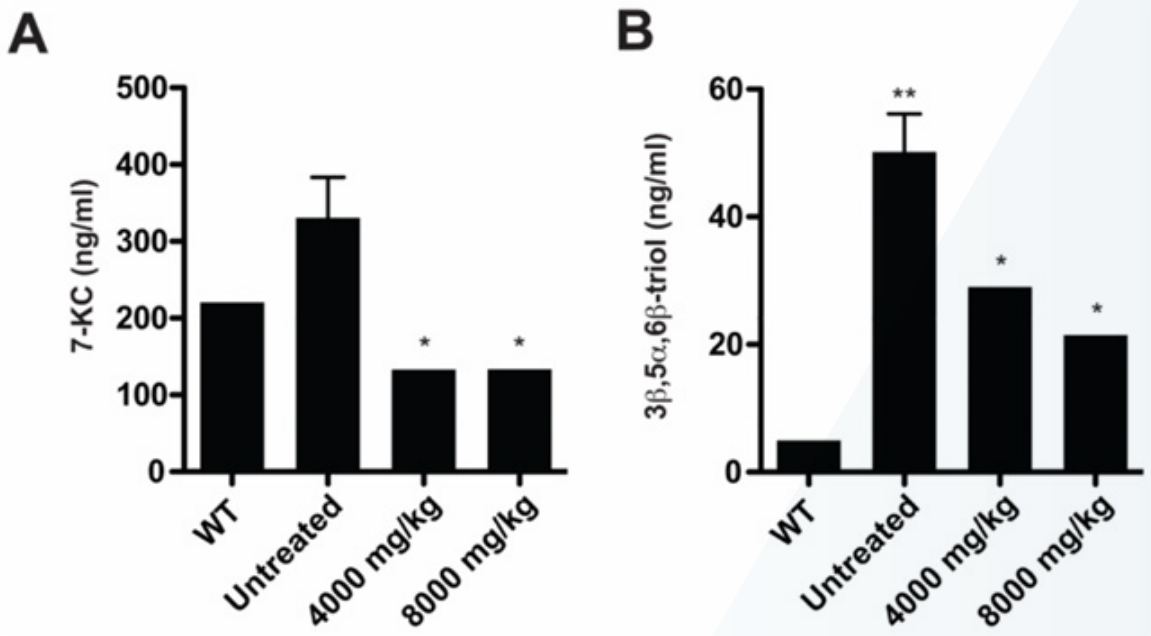


Figure 25. Circulating oxysterol biomarkers are decreased in response to cyclodextrin therapy. (**A**) Serum 7-KC and (**B**) 3β,5α,6β-triol concentrations were measured in untreated WT (4–16 weeks) and NPC1 (16 weeks) cats, and in NPC1 cats (16–18 weeks) treated with a single subcutaneous injection of 4000 or 8000 mg/kg at 3 weeks (n=2–4/group). Image reproduced from Proter et al., *Sci Transl Med*, 2010, licensed under CC BY 4.0

Study Conclusions

- This study shows that oxysterols are highly sensitive and specific biomarkers for NPC1 disease, which addresses a significant unmet need in diagnosis and monitoring of this disease.
- Unlike other diseases where oxysterol increases are modest, NPC1 shows large, disease-specific elevations that enable clear discrimination from controls and other disorders.
- Since oxysterols correlate with disease severity and age of onset, they may also be useful for tracking disease progression. The observed reduction in oxysterol levels following treatment in animal models suggests they could serve as surrogate endpoints in clinical trials to help evaluate therapeutic efficacy.

Case Study 3: Characterizing Gut-Brain Interactions Unique to Persons with Autism Spectrum Disorder

Introduction. Currently, Autism Spectrum Disorder (ASD) is diagnosed based on behavioral criteria rather than biological markers, which contributes to delayed diagnosis and limits early intervention opportunities. The increasing prevalence of ASD and lack of FDA-approved treatments for core symptoms calls for objective molecular biomarkers to enable earlier detection and support personalized therapies. ASD is increasingly associated with metabolic abnormalities, including mitochondrial dysfunction and oxidative stress. However, findings from prior studies have been inconsistent due to small sample sizes, limited metabolite coverage, and variability in study design, which has hindered attempts to identify consistent metabolic signatures. Additional complexity arises from the diet and gut microbiome, which can significantly shape metabolite profiles and may interact with genetic risk factors to influence ASD biology.

Preliminary Data and Study Goals. To address these gaps in knowledge investigators sought to perform comprehensive metabolomics analyses of both plasma and fecal samples from a large cohort of children with ASD alongside a neurotypical control group [14]. The goal of this study was to identify robust metabolic signatures associated with ASD and characterize associations between metabolism and behavioral symptoms.

Methods. Participants were aged 3–12 years. ASD diagnoses were confirmed using standardized clinical tools and neurotypical controls were screened to ensure typical development. A subset of participants also underwent evaluation for gastrointestinal (GI) symptoms to stratify ASD individuals into

groups with and without GI dysfunction for additional analyses. Plasma and feces were analyzed using global metabolomics and a complex lipid panel. Metabolic differences between study groups were determined using ANOVA-based comparisons. Random Forest machine learning was used to determine whether metabolic profiles could distinguish ASD patients from controls. Additionally, fecal microbiota from ASD and neurotypical donors were transplanted into germ-free mice to examine whether microbiome-associated metabolic signatures could be transferred and provide insight into gut-brain interactions.

Results. Hundreds of metabolites differed between groups and metabolite profiles could modestly distinguish ASD from neurotypical individuals, with key discriminatory metabolites including lipids, steroid hormones, and microbially derived compounds. Altered levels of acyl-carnitines and energy-related metabolites suggested impaired cellular energy metabolism and disruptions in amino acid and glutathione pathways consistent with increased oxidative stress were observed (**Figure 26**). Many of these altered metabolic pathways correlated with behavioral severity scores, linking metabolic abnormalities to clinical features of ASD. Several microbially derived metabolites, including 4-ethylphenyl sulfate, were elevated in ASD and some were transferable to mice via fecal microbiota transplantation, suggesting a causal contribution of gut microbes to metabolic changes (**Figure 27**).

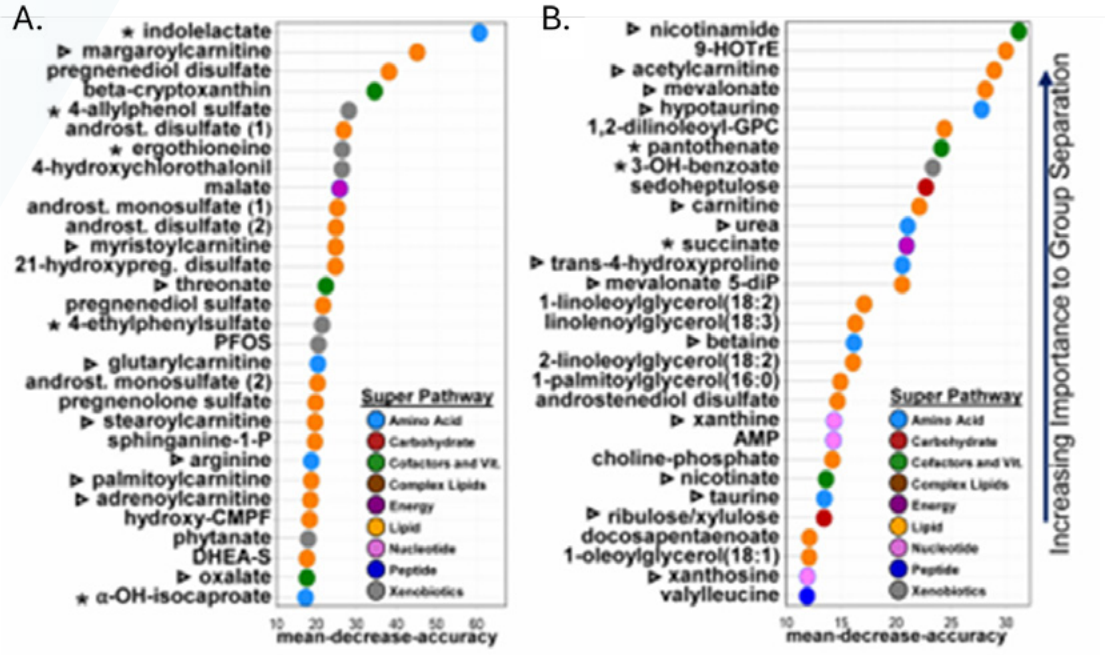


Figure 26. Plasma and Fecal Metabolomes Differ between ASD and neurotypical groups. Top 30 most distinguishing metabolites between each group in (**A**) plasma and (**B**) feces by random forest analysis, with mean decrease accuracy along the x-axis. Image reproduced from Needham et al., *Biol Psychiatry*, 2021, licensed under CC BY 4.0

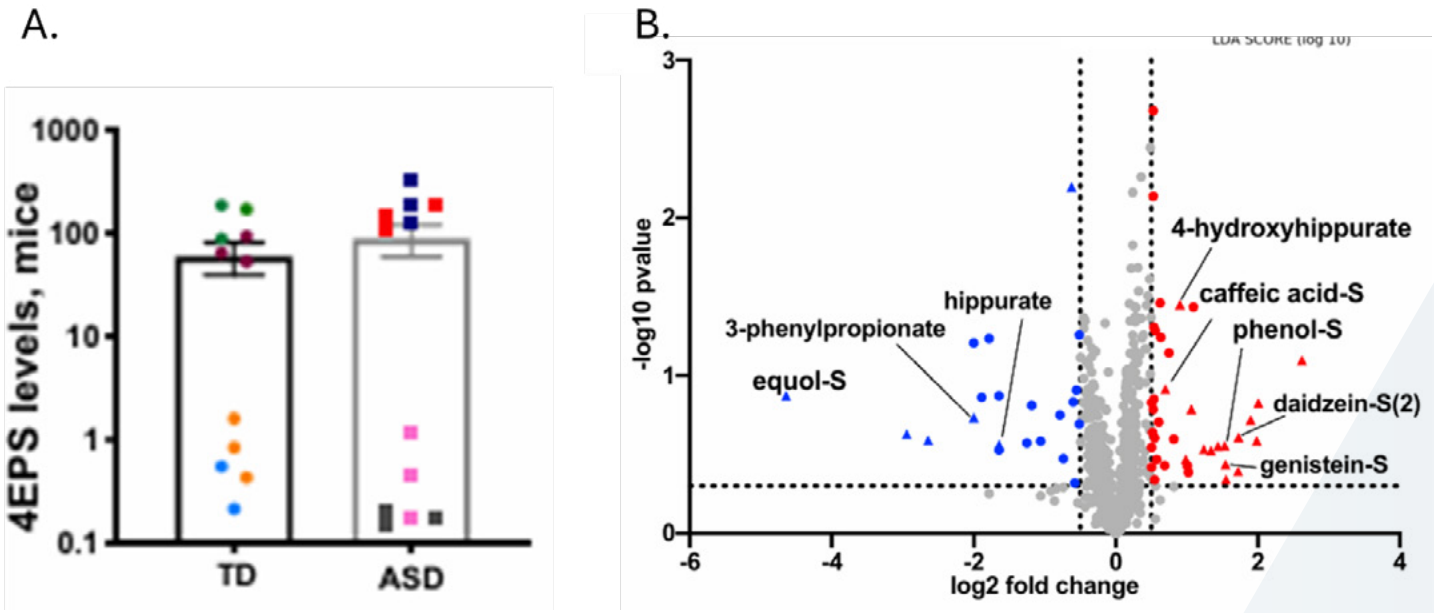


Figure 27. Transfer of human fecal microbiota into mice. (**A**) Scaled intensity values indicating relative levels of 4-ethylphenyl sulfate (4EPS) levels in mice colonized with neurotypical or ASD donors, colored according to donor. (**B**) Scaled intensity values indicating relative levels of 4EPS levels in mice colonized with TD or ASD donors, colored according to donor. Image reproduced from Needham et al., *Biol Psychiatry*, 2021, licensed under CC BY 4.0.

Study Conclusions

- This study revealed diverse metabolic profiles that were linked to gastrointestinal symptoms and behavioral scores, suggesting that ASD cannot be explained by a single mechanism, but rather, it is governed by genetic risk, diet, and the gut microbiome.
- Many altered metabolites were derived from or impacted by gut microbes. Some of those metabolic signatures were shown to be transferable to mice through microbiota transplantation, suggesting that the gut and immune system can impact brain function through circulating metabolites.
- Overall, this study suggests that integrating metabolomics with clinical and environmental data could improve ASD diagnosis and potentially identify novel therapeutic targets.

Case Study 4: Elucidating a Novel Mechanism that Explains why Standard of Care Treatments for Lymphangioleiomyomatosis are Partial and Reversible

Introduction. Lymphangioleiomyomatosis (LAM) is a rare, progressive lung disease that primarily affects women and is characterized by abnormal proliferation of smooth muscle–like cells, leading to cystic lung destruction and respiratory failure. LAM is linked to mutations in the tuberous sclerosis complex genes (TSC1/TSC2), which result in hyperactivation of the mTORC1 signaling pathway, a key regulator of cell growth and metabolism. However, mTORC1 activation alone does not fully explain LAM pathogenesis because clinical benefits from mTOR inhibitors like rapamycin are incomplete and reversible. The striking female predominance of LAM points to a role for estradiol in disease progression.

Preliminary Data and Study Goals. Previous studies have shown that estradiol can enhance tumor growth and metastasis in TSC2-deficient models, suggesting a hormonal contribution to disease biology. Additionally, pathways that involve inflammatory mediators have also been suggested as important mediators of disease biology. Investigators hypothesized that prostaglandin biosynthesis, driven by the COX-2 enzyme, may be a key downstream pathway linking estrogen signaling and TSC2 loss. The goal of this study was to determine how estradiol, TSC2 deficiency, and mTOR signaling interact to regulate COX-2 and prostaglandin production, and whether this pathway could serve as a therapeutic target in LAM [15].

Methods. Mechanistic studies were conducted using TSC2-deficient cell lines and alongside TSC2 “add-back” controls. Cells were treated with estradiol and various pathway inhibitors. Outcomes including COX-2 expression, signaling pathway activation, and prostaglandin production were measured using immunoblotting, ELISA, and RT-PCR. Gene knockdown (e.g., Rictor shRNA) was used to assess the role of mTORC2 signaling. Global metabolomics identified changes in prostaglandin and related lipid metabolites following estradiol treatment. TSC2-deficient and TSC2-restored cells were implanted into Tsc+/- mice to assess tumor growth, prostaglandin production, and treatment responses. Mice were treated with aspirin or celecoxib to evaluate the therapeutic impact of COX-2 inhibition. Lung tissue, serum, urine, and exhaled breath condensate from LAM patients were analyzed to validate findings and assess the relevance of COX-2 activity and prostaglandin signaling in human disease.

Results. Estradiol significantly enhanced prostaglandin production in TSC2-deficient cells, revealing a distinct metabolic signature characterized by increased levels of prostaglandin and related metabolites. In TSC2-deficient

cells MAPK and PI3K–Akt signaling pathways were activated by estradiol, which elevated COX-2 expression to suggest a mechanistic link between estrogen signaling and inflammatory lipid metabolism in LAM. Increased COX-2 expression and prostaglandin biosynthesis occurred independently of mTORC1 signaling. Interestingly, rapamycin inhibited mTORC1 activity but did not reduce COX-2 levels or prostaglandin production while inhibiting mTORC2 significantly reduced COX-2 expression and downstream signaling. In mouse models, pharmacologic inhibition of COX-2 with aspirin or celecoxib reduced prostaglandin levels, suppressed tumor growth, and increased apoptosis in preclinical models (**Figure 28**). In human LAM samples, COX-2 expression and circulating prostaglandins were elevated, and aspirin treatment increased levels of anti-inflammatory lipid mediators, which reduced cell proliferation.

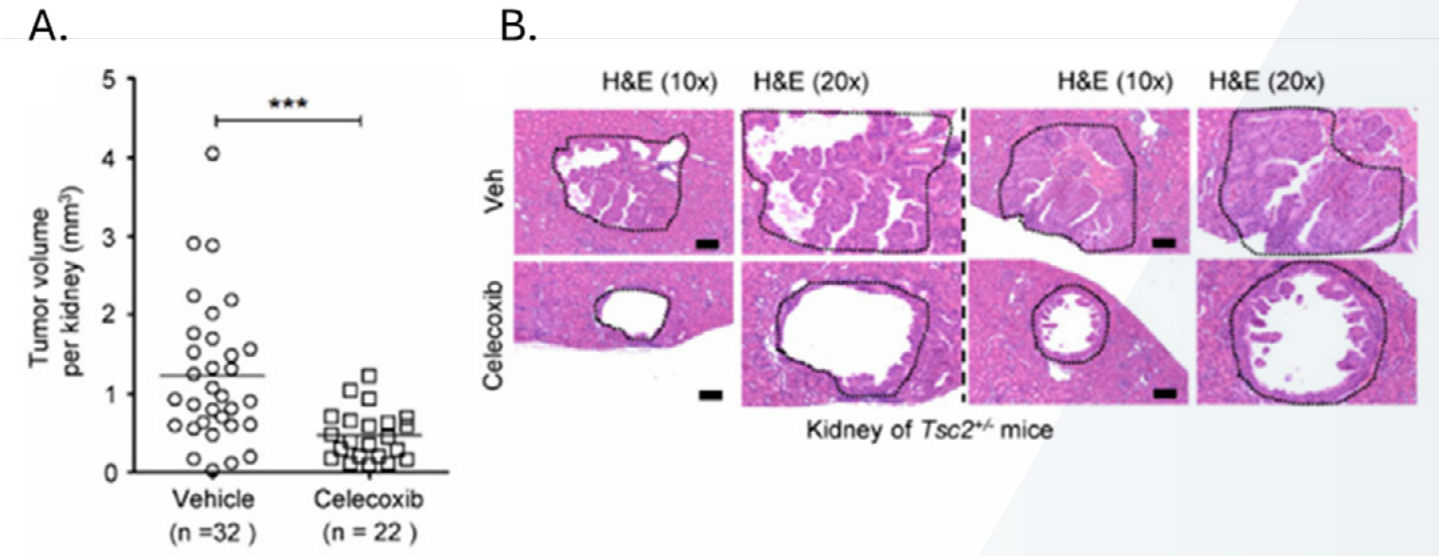
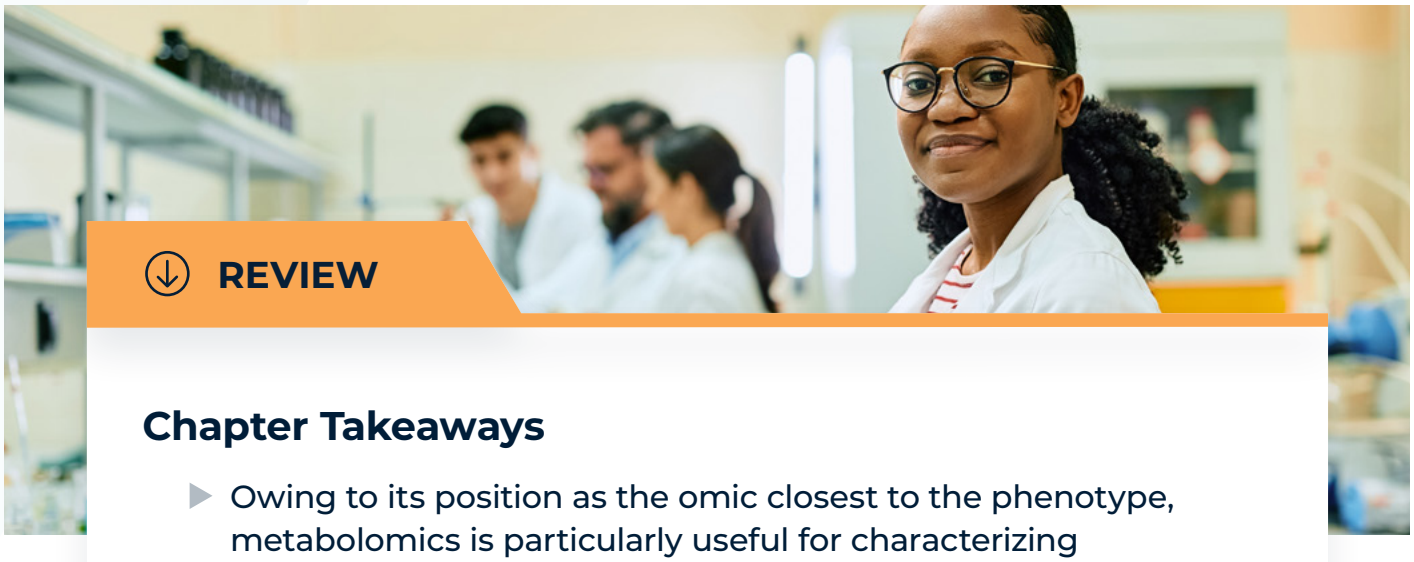


Figure 28. Inhibition of COX-2 suppresses renal tumorigenesis and inhibits the progression of xenograft tumor of TSC2-deficient cells in preclinical models. Tsc2+/2 mice were treated with either vehicle or Celecoxib (0.1% in mouse chow) for one month and then sacrificed for analysis at the end of treatment. Renal cystadenoma histology and microscopic kidney tumor scores were assessed. (**A**) Microscopic kidney tumor scores are plotted on a linear scale (P = 0.0002). Data are analyzed from 16 vehicle and 11 Celecoxib treatment groups. (**B**) Two cystadenomas are shown. Results are representatives of 11 or 16 mice per group. Image reproduced from Li et al., *J Exp Med*, 2014 licensed under CC BY 4.0.

Study Conclusions

- This study identified a novel link between estrogen signaling, TSC2 loss, and prostaglandin metabolism in LAM.
- Therapies that inhibit COX-2 suppressed tumor growth and increased apoptosis in a mouse model of LAM. Reduced cell proliferation was also observed in response to COX-2 suppressing treatments in human cells collected from LAM patients.
- Although LAM was previously thought to be primarily driven by activation of mTORC1, these findings showed that COX-2 mediated prostaglandin production is an mTORC1-independent mechanism that contributes to disease progression, explaining why standard of care treatments such as rapamycin provide only partial clinical benefits.



REVIEW

Chapter Takeaways

- Owing to its position as the omic closest to the phenotype, metabolomics is particularly useful for characterizing biological mechanisms and identifying biomarkers and therapeutic targets in preclinical models that translate remarkably well to humans.
- Applying metabolomics to translational studies may improve the success rate of clinical trials, particularly for conditions for which many therapies have been proposed but eventually failed in clinical studies due to the lack of robust translational models.

Chapter 5

Metabolomics for Basic Science

AT A GLANCE

In this chapter we will address:

- How metabolomics can elucidate mechanisms that are invisible to other omics sciences to transform future therapeutic strategies.
- How metabolomics has revealed key insights that have significantly advanced our understanding and clinical implications of the microbiome-brain axis and population health.
- How metabolomics can generate meaningful data from various sample matrices that are not commonly analyzed.

Overview

Metabolomics has been instrumental in advancing our understanding of basic science principals owing to the deep insight it provides regarding mechanisms that directly impact the phenotype, natural history of disease, and ability to spur discovery and hypothesis-generating findings. In recent years it has also contributed significantly to topics of special interest including microbiome-focused research and population health. Finally, its compatibility with uncommon sample matrices makes it broadly applicable to most areas of the life sciences. Here, we highlight a few studies that show how metabolomics can elucidate mechanisms and be applied to alternative sample types.

MECHANISM

Cardiac Remodeling in Heart Failure

Introduction. Heart failure (HF) is associated with impaired metabolic flexibility, in which the heart cannot augment glucose metabolism in response to stress, resulting in reduced cardiac efficiency and HF progression. In some circumstances, left ventricular (LV) systolic function can be restored if there is surviving myocardium and the underlying cause of HF is successfully treated. Cardiac resynchronization therapy (CRT) is a treatment for HF where a broad left bundle branch block causes dys-synchronous contraction between the septum and lateral wall of the LV. CRT acutely improves cardiac hemodynamics and oxygen efficiency, and further improvement in LV function is seen as remodeling occurs over time. The mechanisms that link CRT to long-term structural cardiac remodeling remain unclear. CRT has been shown to change the circulating metabolome over time, but it is not clear whether this is cause or effect.

Preliminary Data and Study Goals. Ultimately, an improvement in contractile function necessitates greater ATP delivery to the contractile machinery. Therefore, it is plausible that the degree of metabolic flexibility retained by the failing heart may be key to its ability to remodel. This goal of this study was to test this hypothesis by characterizing the degree of left ventricular remodeling in response to 6 months of CRT and determine whether CRT can acutely change substrate uptake away from the metabolic phenotype of HF to ultimately favor remodeling [16].

Methods. This study analyzed patients with HF alongside appropriate comparison groups using clinical phenotyping and global metabolomic profiling. After an overnight fast, patients were stabilized on an insulin/

glucose infusion for at least 1 hour before CRT device implantation. Pressure-volume loop measurements and coronary arteriovenous samples were collected. The insulin/glucose infusion was stopped and an intralipid infusion started for 15 minutes, after which measurements were repeated. Arterial and venus blood samples were analyzed using untargeted metabolomics, and metabolic flexibility was assessed by evaluating uptake of certain metabolites and by calculating myocardial oxygen efficiency. Statistical and pathway analyses were used to identify metabolites associated with impaired metabolic flexibility.

Results. CRT caused a significant uptake in non-esterified fatty acids (NEFA) with no significant uptake in glucose, beta-hydroxybutyrate, or lactate (**Figure 29**). A strong correlation was observed between the acute improvement in cardiac hemodynamic performance induced by QRSd shortening from CRT. There was a strong positive correlation between the increased stroke work and NEFA uptake. The change in substrate uptake in response to CRT correlated with long term reverse remodeling of left ventricular end diastolic volume (**Figure 30**). There was significant correlation between the increase in NEFA uptake and reduction in LVEDV. Lipidomic analysis showed this was driven by increases in both long chain and medium chain fatty acids.

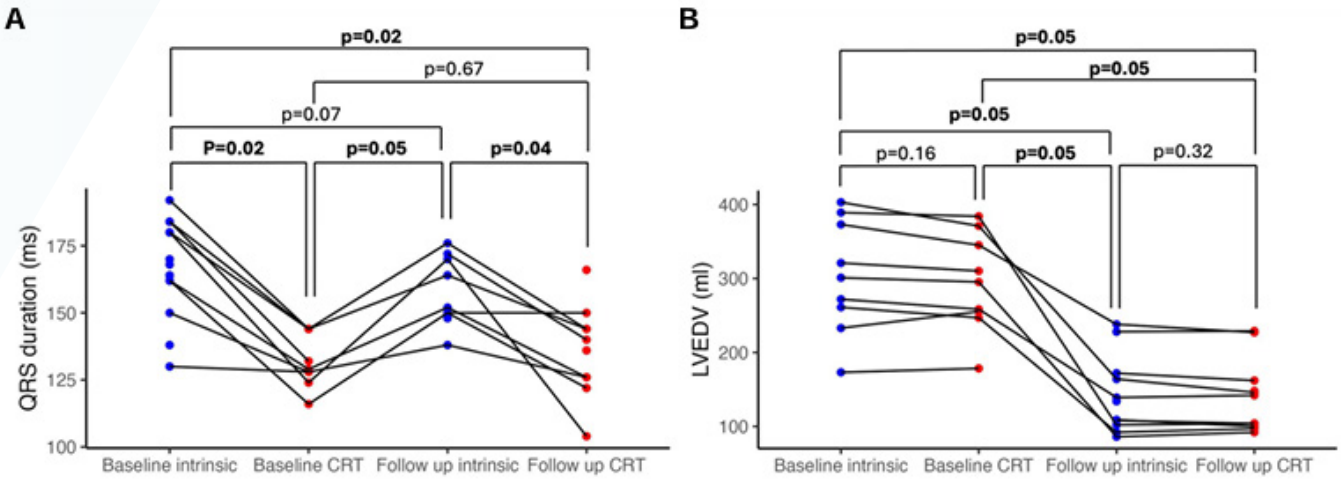


Figure 29. Chronic left ventricular remodeling after 6 months CRT, independent of acute changes in QRS duration. QRS duration (**A, 13 patients**) and left ventricular end diastolic volume (**B, 9 patients**) at baseline implant and 6 month follow up, with intrinsic rhythm and optimized cardiac resynchronization therapy pacing. Image reproduced from Green et al., *Eur Heart J*, 2025, licensed under CC BY 4.0.

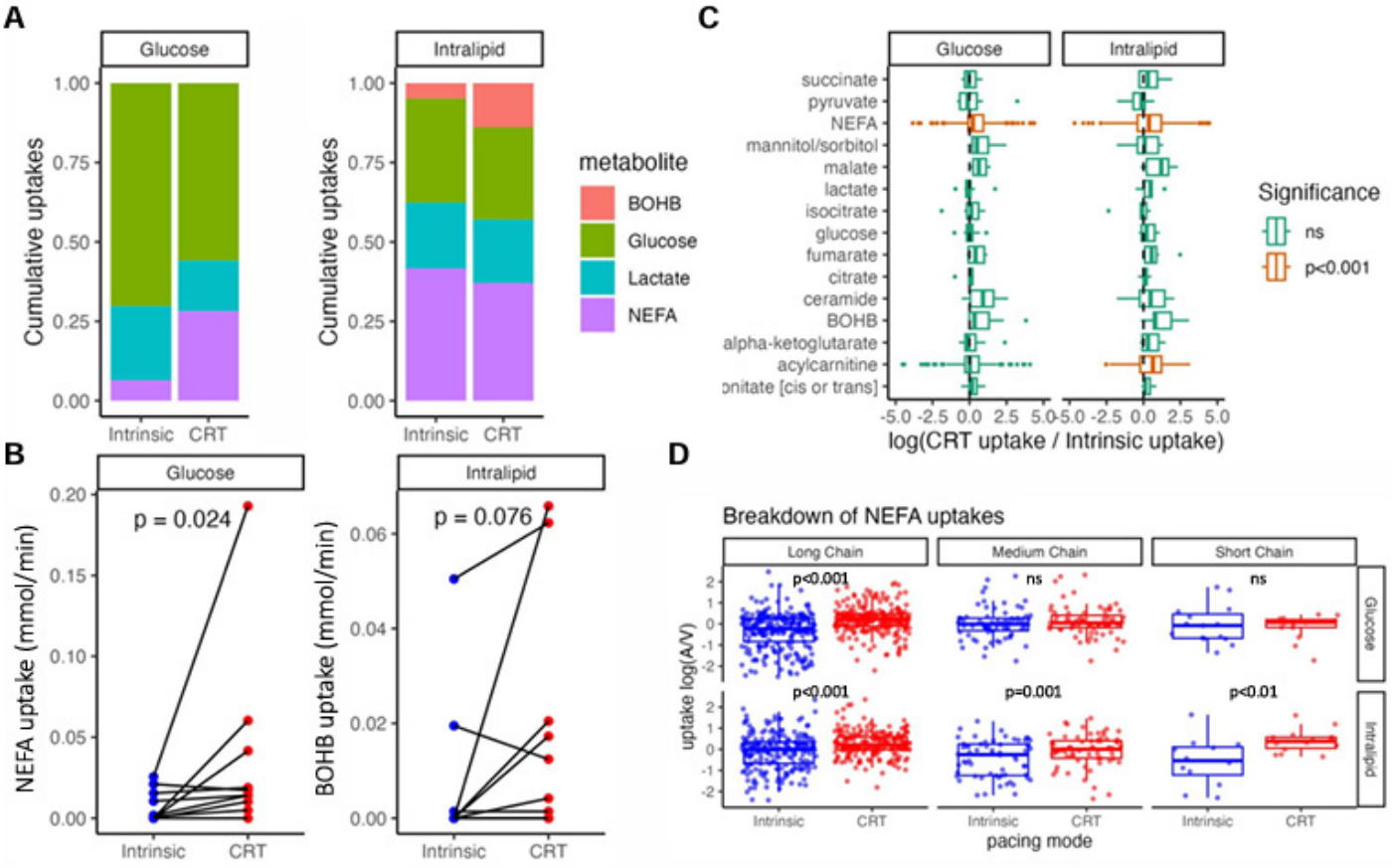
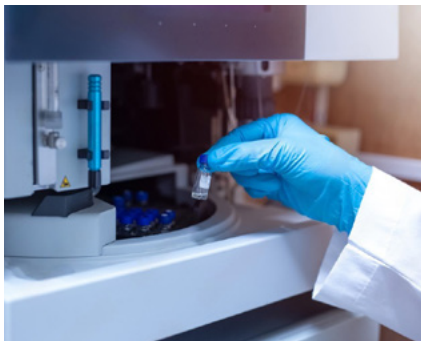


Figure 30. The effect of CRT on cardiac substrate uptake during insulin/glucose intralipid infusions. (**A**) Cumulative proportion of uptake of NEFAs, beta-hydroxybutyrate, lactate, and glucose during intrinsic conduction and optimized CRT while on intralipid and insulin/glucose infusions. (**B**) Illustration of the effect of cardiac resynchronization therapy on the uptake of non-esterified fatty acid and β-hydroxybutyrate while on insulin/glucose and intralipid infusions respectively. (**C**) Metabolomic analysis illustrating the difference in uptake of intermediary metabolites induced by initiating cardiac resynchronization therapy, where positive values indicate increased uptake with cardiac resynchronization therapy. (**D**) Lipidomic analysis illustrating the effect of cardiac resynchronization therapy on the uptakes [defined as log (arterial/venous)] of different chain length non-esterified fatty acid while on insulin/glucose and intralipid infusions. Image reproduced from Li et al., *J Exp Med*, 2014 licensed under CC BY 4.0.

Study Conclusions

- These findings show that in non-ischemic cardiomyopathy the heart retains substantial metabolic flexibility, and CRT is thus able to reverse the metabolic phenotype of HF towards a more physiological phenotype of NEFA uptake.
- The degree of retained metabolic flexibility is correlated with long-term reverse remodeling of left ventricular end diastolic volume.
- In this study, metabolomics revealed that cardiac remodeling in response to CRT may rely on changes in cellular metabolism triggered by CRT rather than acute effects of QRSd narrowing.

Identifying a Novel Therapeutic Target for Diabetic Kidney Disease



Introduction. Diabetic kidney disease (DKD) is a leading cause of chronic kidney disease and kidney failure. Although sodium-glucose cotransporter 2 (SGLT2) inhibitors were originally developed to lower blood glucose by increasing urinary glucose excretion, clinical trials have shown that they also provide strong renal and cardiovascular protection, even in patients without diabetes. However, the biological mechanisms responsible for these protective effects remain unclear, particularly because SGLT2 is expressed only in proximal tubular cells (PTCs) of the kidney, raising the question of how targeting a transporter in a limited cell population can improve overall kidney function.

Preliminary Data and Study Goals. Prior studies suggest that diabetic kidneys exhibit substantial metabolic alterations, including increased glycolysis and mitochondrial dysfunction, which SGLT2 inhibition may normalize. Thus, study investigators hypothesized that loss or inhibition of SGLT2 alters metabolic pathways within kidney cells to produce metabolites that protect the kidney. The goal of this study was to test this hypothesis by using metabolomic, transcriptomic, and epigenetic analyses to identify metabolic changes in PTCs lacking SGLT2 function and determine whether these pathways contribute to kidney protection [17].

Methods. Male wildtype (WT) and SGLT2 loss-of-function "Sweet Pee" (SP) mice were fed either a normal diet or a high-fat diet (HFD) for up to 18 weeks to induce metabolic stress and early diabetic kidney disease. Kidney function, histology, and metabolic parameters were assessed by measuring serum creatinine and urine albumin-to-creatinine ratio, and by immunostaining kidney tissue for markers of injury and fibrosis. Single-cell RNA sequencing (scRNA-seq) was performed in proximal tubular cells, and untargeted metabolomic profiling was performed on kidney cortex and serum. CUT&RUN epigenetic profiling was performed to measure histone methylation changes associated with altered metabolite levels.

Results. In mice fed a high-fat diet, those lacking SGLT2 function showed less kidney damage, inflammation, fibrosis, and proteinuria than wild type mice, despite similar metabolic stress. Single-cell RNA sequencing identified a population of injured proximal tubular cells that emerged in wild type kidneys under high-fat diet conditions but was largely suppressed in the absence of SGLT2 function. Metabolomic analysis revealed that kidneys lacking SGLT2 had increased activity of methionine metabolism, particularly higher levels of the metabolite S-adenosylmethionine (SAM) (**Figure 31**). Functional experiments showed that blocking the SAM-producing enzyme MAT2A eliminated the protective effects of SGLT2 loss, while SAM supplementation reduced inflammatory responses in cultured kidney cells

exposed to metabolic stress (**Figure 32**). Pharmacologic SGLT2 inhibition produced similar protective effects as genetic SGLT2 loss. Finally, epigenetic analyses showed that elevated SAM increased repressive histone methylation (H3K27me3) at promoters of inflammatory genes, including those in the NF-κB pathway, leading to reduced inflammatory signaling. Together, these results indicate that SGLT2 inhibition promotes kidney protection by increasing SAM-mediated epigenetic repression of inflammatory pathways during metabolic stress.

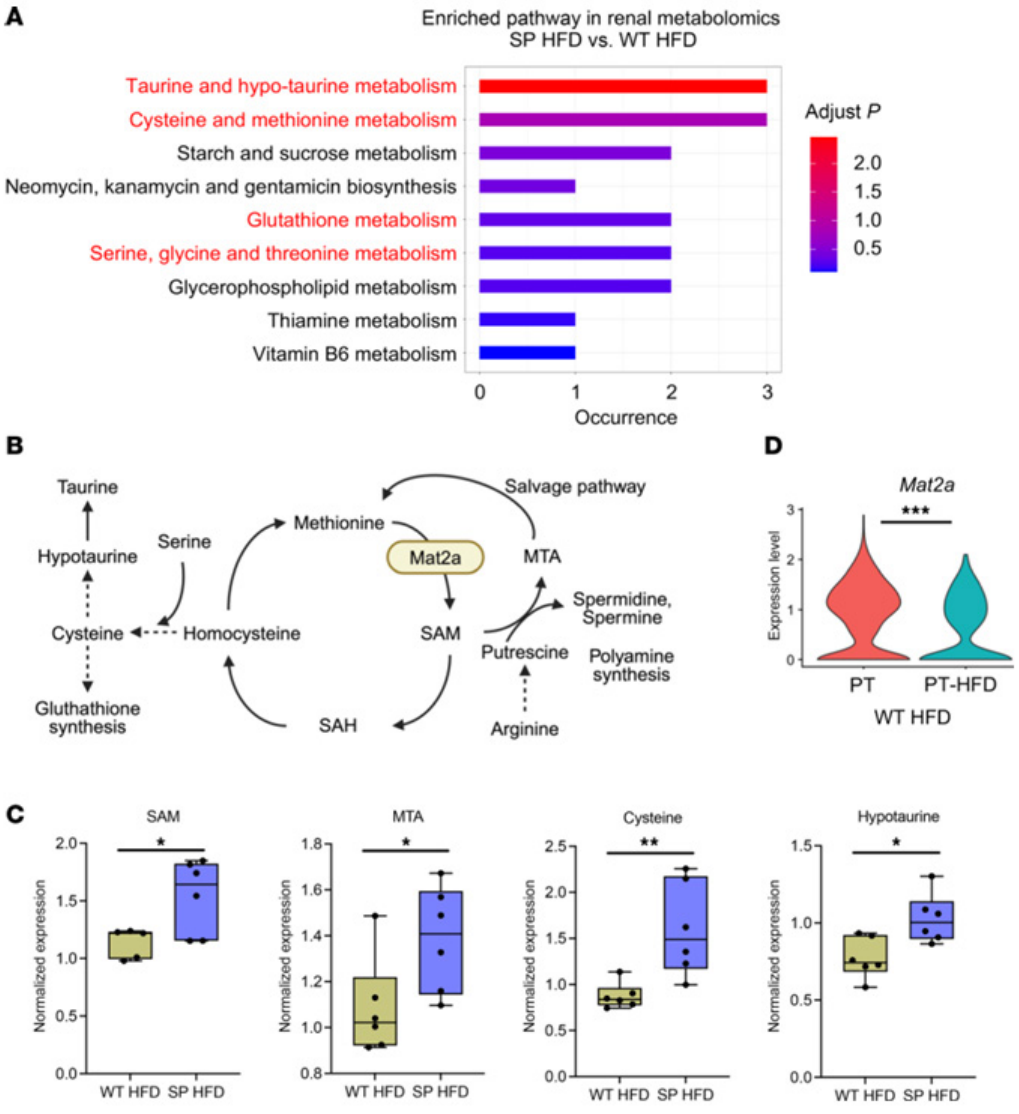


Figure 31. Metabolic profiles in the kidney. (**A**) Metabolic enrichment pathway analysis in tissue isolated from the renal cortex. Color indicates adjusted P value. (**B**) Methionine metabolism and network pathways. (**C**) Relative expression of SAM, MTA, and metabolites related to cysteine and taurine metabolism in renal cortex (n=6 per group). Boxes show the 25th to 75th percentiles, center lines indicate medians, whiskers extend to min and max, and all data points are shown. (**D**) Violin plot showing proximal tubule expression of Mat2a in normal proximal tubule cells (PT) and the subpopulation of PTC (PT-HFD) that emerged in wild-type mice fed a high-fat diet (WT-HFD). Image reproduced from Maekawa et al., *J Clin Invest*, 2025, licensed under CC BY 4.0.

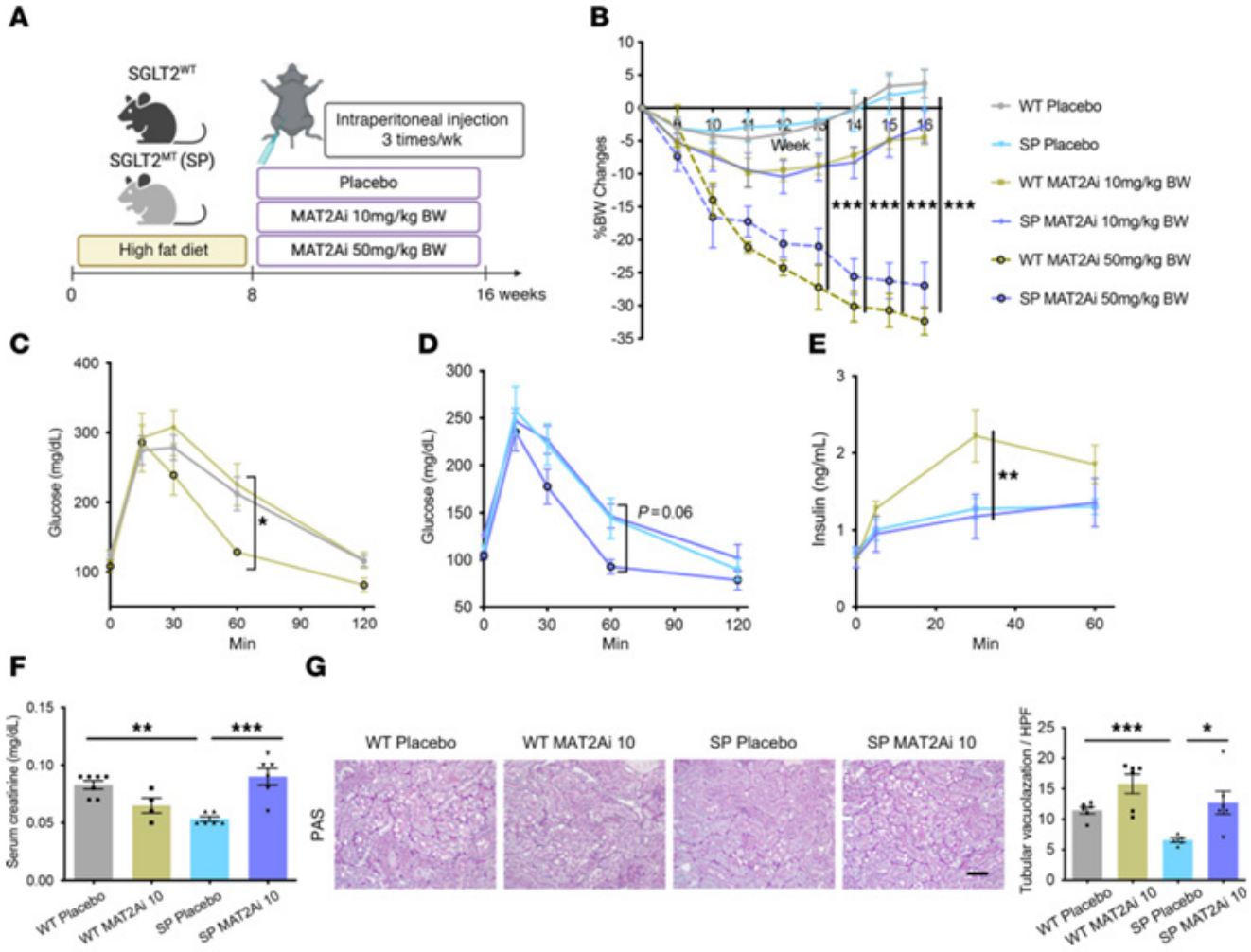


Figure 32. Inhibition of methionine enzyme, MAT2A, abrogates kidney protection in SPHFD mice. (**A**) Schematic protocol. (**B**) Chronological changes in percentage of BW change. (**C–E**) Low-dose MAT2Ai does not alter glucose tolerance and insulin secretion capacity, but high dose MAT2Ai does lower them. (**F**) Serum creatinine level. (**G**) Representative images of PAS staining. Image reproduced from Maekawa et al., *J Clin Invest*, 2025, licensed under CC BY 4.0.

Study Conclusions

- This study provides evidence that metabolites play an active regulatory role in progression of chronic kidney disease and suggest that targeting methionine metabolism or SAM-related pathways could be a viable therapeutic strategy for patients who cannot tolerate SGLT2 inhibitors.
- Metabolomics was crucial in characterizing the relationship between SGLT2 inhibition, kidney metabolism, and the inflammatory response in mice, which can serve as a springboard for future human studies.

Identifying a Potentially Preventative Treatment for an Aggressive Malignancy

Introduction. Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer death worldwide and one of the few cancers that is becoming more prevalent globally. Currently, the five-year survival rate sits at 13%, largely due to most diagnoses occurring at a later stage after the tumor has metastasized and resection is no longer an option. More than 90% of PDAC tumors contain activating KRAS mutations, making KRAS a central driver of tumor growth and an attractive therapeutic target. However, clinical responses to KRAS inhibitors and drugs targeting the KRAS signaling pathway (such as RAF/MEK/ERK inhibitors) have been limited, with only partial responses and rapid development of drug resistance. Most current combination strategies focus on components within the canonical KRAS signaling pathway, but cancer cells can bypass these targets through compensatory mechanisms, highlighting the need to identify new vulnerabilities outside the traditional KRAS pathway.

Preliminary Data and Study Goals. To address this unmet need, study investigators sought to identify genes that are essential for KRAS-mutant PDAC cell survival with the goal of discovering novel therapeutic targets and combination strategies that could potentially improve the effectiveness of KRAS inhibitors in pancreatic cancer [18].

Methods. Genome-wide CRISPR/Cas9 knockout screens, RNA interference datasets, and large cancer dependency databases were used to identify genes that are selectively essential for KRAS-mutant PDAC cells. Drug sensitivity screens were performed to identify compounds that preferentially inhibit KRAS-mutant cells. Candidate genes were validated using gene knockdown or knockout approaches and cell viability assays in PDAC cell lines. RNA sequencing, metabolomics, proteomics, and stable isotope tracing were used to analyze changes in nucleotide metabolism and cellular pathways after gene inhibition or KRAS blockade. The functional importance of targets was further tested using genetically engineered mouse models and orthotopic tumor transplantation models. Proteolysis-targeting chimera (PROTAC) compounds that selectively degrade IMPDH2 were used to test their therapeutic potential in patient-derived organoids and xenograft tumor models.

Results. De novo guanine nucleotide biosynthesis (DNGB) was identified as a key metabolic vulnerability in KRAS-mutant PDAC cells with the critical gene being *IMPDH2*, which encodes an enzyme critical to DNGB. Pharmacological suppression of IMPDH2 or other DNGB enzyme activity significantly reduced PDAC cell growth. In mouse models, genetic deletion of *Impdh2* reduced pancreatic tumor initiation, slowed disease progression, and improved

survival (**Figure 33**). Metabolomic analyses showed that mutant KRAS stimulates purine biosynthesis to preferentially increase production of adenine nucleotides over guanine nucleotides, creating a metabolic "bottleneck" for guanine synthesis (**Figure 34**). As a result, PDAC cells become dependent on IMPDH2 to maintain guanine nucleotide levels and continue proliferating. Inhibiting IMPDH2 depleted guanine nucleotides, which impaired PDAC cell growth. Importantly, IMPDH2 expression was shown to not be regulated by the canonical KRAS signaling pathway, meaning that cancer cells could not compensate for IMPDH2 inhibition through KRAS signaling. A selective IMPDH2-degrading compound (MED-B-4) suppressed growth of PDAC cell lines with greater potency than existing IMPDH inhibitors.

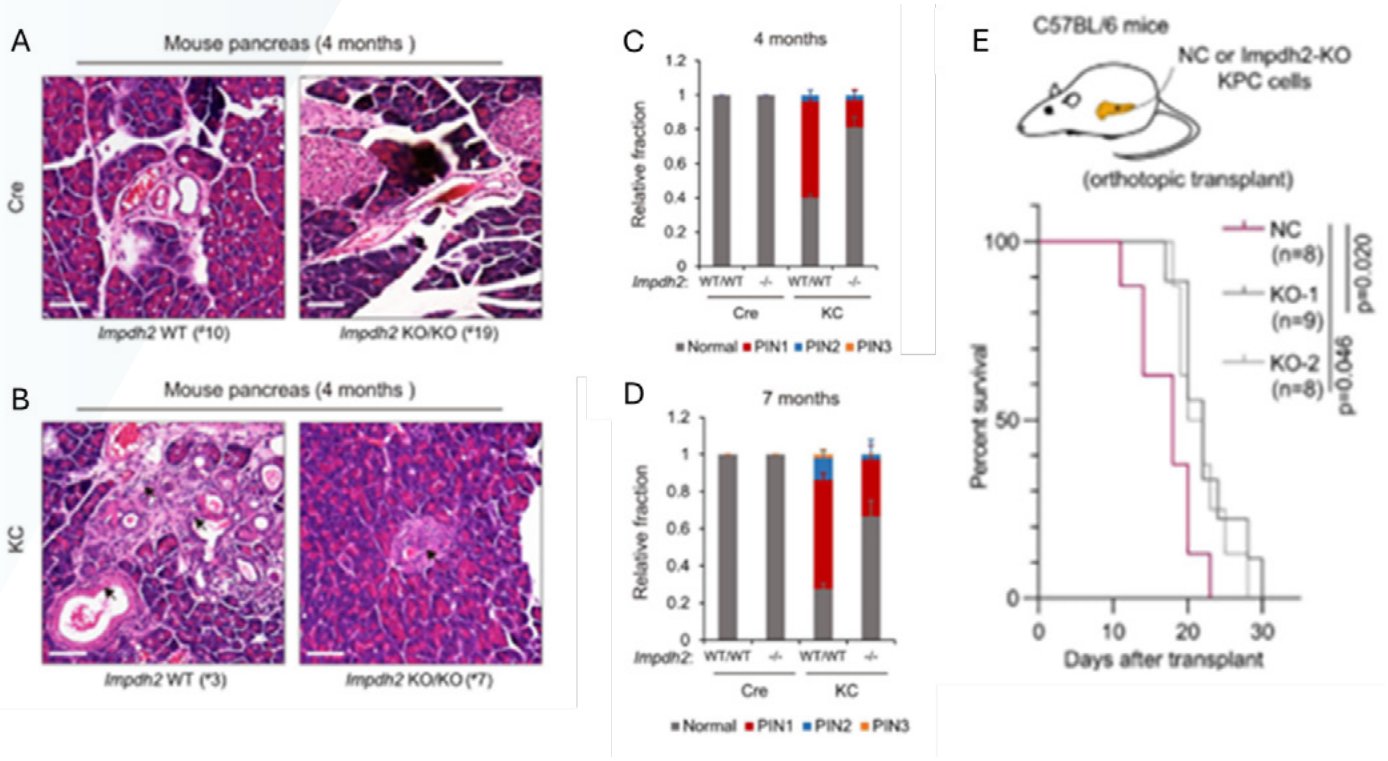


Figure 33. DNGB inhibition or conditioned Impdh2 knockout inhibits pancreatic carcinogenesis and cancer progression in vivo. Representative images of H&E staining in pancreatic tissue specimens from control and (Cre; Impdh2-/-) mice (**A**), or from KC and (KC; Impdh2-/-) mice (**B**). Frequency of pancreatic intraepithelial neoplasia (PanIN or PIN) lesion stages (PIN 1, PIN 2 and PIN 3) in different genotype mice 4 months of age (**C**) or 7 months of age (**D**). Overall survival plot of the mice orthotopically transplanted with negative control KPC cells, a transformed PDAC cell line, or *Impdh2* knockout KPC cells (**E**). Image reproduced from Wu et al., *Gut*, 2026, licensed under CC BY 4.0.

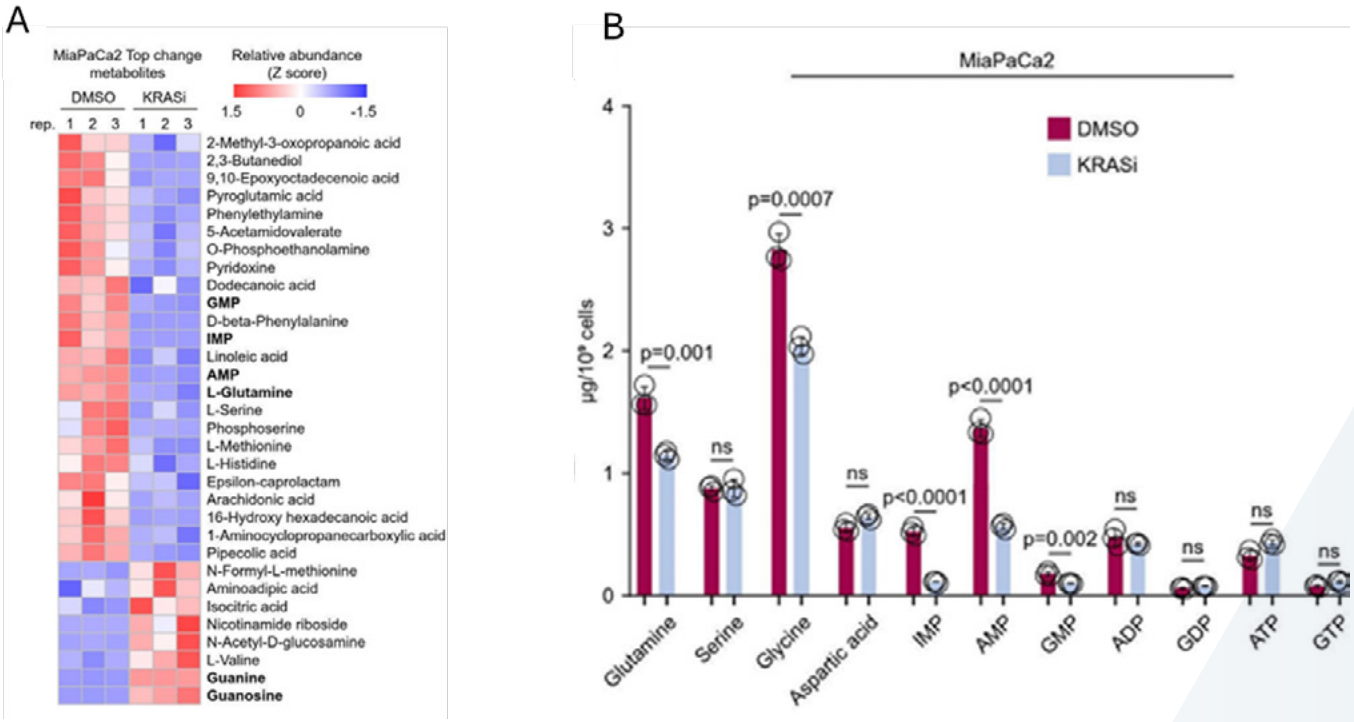


Figure 34. Mutant KRAS stimulates de novo biosynthesis of adenine more than guanine nucleotides. (**A**) Heat map showing the metabolites which are significantly and consistently changed on acute inhibition of mutant KRAS(G12C) in the transformed PDAC cell line, MiaPaCa2 cells as determined by untargeted metabolomics assays. (**B**) Quantification of amino acids and purine nucleotides using targeted LC-MS/MS In the control and KRAS inhibition cells (control: DMSO for 120 min; KRAS inhibition: 100 nM AMG510 for 120 min). Image reproduced from Wu et al., *Gut*, 2026, licensed under CC BY 4.0.

Study Conclusions

- Prior to this study de novo guanine nucleotide biosynthesis was an underappreciated metabolic vulnerability in KRAS-mutant PDAC. Metabolomics played an important role in showing that KRAS uniquely enhances adenine nucleotide production to create a shortage of guanine nucleotides, making PDAC cells dependent on IMPDH2, the key enzyme responsible for converting inosine monophosphate to guanine nucleotides.
- Importantly, since IMPDH2 is not regulated by the canonical KRAS signaling pathway, PDAC cells cannot compensate for its inhibition through typical KRAS pathway feedback mechanisms. This led to the discovery that blocking IMPDH2 causes irreversible guanine nucleotide depletion, which disrupts DNA/RNA synthesis and suppresses tumor growth.
- These findings have implications for future therapies, as they suggest that selective degradation of IMPDH, rather than broad inhibition, could provide a more precise strategy for PDAC treatment.

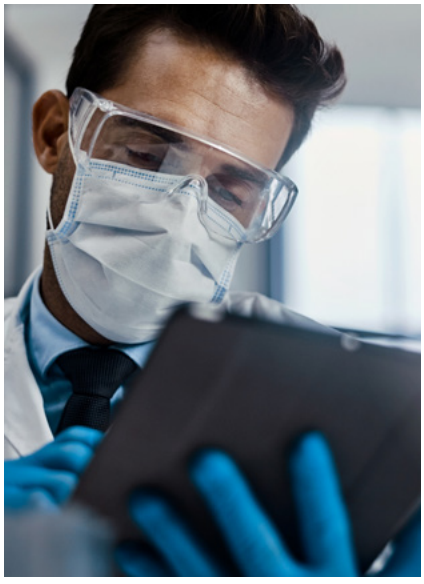
MICROBIOME

Elucidating Mechanisms that Link the Microbiome to Multiple Sclerosis

Introduction. Microbial metabolites have been shown to influence immune regulation, inflammation, and neurological function, suggesting they may contribute to disease activity or progression. Various studies have identified abnormalities in the gut microbiomes of patients with multiple sclerosis (MS), but a role for intestinal microbes and their metabolites in MS pathology has yet to be established.

Preliminary Data and Study Goals. The goal of this study was to determine whether specific gut microbes and circulating stool metabolites are associated with worsening MS or the transition to progressive disease by analyzing longitudinal clinical, microbiome, and metabolomics from participants in a well-characterized MS cohort [19].

Methods. This study analyzed participants from the Comprehensive Longitudinal Investigation of Multiple Sclerosis (CLIMB) cohort, and associated clinical data, MRI imaging, and banked plasma and feces. Investigators followed patients for approximately two years and categorized them into groups defined by: 1) stable disease, 2) worsening disability, and 3) having transitioned from relapsing-remitting MS to progressive MS. Shotgun metagenomic sequencing was performed on stool samples to characterize gut microbiota. Both stool and serum samples were analyzed using global metabolomics profiling to measure microbial and host-derived metabolites. Statistical models were used to identify associations between microbial taxa, metabolite levels, and changes in clinical outcomes including disability progression, MRI findings, and quality of life measures.



Results. Significant associations between the composition of gut microbiota, metabolite profiles, and MS disease progression were identified. Individuals who experienced worsening disease or a transition to progressive MS showed reduced levels of several beneficial microbial taxa, including species known to produce short-chain fatty acids and other anti-inflammatory metabolites (**Figure 35**). By contrast, higher levels of metabolites produced by these beneficial microbes were associated with stable disease. Microbial features and metabolite levels were associated with disability score, MRI indicators of disease activity, and patient-reported outcomes, indicating that microbiome-derived metabolic changes may play a direct role in MS disease progression (**Figure 36**).

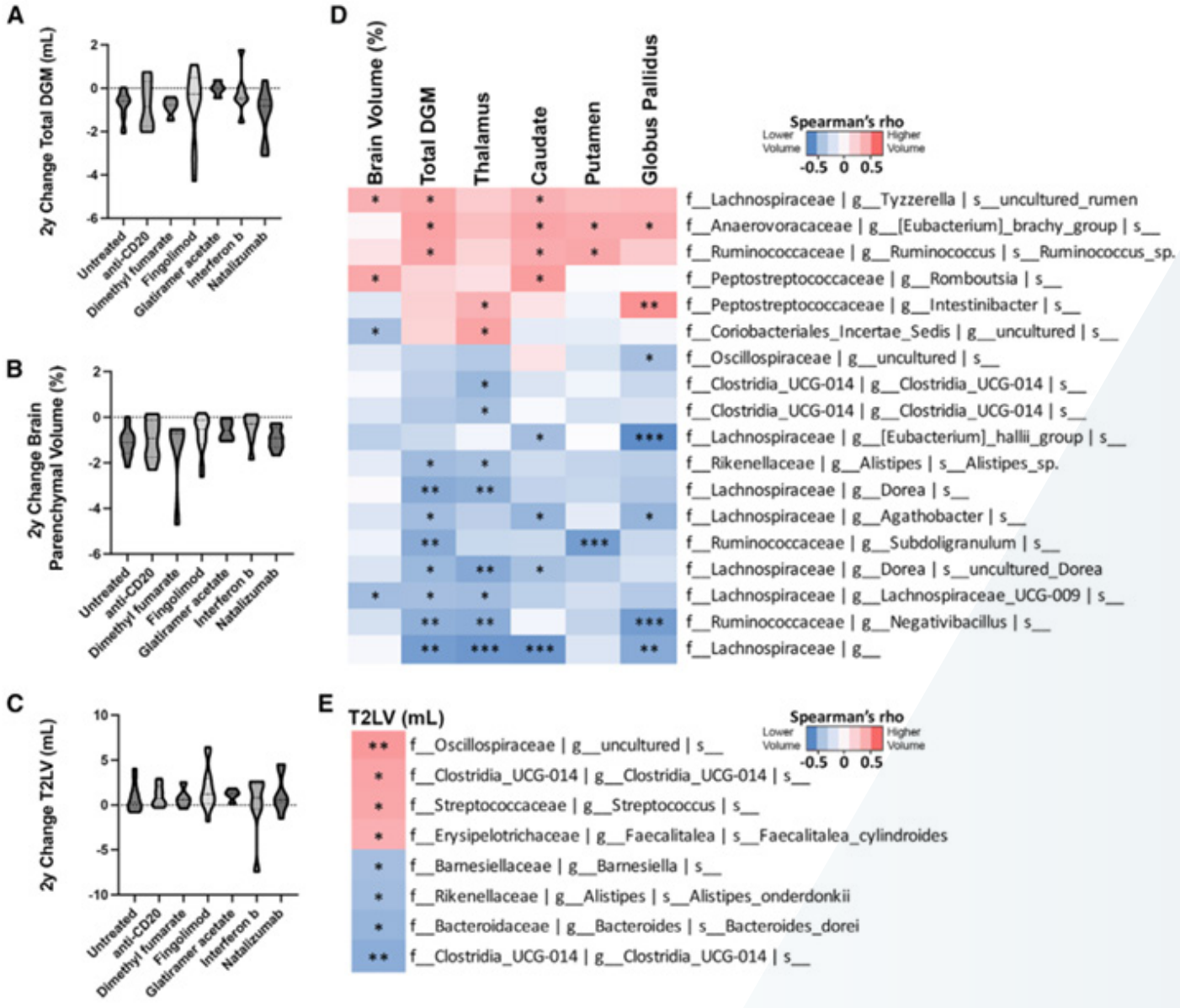


Figure 35. Gut microbes correlate with 2-year changes in 3T MRI. Changes in DGM (**A**), whole brain parenchymal volume (**B**), and T2LV stratified by DMT (**C**). Spearman's correlations demonstrate microbial taxa significantly positively (red) or negatively (blue) correlated with 2-year changes in 3T volumetric measures (**D**). Spearman's correlation demonstrates taxa positively (red) or negatively (blue) correlated with T2LV (**E**). Image reproduced from Schwerdtfeger et al., *Ce ll Rep Med*, 2025, licensed under CC BY 4.0.

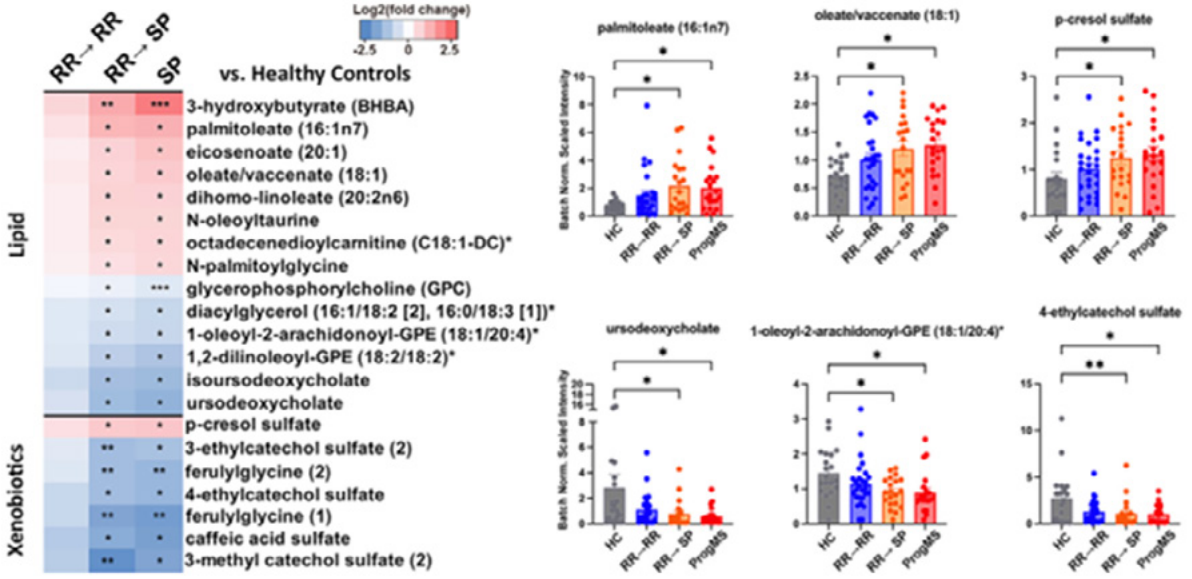


Figure 36. Serum metabolites linked to development of progressive MS. (**A**) All serum metabolites that were significantly altered in patients who transitioned from relapse-remitting (RR) disease to stable progressive (SP) disease. (**B**) Bar graphs with individual values of biologically relevant metabolic alterations by disease status. Image reproduced from Schwerdtfeger et al., *Cell Rep Med*, 2025, licensed under CC BY 4.0.

Study Conclusions

- The findings of this study support the idea that the gut microbiome may influence MS through metabolic signaling pathways and immune modulation, and the microbial taxa and metabolites identified may serve as biomarkers of disease progression or novel therapeutic targets that could be modulated by diet.
- Without metabolomics, investigators would have only been able to observe shifts in microbial species and would not have learned which metabolic pathways were disrupted, which bioactive molecules might influence immune signaling and neuroinflammation, or which molecules could be further investigated as potential therapeutic targets.

Understanding the Microbiome's Influence on Type 2 Diabetes Pathophysiology

Introduction. Type 2 diabetes (T2D) is a complex metabolic disease influenced by genetic, environmental, and microbial factors. Increasing evidence suggests that the gut microbiome contributes to metabolic health by producing bioactive metabolites that affect host physiology, including glucose metabolism and inflammation. Although microbiome sequencing studies have identified microbial taxa associated with T2D, they do not fully explain the functional biochemical mechanisms linking gut microbes to metabolic disease.

Preliminary Data and Study Goals. Given that microbial metabolites act as signaling molecules that influence host metabolism, investigators attempted to characterize how microbial metabolic activity contributes to T2D risk and progression by performing metagenomic sequencing and global metabolomics analyses [20].

Methods. This study analyzed cohorts with known diabetes status and metabolic phenotyping data. Metagenomic sequencing was used to characterize the composition and abundance of the gut microbiome. Plasma and stool were analyzed by global metabolomics profiling to measure a broad range of host- and microbe-derived metabolites. Statistical analyses were used to identify metabolite and microbial taxa associated with T2D and related metabolic traits including insulin resistance and glycemic control. Integrative analyses were used to identify connections between specific microbes and metabolites to determine how microbial metabolic pathways influence host metabolic phenotypes.

Results. Individuals with T2D had distinct microbiome and metabolite profiles compared to healthy individuals. Several microbial taxa were associated with altered concentrations of metabolites involved in amino acid metabolism, lipid metabolism, and microbial fermentation pathways. Metabolomics revealed specific microbial-derived metabolites that were significantly correlated with measures of insulin resistance and glucose regulation (**Figure 37**). Interestingly, metabolites associated with T2D were shown to reverse in response to diet or exercise intervention (**Figure 38**). Some metabolites showed stronger associations with T2D risk than the microbial taxa themselves, suggesting that microbial metabolic activity rather than simple microbial abundance may be a key driver of disease-related metabolic changes.

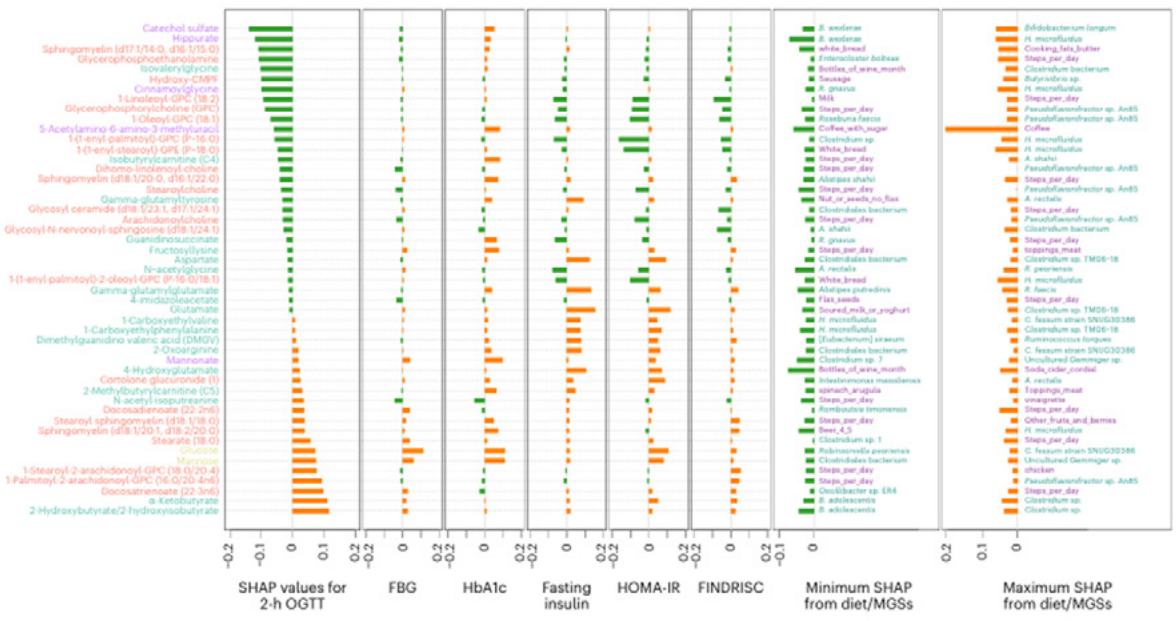


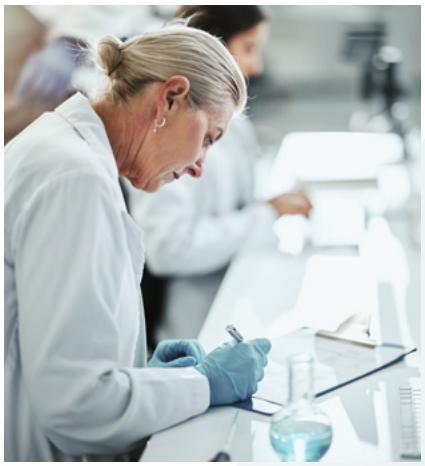
Figure 37. Gut microbial features explaining glucose intolerance. Top ten metabolites identified as important features in the 2-hour oral glucose tolerance test (OGTT), fasting blood glucose (FBG), AbA1c, fasting insulin, homeostatic model assessment of insulin resistance (HOMA-IR) or Finnish Diabetes Risk Score (FINDRISC) (n=49). Image reproduced from Wu et al., *Nat Med*, 2025, licensed under CC BY 4.0.

Figure 38. Responses of prediabetes and T2D-associated metabolites to a 2-week diet intervention or before and after exercise. Heatmap showing the overlapping metabolites involved in amino acid, lipid and xenobiotic metabolism (n=123) in two clinical trials of either diet (14 days) or exercise for 1-h (before, 120 and 180 min after exercise) interventions with those 502 altered metabolites in prediabetes and T2D. Responses reversed (Y, yes; N, no) by either diet (D) or exercise (E) or both (B) were clustered and are shown in distinct colors beside the row clustering branches. Representative metabolites are labeled in red, and five others in black. Image reproduced from Wu et al., *Nat Med*, 2025, licensed under CC BY 4.0.

Study Conclusions

- By directly measuring hundreds of host- and microbial-derived metabolites, metabolomics identified specific metabolic compounds linked to insulin resistance and glucose regulation, demonstrating that microbial metabolic activity, rather than composition, plays a key role in T2D progression.
- In this study, metabolomics provided the critical functional evidence that connected gut microbial metabolism to T2D pathophysiology.

An Understudied Link Between Symptoms of Irritable Bowel Syndrome and Cognition



Introduction. Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by recurrent abdominal pain and altered bowel habits. Brain-gut-microbiome interactions are becoming recognized as important regulators of gastrointestinal function, symptom perception, and mood, which have marked them as targets for therapeutic intervention in IBS. Cognitive behavioral therapy (CBT) is an effective brain-targeted intervention that teaches information processing skills to address psychological factors known to exacerbate abdominal symptoms including maladaptive coping, intense worry, and stress reactivity.

Preliminary Data and Study Goals. One research group previously showed in a large randomized clinical trial that two CBT programs tailored for IBS were effective in producing sustained gastrointestinal symptom improvement compared to an IBS education program that controlled for nonspecific effects from undergoing treatment. Based on these findings the study team theorized that CBT alleviates symptoms by modulating primarily the brain component of the brain-gut-microbiome (BGM) axis and that microbial signals to the brain in the form of neuroactive metabolites including short-chain fatty acids and serotonin could modulate responsiveness to the biological effects of CBT. Thus, the goal of this study was to determine whether metabolic signatures in IBS patients are associated with cognitive performance and symptoms related to the BGM [21].

Methods. Eligible patients were randomized to receive 10 sessions of clinic-based CBT or 4 sessions of largely home-based CBT with minimal therapist contact over a 10-week acute phase. All participants underwent baseline and post-treatment MRI imaging sessions. Clinical questionnaires were used to assess the severity of gastrointestinal symptoms. Stool samples were analyzed using 16S rRNA gene sequencing and untargeted metabolomics. Statistical analyses identified metabolites associated with IBS status, cognitive performance, and symptom severity.

Results. Of the 84 IBS participants who underwent neuroimaging, 58 were classified as CBT responders while 26 were classified as non-responders based on 50-point or greater decrease on the IBS Symptom Severity Scale post-treatment. Microbial beta diversity was significantly different between responders and non-responders, and the abundance of the microbiome-derived neurotransmitter serotonin was significantly increased in responders. Following CBT, responders showed a decrease in connectivity between multiple regions associated with specific brain networks related to emotional regulation (**Figure 39**). Notably, these changes were accompanied by a significant decrease in both abdominal pain and perceived stress.

Amongst patients who responded to CBT, *Bacteroides* were higher at baseline compared to non-responders (**Figure 40**). A random forest classifier containing 11 bacterial genera was used to predict CBT response. This classifier was able to accurately predict CBT response, demonstrating that the microbiome can serve as an effective biomarker for evaluating a patient's potential response to treatment and prioritizing likely positive responders for CBT.

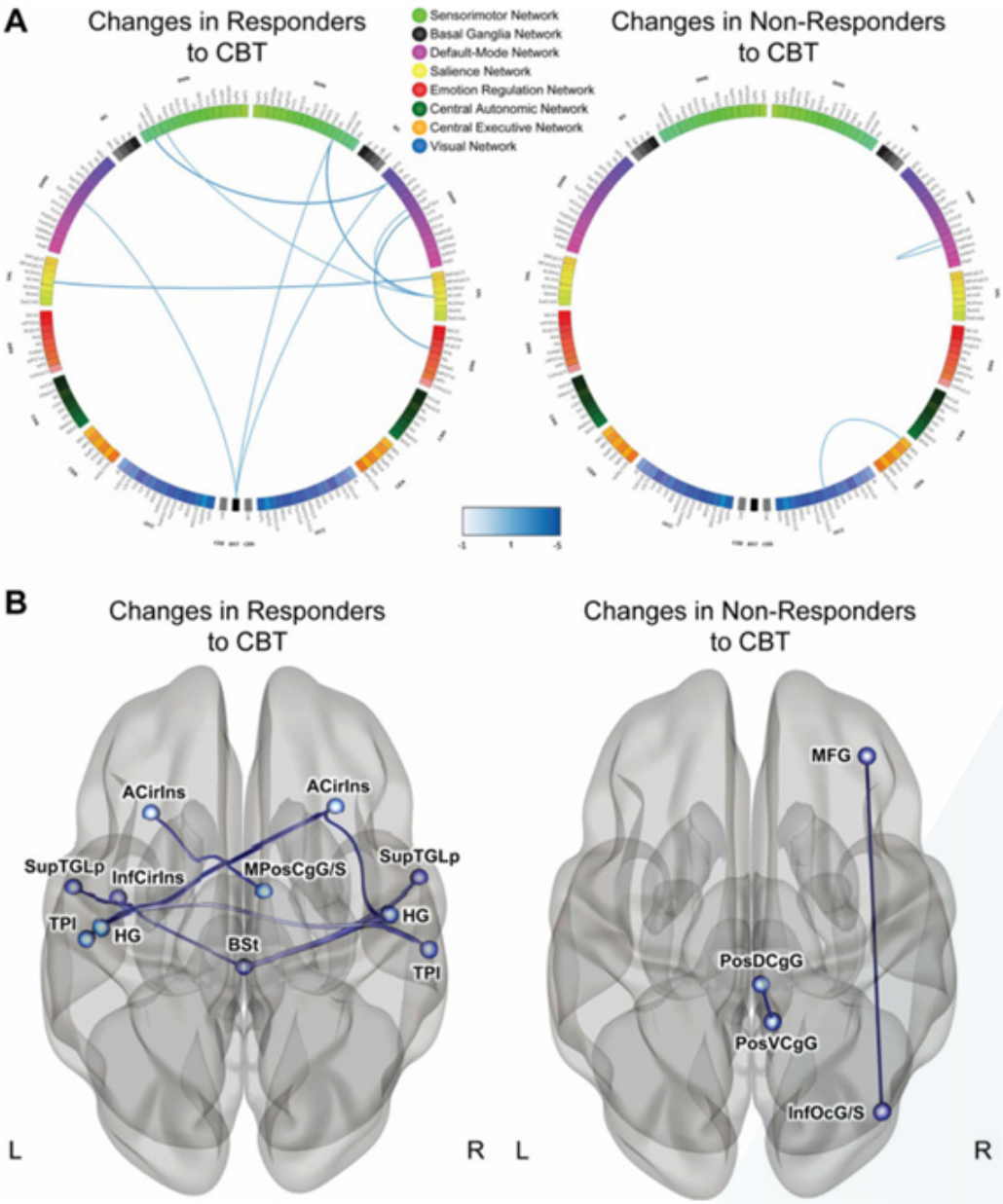


Figure 39. Changes in functional connectivity in responders and non-responders to CBT. (**A**) Connectograms showing pair-wise connectivity differences between groups. Blue lines indicate significant decreases in connectivity. (**B**) Regions that significantly differed between responders and non-responders to CBT. Image reproduced from Jacobs et al., *Microbiome*, 2021, licensed under CC BY 4.0.

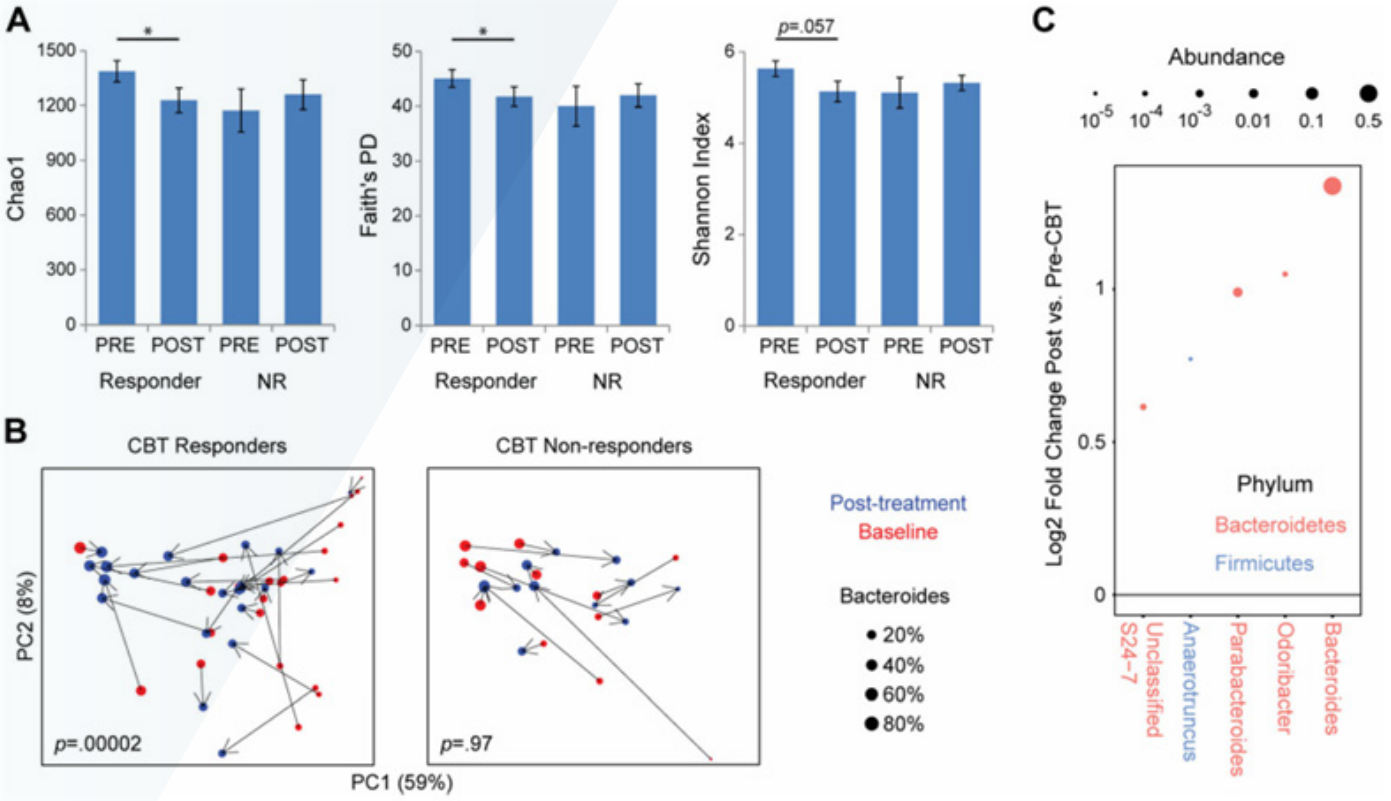


Figure 40. CBT responders have altered intestinal microbiome composition after CBT characterized by *Bacteroides* expansion. (**A**) Fecal microbial alpha diversity is shown for CBT responders and non-responders (NR) at baseline (PRE) after CBT (POST). (**B**) Principal coordinates analysis of 16S rRNA sequence data before and after CBT, stratified by CBT response status. Each dot represents a sample, colored by time point (red= baseline, blue= post-CBT) and sized by Bacteroides abundance. (**C**) Microbial genera with statistically significant association with CBT responder status are shown. Image reproduced from Jacobs et al., *Microbiome*, 2021, licensed under CC BY 4.0.

Study Conclusions

- These findings suggest a microbiome-based mechanism by which IBS patients experience pain in the absence of pathology and provide novel avenues for components of the BGM axis as biomarkers for IBS treatment.
- Metabolomics was essential for this study because it enabled direct measurement of gut-brain signaling molecules that link gastrointestinal dysfunction to cognitive symptoms in IBS, helping to unravel the complex connection between the microbiome and human health.

POPULATION HEALTH

Finding Novel Associations Between Biochemical Pathways and Noncommunicable Diseases to Explain Multimorbidity

Introduction. Multimorbidity, the co-occurrence of multiple chronic diseases, is a substantial global health burden that is becoming more prevalent. Before this problem can be adequately addressed, we must gain a better understanding of disease risk factors and mechanisms of disease progression. Although many chronic conditions share risk factors and biological mechanisms, the majority of studies are limited to single diseases. Molecular profiling could identify common biological pathways that predispose individuals to multiple diseases. To this end, investigators performed untargeted metabolomic profiling on plasma samples collected from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort [22].

Preliminary Data and Study Goals. The goal of this study was to identify shared metabolic pathways, determine which associations are driven by modifiable risk factors, and highlight metabolites linked to the development of multiple chronic conditions.

Methods. Plasma samples from 11,966 individuals from the EPIC-Norfolk cohort were profiled using untargeted metabolomics. Incident diseases and mortality were identified through long-term follow-up using linked electronic health records, hospitalization data, and cancer registries that covered over 219,000 person-years. Associations between metabolite levels and disease incidence were assessed using Cox proportional hazards models adjusted for age and sex. Linear regression was used to examine metabolite associations with multimorbidity.

Results. Findings revealed 458 metabolites associated with at least one disease outcome, which produced 1226 metabolite-disease associations overall. 65.5% of significant metabolites were associated with two or more diseases, indicating extensive biological overlap among chronic conditions. Strong connections were observed across cardiometabolic, respiratory, renal, and liver diseases, and many metabolites showed consistent directions of association across multiple conditions (**Figure 41**). Mediation analyses showed that many metabolite-disease relationships were associated with common risk factors including obesity, smoking, inflammation, glucose resistance, and elevated lipid levels. Some metabolites were uniquely associated with specific diseases, while 30 metabolites were significantly linked to the development of multimorbidity (**Figure 42**).

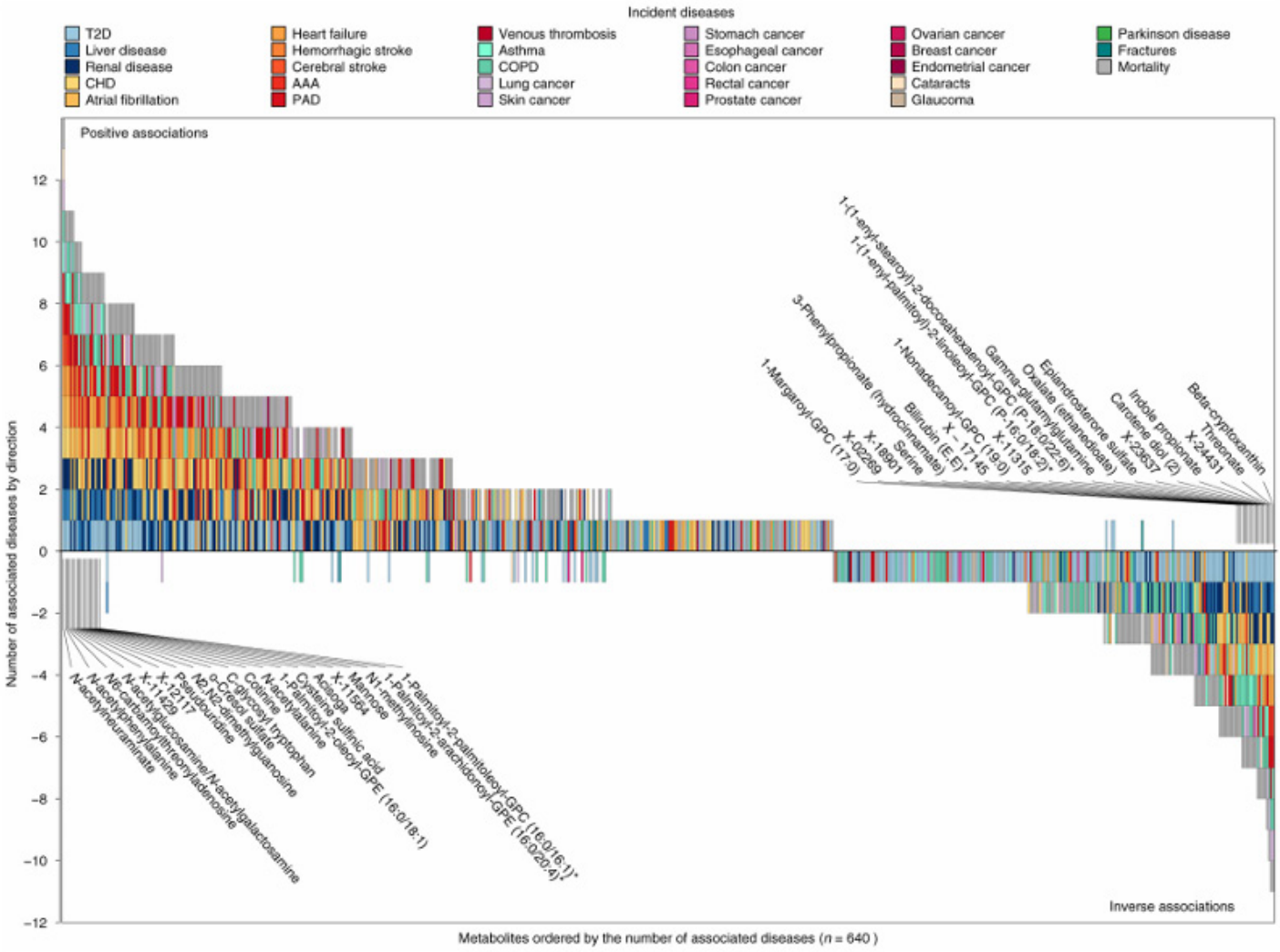


Figure 41. Brick plot showing the ranking of metabolites based on the number of associated disease end points. P < 0.001 was considered significant accounting for 28 diseases tested for each metabolite. The x axis shows the rank of each metabolite according to the number of associated metabolites, counting inverse associations as negative numbers to simplify the representation of results. The y axis shows the number of associated metabolites, whereby positive numbers indicate positive associations, and negative numbers indicate inverse associations. The colors of each box indicate the associated disease end point. Selected metabolites with multiple associated end points are annotated. Image reproduced from Pietzner et al., *Nat Med*, 2021, licensed under CC BY 4.0.

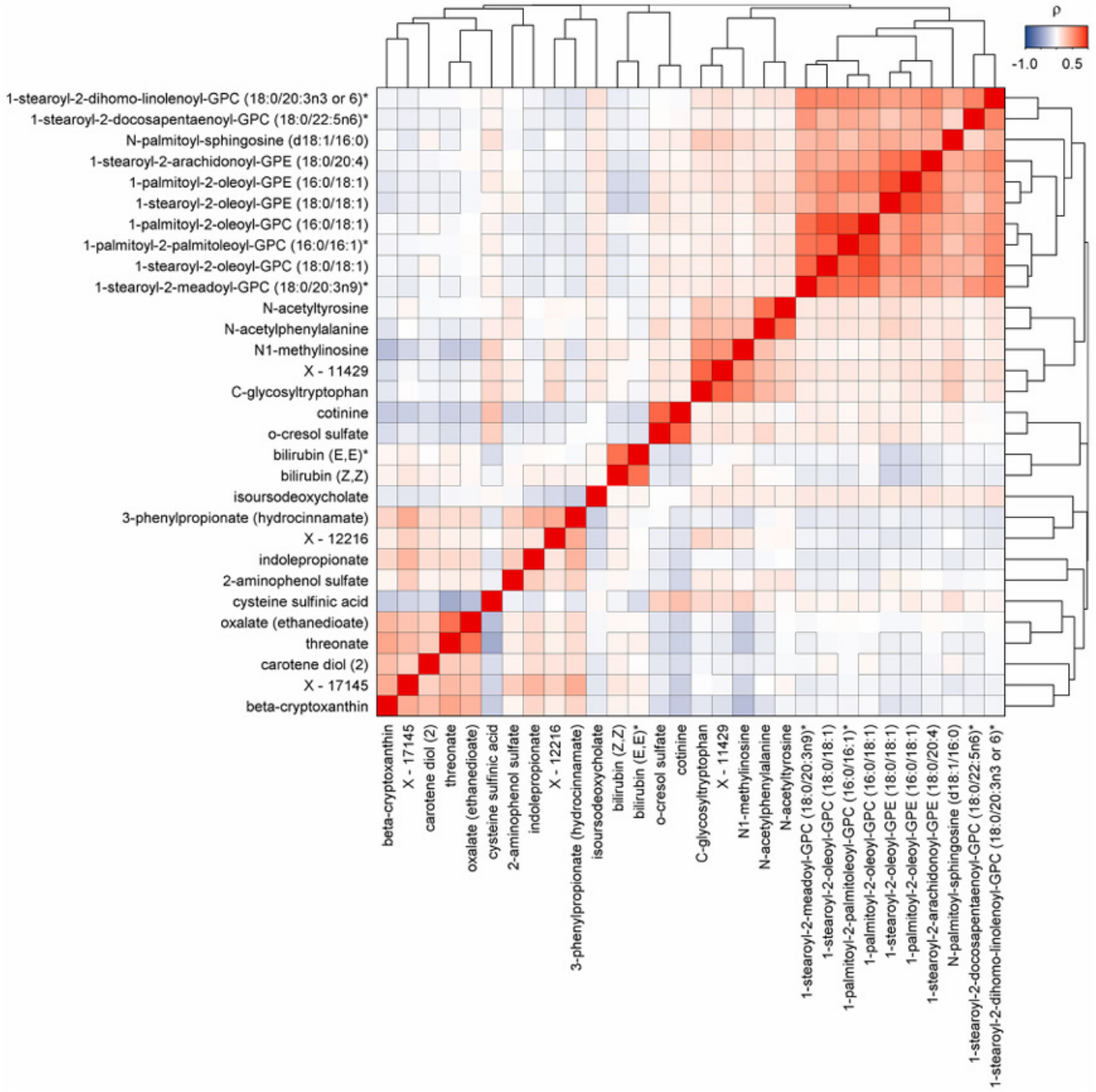


Figure 42. Pairwise correlation heatmap of multimorbidity candidate metabolites. Pairwise correlation matrix of plasma metabolites significantly associated with the incidence of noncommunicable disease multimorbidity. Colors indicate positive (red) or inverse (blue) correlations and black frames indicate statistical significance after correction for multiple testing. Image reproduced from Pietzner et al., *Nat Med*, 2021, licensed under CC BY 4.0.

Study Conclusions

- In this study untargeted metabolomics was instrumental in identifying biochemical pathway associations shared across multiple diseases, which provided both mechanistic insight into disease risk and practical insight for development of future therapies.
- In many cases, pathways that were associated with more than one disease demonstrated the same directions of association with each disease individually. This suggests that future interventions could target shared pathways to prevent multiple illnesses.
- This study's findings could also guide the identification of subtypes of multimorbidity by investigating how those pathways associate with co-occurrence of seemingly unrelated diseases.

Predicting Biological Age to Better Assess Disease Risk

Introduction. Chronological age is a major risk factor for numerous diseases, but it does not capture the complex biological aging process or the substantial variability of biological aging between individuals. The difference between chronological aging and biologically driven aging could be a more informative reflection of health status than chronological aging alone. Biological aging reflects complex interactions among genetic, lifestyle, and environmental factors, and identifying biomarkers that better represent this process could improve understanding of disease risk and health trajectories. Metabolomics is a promising approach because circulating metabolites reflect both endogenous metabolic activity and external exposures including diet, medications, and environmental factors.

Preliminary Data and Study Goals. Previous metabolic age prediction studies have been limited by small sample sizes, targeted metabolite panels, or restricted age ranges, which may reduce predictive accuracy and generalizability. Therefore, the goals of this study were to develop a robust metabolomic age prediction model by performing untargeted metabolomics profiling on INTERVAL, a large population-based cohort, and to evaluate whether the resulting metabolomic age estimates were associated with health outcomes [23].

Methods. The INTERVAL study is a prospective cohort study of approximately 50,000 participants nested within a randomized sample of blood donors. In this study, plasma samples from 12,000 health blood donors, aged 18-75 years, were analyzed using global metabolomics. Ridge regression combined with bootstrapping was used to build and internally

validate models that predicted chronological age based on metabolite profiles. Separate models were also constructed using only endogenous metabolites and using sex-specific data for men and women. The resulting metabolomic age predictions were then tested in the Netherlands Epidemiology of Obesity (NEO) study to determine whether differences between predicted metabolomic age and chronological age were associated with health characteristics.

Results. The metabolomic age prediction models demonstrated strong performance, explaining more than 80% of the variation in chronological age when both endogenous and xenobiotic metabolites were included (**Figure 43**). Key metabolites that strongly contributed to age prediction included hydroxyasparagine, vanillylmandelate, and 5,6-dihydrouridine. When the model was applied to the NEO cohort, higher metabolomic age relative to chronological age was associated with obesity and cardiovascular disease, partly due to the xenobiotic metabolites reflecting the use of medication and environmental factors such as cotinine (**Figure 44**). The study also identified 163 metabolites that differed between men and women, and sex-specific models showed high predictive performance but only moderate correlation with each other, suggesting that these models captured meaningful sex differences in age-related metabolic patterns.

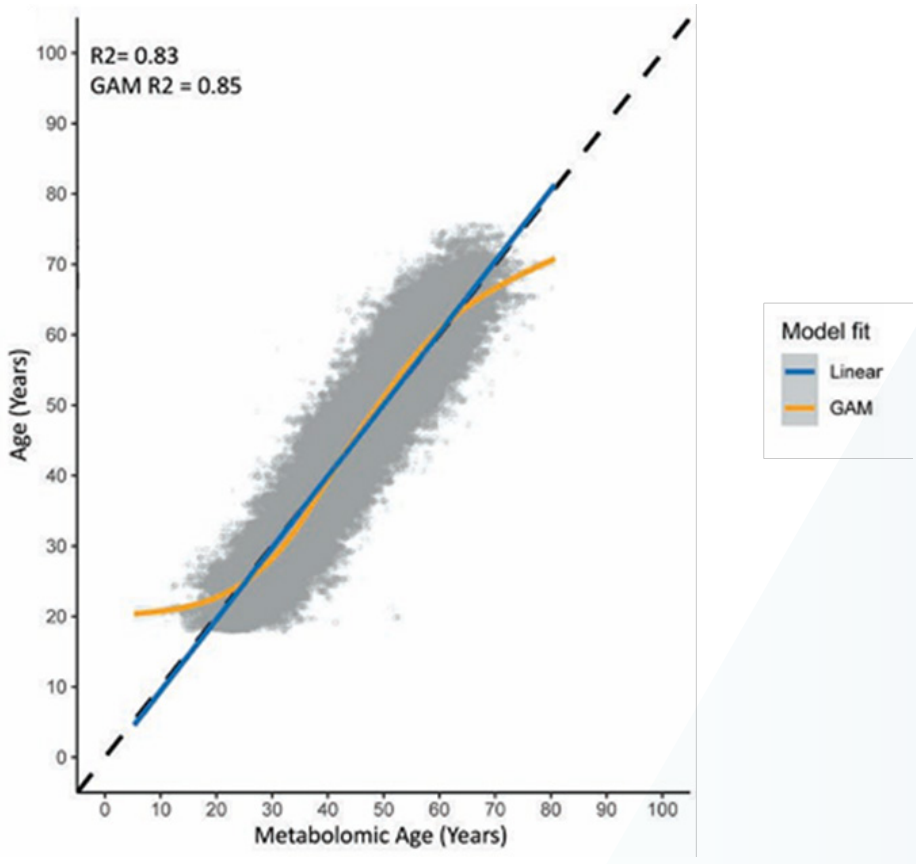


Figure 43. Correlation plot of the Metabolomic age (predicted age) on the x axis and the chronological age on the y axis for all endogenous and xenobiotic metabolites measured. Image reproduced from Faquih et al., *J Gerontol A Biol Sci Med Sci*, 2025, licensed under CC BY 4.0.

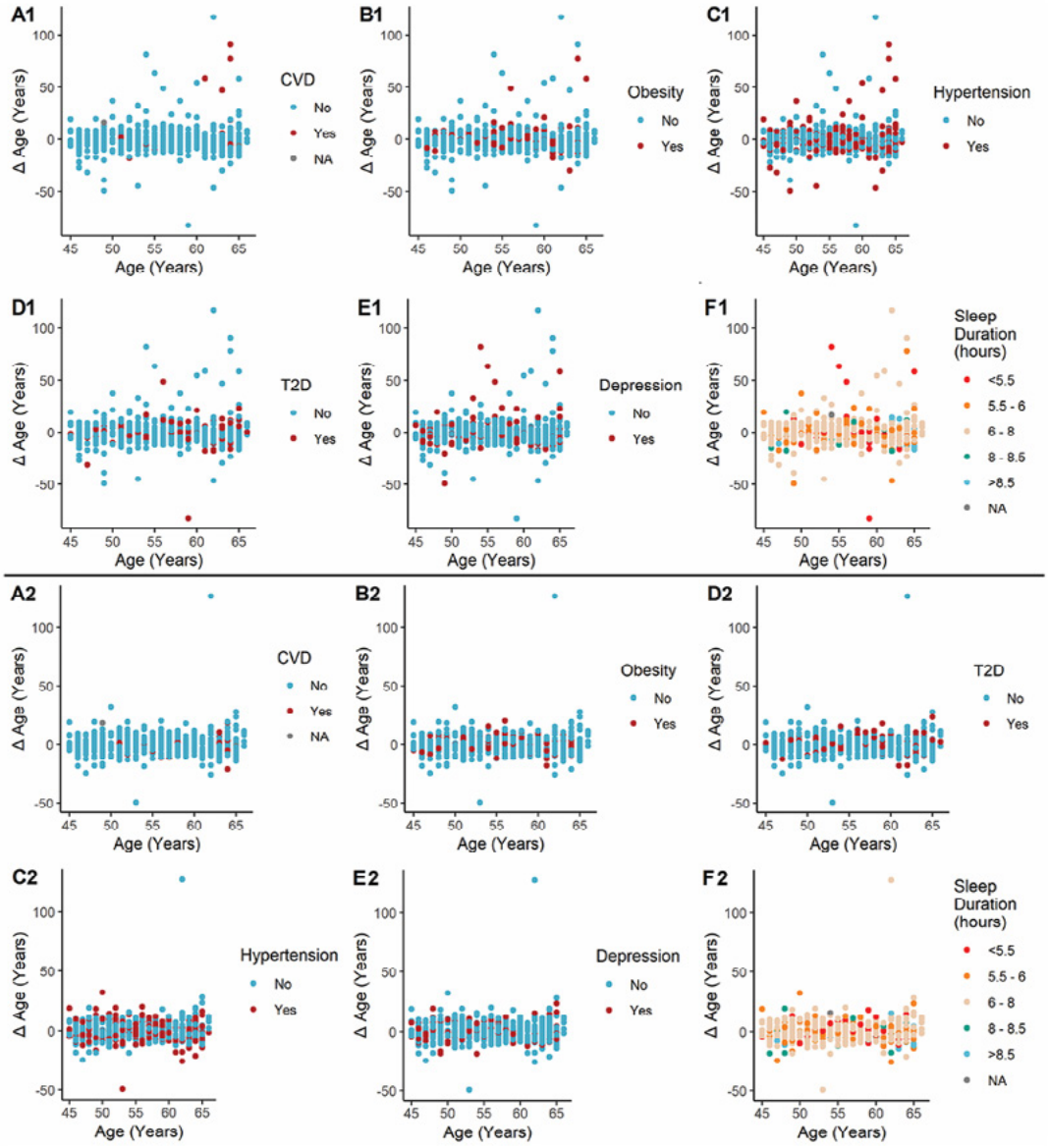


Figure 44.
Scatterplots showing the age difference (Δ age) as predicted using the endogenous + xenobiotic metabolites model (**A1-F1**) and the endogenous metabolites only model (**A2-F2**). NEO= Netherlands Epidemiology of Obesity. Image reproduced from Faquih et al., *J Gerontol A Biol Sci Med Sci*, 2025, licensed under CC BY 4.0.

Study Conclusions

- Incorporating a broad range of endogenous and xenobiotic metabolites measured using global metabolomics produced a robust metabolomic age model that outperformed many previous approaches.
- These findings show that metabolomic age prediction models have potential as tools for studying aging biology and identifying individuals at increased risk of age-related diseases.

Linking Heterozygous Pathogenic Variants to Disease Outcomes

Introduction. Although genome-wide association studies (GWAS) of common variants have identified many loci linked to metabolite levels, these variants often have modest effects and do not directly indicate gene function. Rare damaging variants identified through whole-exome sequencing may provide stronger functional insights because substantially alter protein activity. However, most rare variants occur in the heterozygous state, and their physiological consequences are not well understood.

Preliminary Data and Study Goals. In this study, investigators aimed to integrate metabolomics with exome sequencing to systematically determine how rare heterozygous variants influence metabolite levels and human traits, and to determine whether these variants can reveal graded effects on gene function and metabolic pathways [24].

Methods. Untargeted metabolomics and whole-exome sequencing were used to identify associations between metabolites and rare damaging variants. Plasma and urine metabolite levels were measured for a wide range of metabolites in large population cohorts including UK Biobank (UKB) and German Chronic Kidney Disease (GCKD). Rare variant aggregation tests were performed to assess whether groups of rare, predicted damaging variants within a gene were associated with metabolite levels. Computational simulations using genome-scale metabolic network models were used to validate findings.

Results. 235 significant gene-metabolite associations involving rare damaging variants were identified across multiple metabolic pathways, many of which had not been previously reported. These associations highlighted genes that encode enzymes and transporters that influence metabolite concentrations in plasma or urine. Several gene variants with different predicted functional impacts produced graded changes in metabolite levels, which provided evidence of dose-dependent effects on metabolic function (**Figure 45**). Notably, rare variants in sulfate transporter genes such as SCL13A1 and SLC26A1 were strongly associated with circulating sulfate concentrations and were also linked to height and musculoskeletal traits (**Figure 46**). Integrating genetic evidence with computational metabolic modeling supported the functional relevance of many associations.

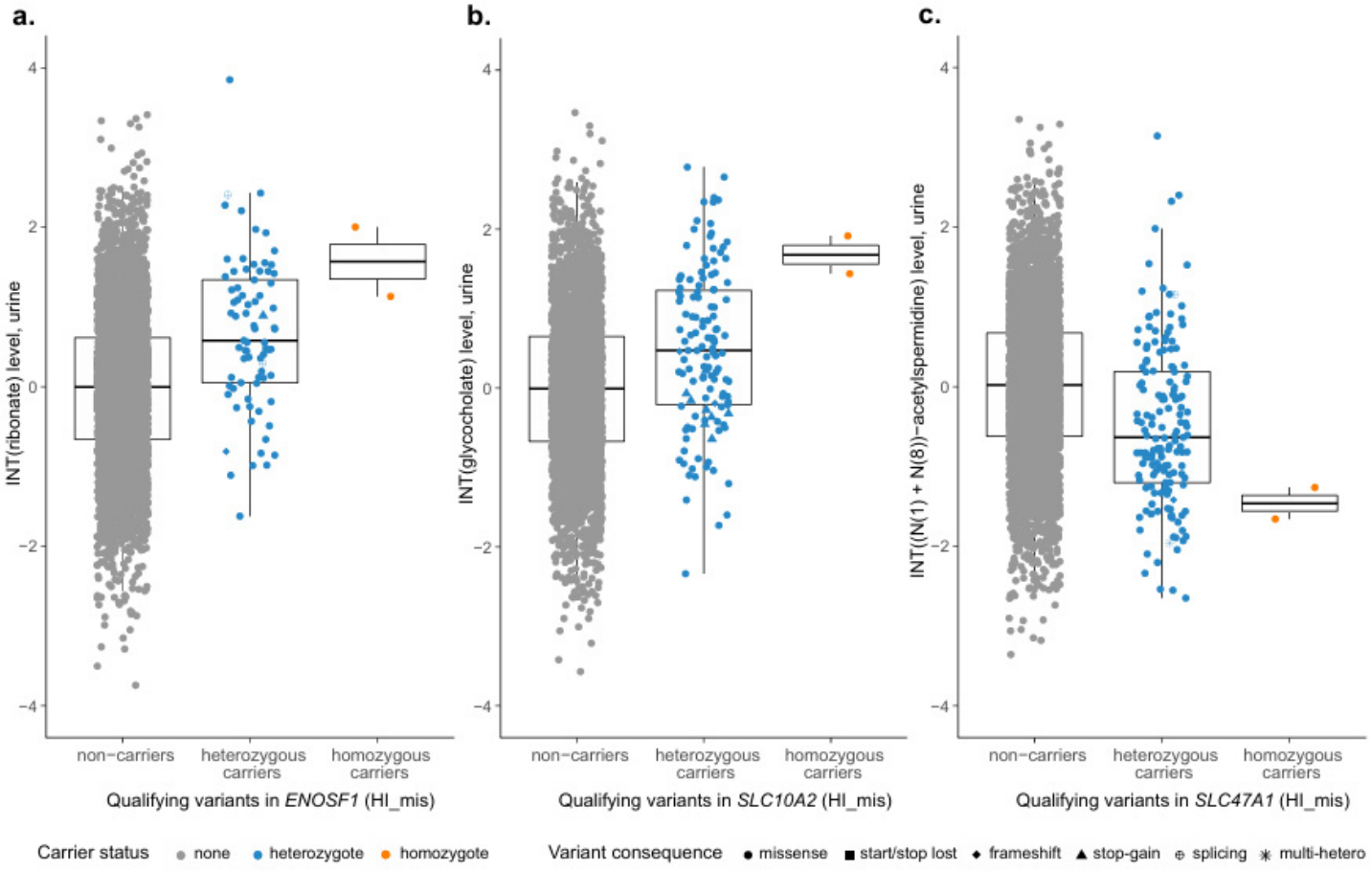


Figure 45. Metabolite levels by qualifying variant carrier status for significantly associated genes with more than one homozygous carrier. Urine metabolite levels after inverse normal transformation and covariate-adjustment are shown on the y axis, among non-carriers and heterozygous carriers of qualifying variants in the HI_mis mask on the x axis. Symbol color and shape indicate a variant's carrier status and consequence, respectively. Carriers of multiple heterozygous qualifying variants are denoted by as asterisk. Boxes range from the 25th to the 75th percentile of metabolite levels, the median is indicated by a line, and whiskers end at the last observed value within 1.5*(interquartile range) away from the box. The medial levels of (**A**) ribonate (n=4,618), (**B**) glycocholate (n=3,753), and (**C**) (N(1) + (N(8))-acetylspermidine (n=4,619) are all more extreme for the homozygous than the heterozygous carriers, reflecting a dose-response effect. Image reproduced from Scherer et al., *Nat Genet*, 2025, licensed under CC BY 4.0.

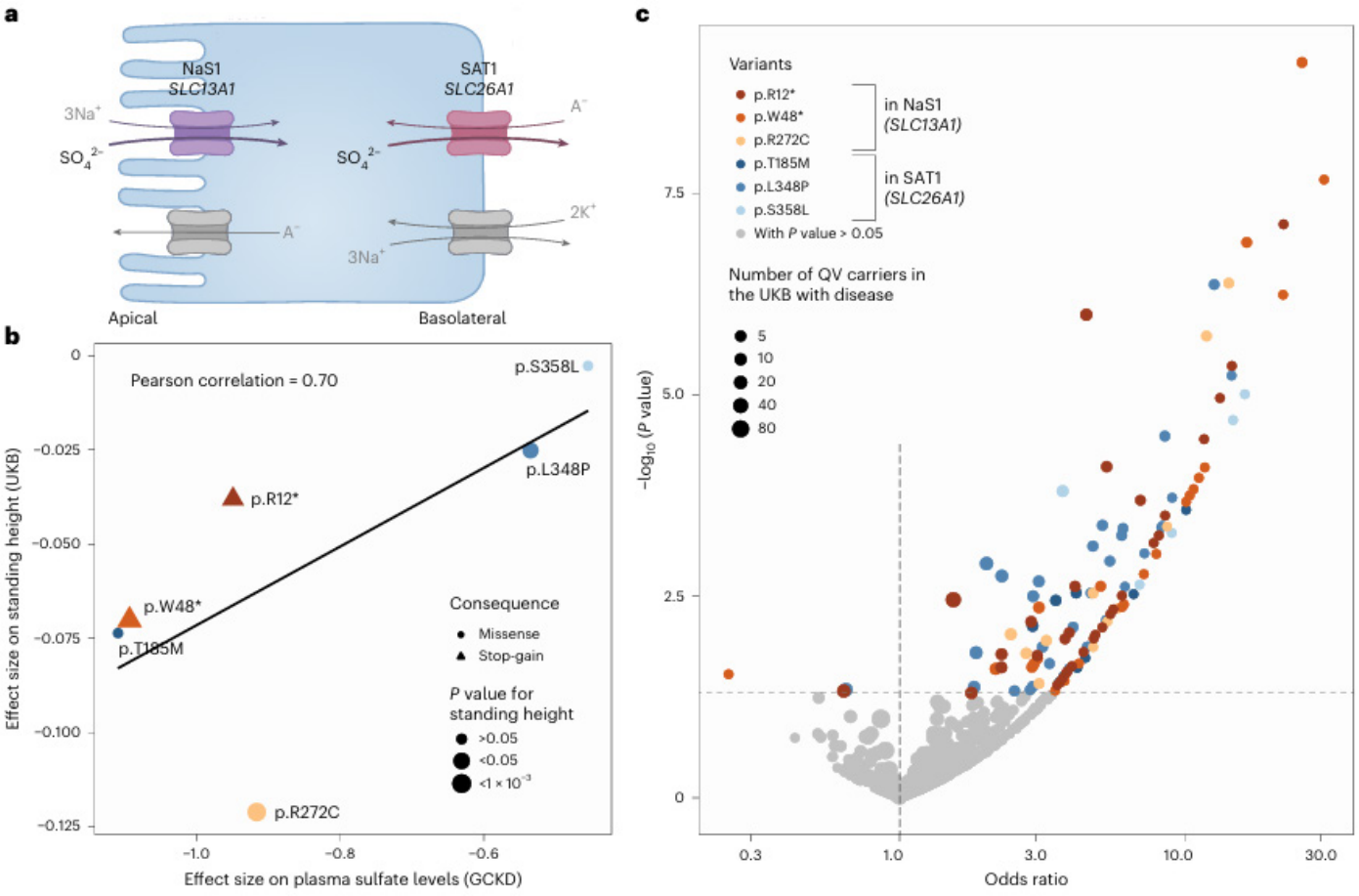


Figure 46. Impact of functional qualifying variants in SLC13A1 and SLC26A1 on height, musculoskeletal traits and fractures supports the role of plasma sulfate as an intermediate readout. (**A**) Schematic representation of the sulfate reabsorption mechanism involving NaS1 encoded by SLC13A1 at the apical membrane and SAT1 encoded by SLC26A1 at the basolateral membrane of epithelial cells. (**B**) Scatterplot shows the relation between the effect sizes of six qualifying variants on plasma sulfate levels in the GCKD cohort (x axis) and on standing height in the UKB cohort (y axis). (**C**) Volcano plot showing odds ratios (x axis) and -log10 (p-values) (y axis) for associations of the six qualifying variants with musculoskeletal diseases and fractures in the UKB. Image reproduced from Scherer et al., *Nat Genet*, 2025, licensed under CC BY 4.0.

Study Conclusions

- Metabolomics was integral into showing that even heterozygous damaging variants can produce measurable metabolic changes.
- The graded phenotypic effects that were observed show how genetic variation shapes metabolism and human traits.
- Overall, this study's findings demonstrate the value of metabolomics in gaining a functional readout by which to interpret rare genetic variants and show how combining large-scale metabolic profiling with sequencing can reveal novel insights into the complex link between genetic variation and disease.

ALTERNATIVE SAMPLE MATRICES

Breast Milk

Introduction. Understanding the human milk metabolome can help inform infant nutrition and health, but the high variability of milk composition between individuals and within the same individual over time, making it difficult to define standard concentrations for many components. Previous metabolomics studies have examined variation in milk composition across lactation stages, maternal health conditions, and populations, but relatively few have focused on identifying the core metabolites consistently present across diverse mothers.

Preliminary Data and Study Goals. The goal of this study was to characterize the breast milk metabolome in healthy lactating women from diverse backgrounds in order to define human milk consistently across lactating mothers and identify or substantiate what is crucial for infant nutrition, growth, and development [25].

Methods. Breast milk samples were collected from 31 women who represented diverse racial, ethnic, and dietary backgrounds. Participants also completed a health and lifestyle questionnaire. Milk samples were analyzed using global metabolomics profiling and relationships between metabolic profiles and various maternal or infant characteristics including maternal age, BMI, number of life births, infant age, and lifestyle factors. Metabolic pathways associated with key variables were identified through enrichment analysis.

Results. 389 metabolites were present in all samples. In general, the abundance of these shared metabolites varied widely among study participants. When grouped by biological pathways, xenobiotics showed the greatest variability across the study population, while nucleotides were the least variable. Infant age, maternal age, number of previous live births, and pre-pregnancy BMI were associated with differences in the milk metabolome (**Figure 47**). Sterol lipids and carbohydrates varied with infant age, as cholesterol and cholesterol sulfate increased as infants grew older. Maternal age was associated with changes in organic oxygen compounds, and the number of live births was linked to variation in organic acids, nucleic acids, carbohydrates, and lipids.

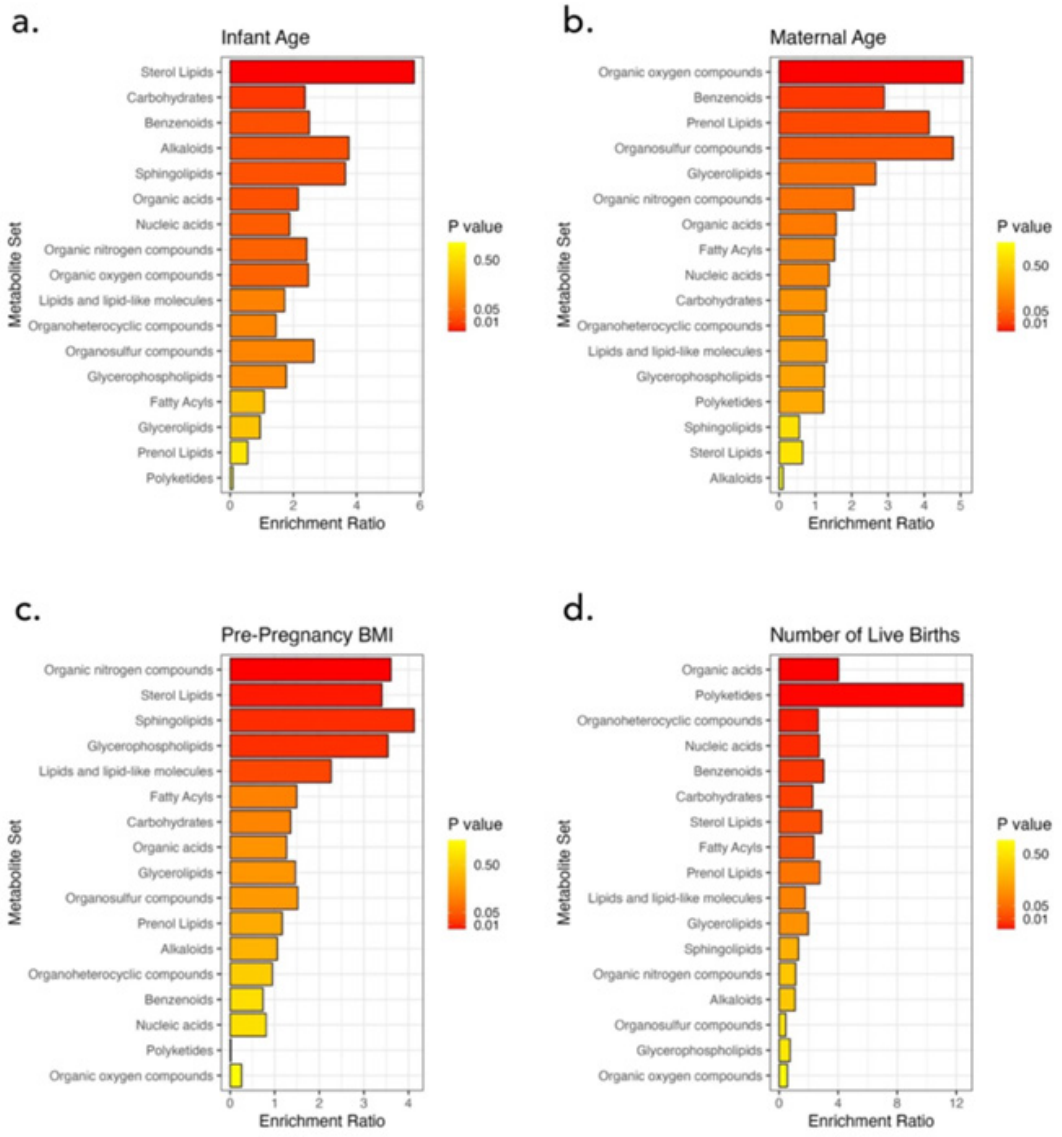


Figure 47. Quantitative Metabolite Set Enrichment Analysis (MSEA) by (**A**) infant age, (**B**) maternal age, (**C**) number of live births, and (**D**) pre-pregnancy BMI. Image reproduced from Holmes et al., *Sci Rep*, 2024, licensed under CC BY 4.0.

Study Conclusions

- This study suggests that human breast milk contains a core set of conserved metabolites that likely play essential roles in infant nutrition and psychological development.
- Metabolites involved in fundamental cellular functions, including nucleotides, lactose, creatinine, and glutamate, showed the least variability suggesting that molecules that contribute to critical processes such as energy metabolism are regulated the most tightly.
- This study is another example showing how metabolomics can provide scientific insight on uncommon sample matrices.

Aqueous Humor

Introduction. Aqueous humor (AH) holds valuable molecular information about retinal diseases and can be collected non-invasively, making it a practical source for biomarker discovery and disease mechanism studies. Previous studies have used metabolomics and proteomics to investigate eye diseases including glaucoma, neovascular age-related macular degeneration (nAMD), and diabetic macular edema (DME). However, interpreting molecular data collected from AH can be biased by confounding factors, including variations in total protein concentration and lens status (phakic (eye contains a natural crystalline lens) vs. pseudophakic (natural lens replaced with an artificial lens to treat cataracts).

Preliminary Data and Study Goals. The goal of this study was to determine how these ocular confounding factors impact proteomic and metabolomic profiles of AH, using samples from patients with nAMD and DME with the long-term goal of improving the reliability of AH-based biomarker discovery [26].

Methods. This prospective, cross-sectional clinical investigation included 102 participants with (nAMD), 18 with (DME), and 18 cataract patients without retinal disease as controls. Aqueous humor (AH) samples were collected through anterior chamber paracentesis and frozen for analysis. The Olink Target 96 proximity extension assay platform was used to measure proteins related to inflammatory, metabolic, neurologic, and cardiovascular processes. Total AH protein concentration was measured separately. Metabolites were analyzed using Metabolon's Global Discovery Panel. Linear models with multiple-testing correction were used to identify differentially abundant proteins and metabolites between groups, while also accounting for potential ocular confounders including lens status, total protein concentration, and exposure to pupil-dilating drugs. Correlation analyses, protein profile clustering, and gene-set enrichment were used to identify biological pathways associated with observed molecular patterns.

Results. AH from patients with nAMD and DME contained significantly higher total protein concentrations than controls. Pseudophakic eyes (artificial lens) had higher AH protein concentrations than phakic eyes (natural lens) and showed increased levels of proteins related to extracellular matrix remodeling and signaling pathways (**Figure 48**). When adjusted for confounding factors the number of differential proteins between disease groups and controls decreased substantially to reveal disease-associated protein signatures of greater biological relevance. Protein clusters linked to neuronal signaling, antimicrobial peptides, metabolic enzymes, and oxidative stress responses were identified and showed enrichment of proteins previously associated with age-related macular degeneration nAMD and DME. Metabolomic profiles were less affected by these confounders.

Metabolomics analysis led to the unexpected discovery that pupil-dilating drops containing phenylephrine/tropicamide were associated with increased levels of several inflammatory proteins, particularly interleukin-6 (IL-6), likely through the presence of the phenylephrine metabolite 3-hydroxymandelate in AH (**Figure 49**). Metabolomics also provided insight into disease phenotypes. For example, elevated glucose and fructose and reduced 1,5-anhydroglucitol in DME samples indicated poor glycemic control and chronic hyperglycemia.

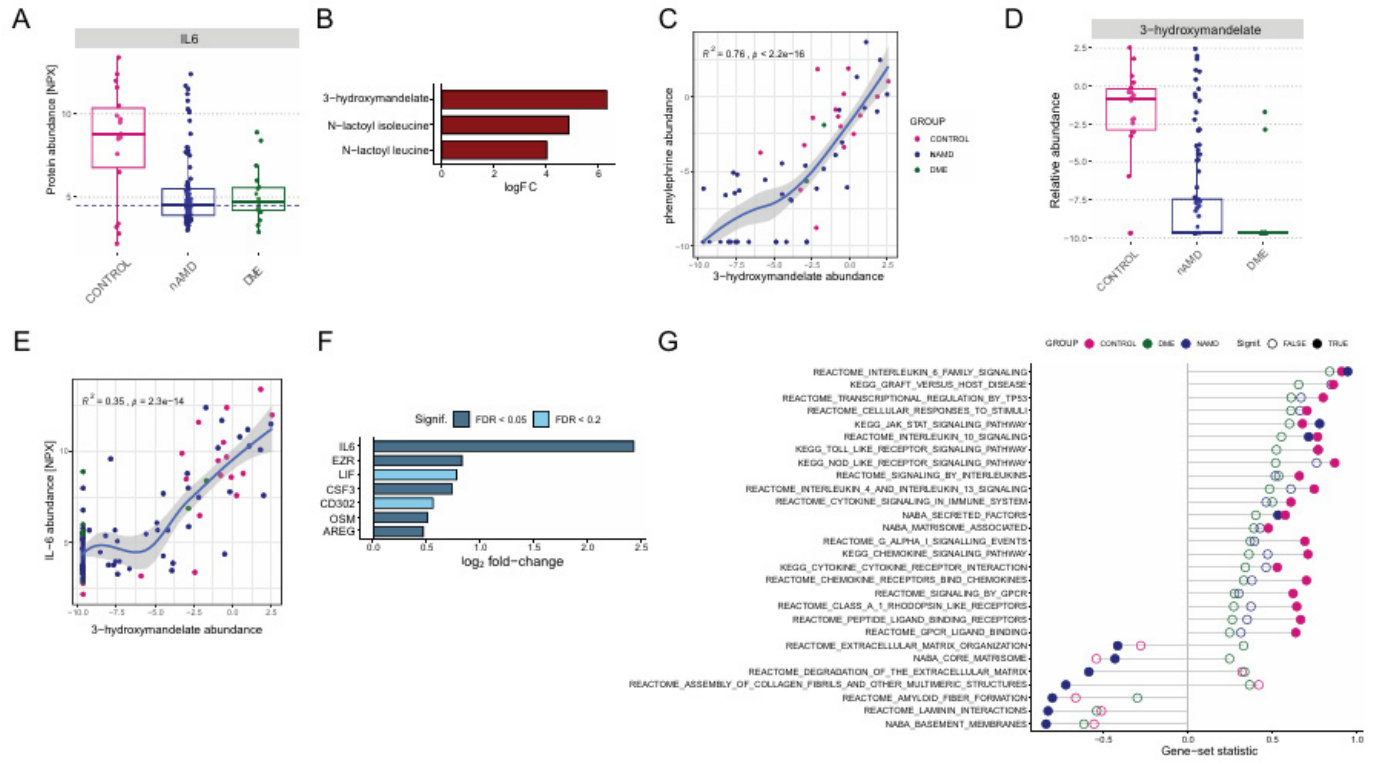


Figure 48. Association of phenylephrine/3-hydroxymandelate exposure with AH protein profiles. (**A**) Box plots display the relative protein abundance values (NPX) for IL-6 across the three patient groups. The dotted line represents the threshold used to split the control group for subsequent differential abundance analysis. (**B**) Bar plot of the metabolites significantly associated with high versus low IL-6 levels within the control group (FDR < 0.05). (**C**) Correlation between 3-hydroxymandelate and phenylephrine levels. (**D**) Box plots display the relative metabolite abundance values for 3-hydroxymandelate across the three patient groups. (**E**) As in (**C**) but for the correlation between 3-hydroxymandelate and IL-6 levels. (**F**) Bar plot of the proteins associated with high versus low 3-hydroxymandelate levels in the nAMD group, with log2 fold changes indicated and significance levels represented by the color scale. (**G**) GSEA results for high versus low 3-hydroxymandelate levels in the nAMD group are shown. Image reproduced from Titz et al., *Transl Vis Sci Technol*, 2024, licensed under CC BY 4.0.

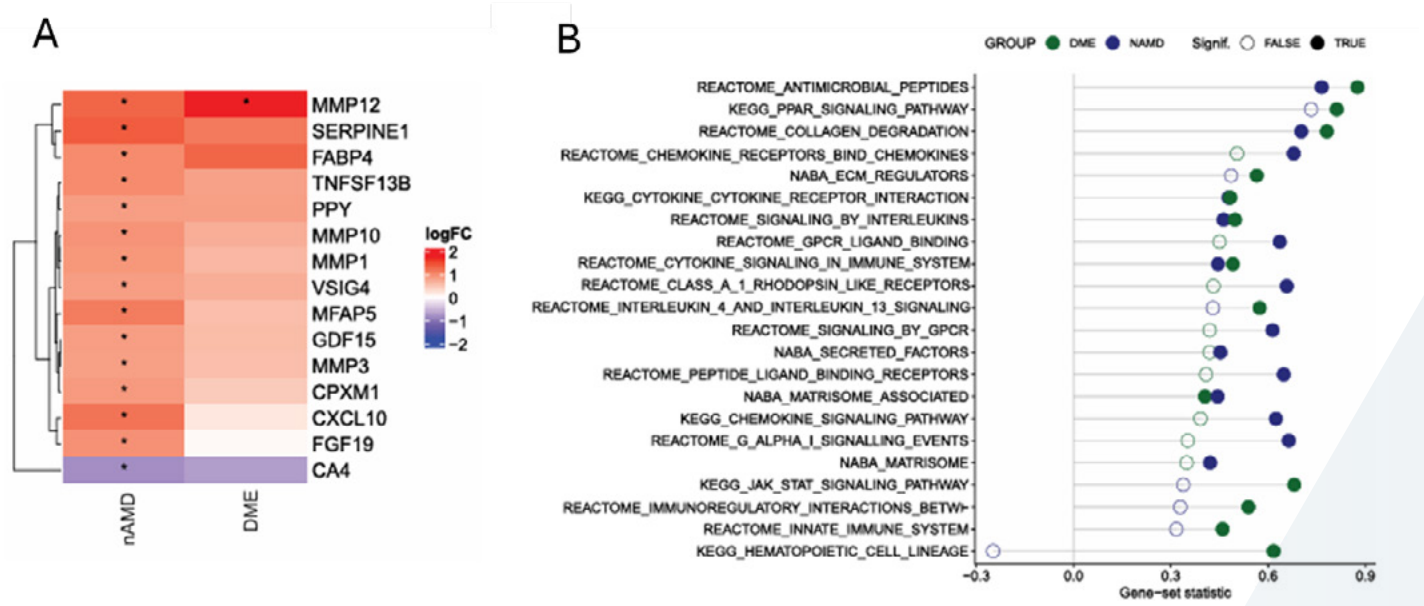


Figure 49. Influence of lens status on aqueous humor protein profiles. (**A**) Heatmap displaying the differential abundance of proteins, adjusted for protein concentration, between pseudophakic and phakic eyes within the nAMD and DME patient groups. The $\log_2$ fold changes are visualized with a *color gradient*, and proteins with statistically significant differences are marked (*P < 0.05, FDR-adjusted). The heatmap is confined to the top 15 proteins ranked by the absolute value of logFC. (**B**) Results from GSEA for lens status (pseudophakic vs. phakic) are presented. Image reproduced from Titz et al., *Transl Vis Sci Technol*, 2024, licensed under CC BY 4.0.

Study Conclusions

- This study's findings show that proteomic analysis of AH can be strongly influenced by ocular and procedural confounding factors, but metabolomics is less affected by them.
- In this study, metabolomics identified phenylephrine-containing pupil-dilating eye drops as a previously unrecognized confounder. The 3-hydroxymandelate in these drops was associated with increased levels of inflammatory proteins. Not only did this uncover a novel mechanism in eye biology, it also shows that correcting for phenylephrine exposure should be done in future studies to accurately interpret AH omics data.
- This study also serves as an example of how metabolomics can reveal crucial scientific insight from an uncommon sample matrix.

Cervical Swabs

Introduction. The vaginal microbiota plays a critical role in women's reproductive health. Healthy vaginal environments are typically dominated by Lactobacillus species, particularly *Lactobacillus crispatus*, which are associated with protective effects, while more diverse, anaerobe-rich microbial communities are linked to bacterial vaginosis (BV). *Lactobacillus iners* has a paradoxical role in BV because unlike other Lactobacillus species, it is associated with poorer clinical outcomes and increased risk of transitioning to BV, despite being a dominant organism in most women. Standard BV treatment with metronidazole often results in *L. iners*–dominated communities, which are unstable and prone to relapse. Addressing this clinical challenge requires new strategies that can modulate the vaginal microbiome to result in growth of durable, health-associated microbiota.

Preliminary Data and Study Goals. The biology of *L. iners* is poorly understood primarily because it is difficult to culture and study in vitro. Compared to other Lactobacillus species, it has a smaller genome and limited metabolic capacity, suggesting reliance on external nutrients. Based on these previous findings, the goal of this study was to identify key metabolic dependencies of *L. iners* and determine whether this vulnerability could be targeted to shift the vaginal microbiota toward more beneficial compositions and improve treatment outcomes for BV [27].

Methods. Clinical isolates were obtained from a large cohort of South African and American women that contained both BV and non-BV cases. An extensive genome catalog was built from these isolates to enable comparison of metabolic capabilities across microbial species. Cervicovaginal lavage samples were analyzed using global metabolomics and microbiota composition was profiled using 16S rRNA gene sequencing. Statistical analyses were used to correlate metabolite levels with bacterial community structure (e.g., Lactobacillus-dominant vs BV-associated communities).

Results. Findings revealed the growth of *L. iners* to be uniquely dependent on cysteine. Robust growth of diverse *L. iners* strains was observed in L-cystine-supplemented culture media, while no growth occurred in L-cystine poor cultures. *L. iners* was shown to have limited ability to utilize complex cysteine sources, explaining its specific growth requirement and highlighting a key metabolic vulnerability. In clinical samples, cysteine levels were significantly higher in women without bacterial vaginosis (BV) and positively correlated with Lactobacillus abundance, while negatively correlating with BV-associated bacteria (**Figure 50**). Genomic analyses revealed that *L. iners* lacks many of the transport systems for cysteine and cysteine-containing molecules that are present in other Lactobacillus species, suggesting that it has limited metabolic flexibility. Cystine uptake inhibitors selectively suppressed *L. iners* growth without significantly affecting other Lactobacillus

species, and in competition experiments, these inhibitors shifted microbial balance toward the health-associated species *L. crispatus*. Combining a cystine uptake inhibitor with metronidazole enhanced *L. crispatus* dominance more effectively than antibiotic treatment alone.

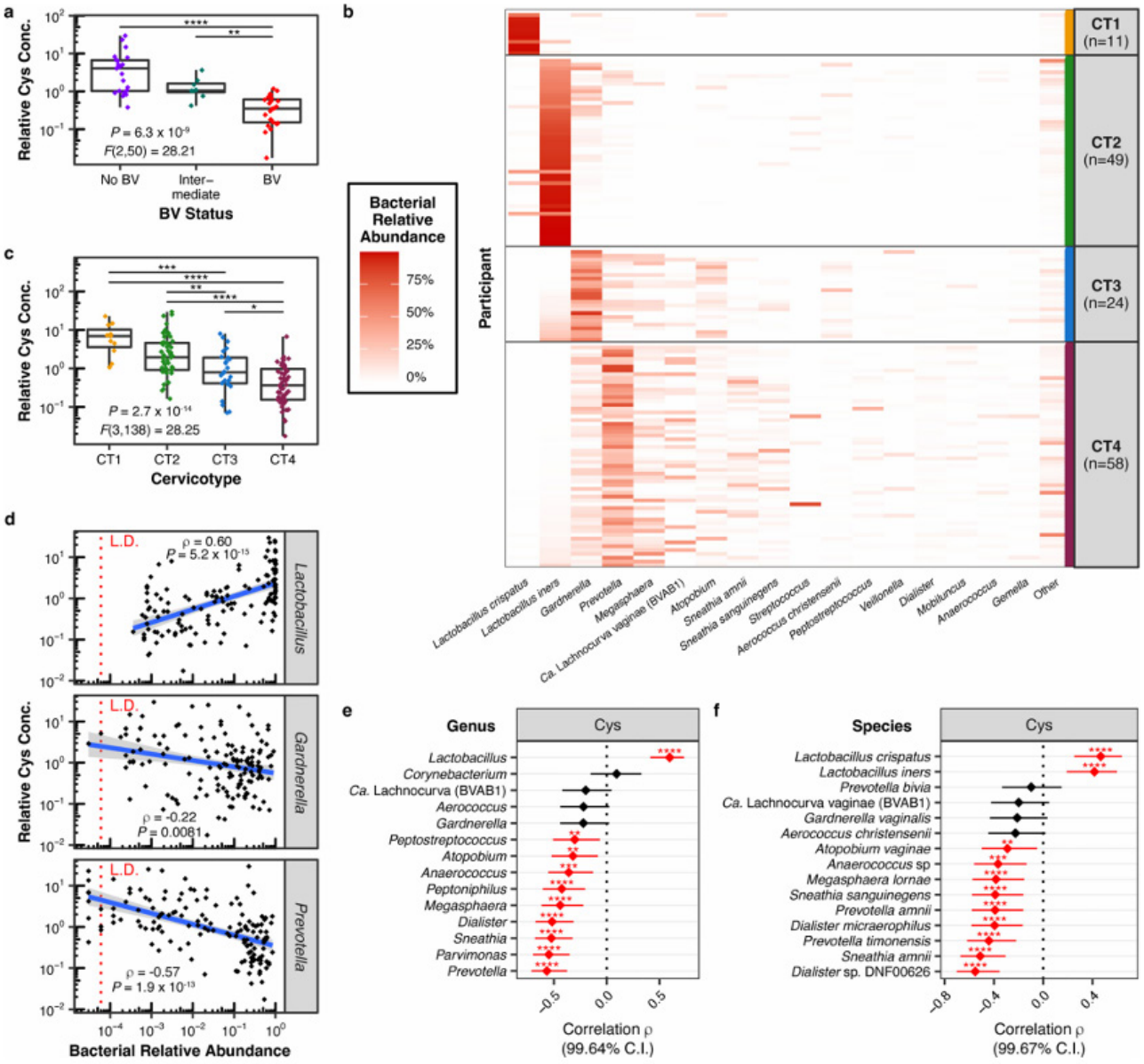


Figure 50. Vaginal cystine (Cys) concentrations are higher in women without BV and correlate with Lactobacillus-dominant microbiota. (**A**), Relative Cys concentration by BV status in cervicovaginal lavage (CVL) fluid from 53 South African women (21 without BV, 24 with BV, and 8 intermediate). (**B**) FGT bacterial microbiota composition among 142 HIV-uninfected South African women (including the 53 from A), determined by bacterial 16S rRNA gene sequencing. (**C**) Relative Cys concentrations per CT in cervical (CTL) fluid from women in (**B**). (**D**) Two-tailed Spearman rank correlation between Cys concentrations and bacterial relative abundances of the genera Lactobacillus, Gardnerella, and Prevotella. (**E, F**) Two-tailed Spearman correlation coefficients (ρ) with adjusted confidence intervals between Cys concentrations and relative abundances of each genus (**E**) or species (**F**) detected at >50% prevalence in cohort (n=142). Image reproduced from Bloom et al., *Nat Microbiol*, 2022, licensed under CC BY 4.0.

Study Conclusions

- This study identified cysteine dependence as a defining biological feature of *L. iners*, which offers an explanation regarding the challenges associated with culturing this species.
- *L. iners* is uniquely limited in its ability to transport and utilize diverse cysteine sources, making it especially reliant on specific forms of exogenous cysteine. This metabolic constraint helps explain its ecological behavior and distinguishes it from more health-associated species like *L. crispatus*.
- The study findings also suggest that host-derived cysteine levels may influence whether Lactobacillus-dominated or BV-associated communities prevail, suggesting that metabolite availability may be a driver of microbial community structure in the vaginal environment.
- Overall, this study demonstrated proof-of-concept that cystine uptake inhibitors may actively suppress *L. iners* and enhance effectiveness of metronidazole, suggesting a novel approach to the treatment of BV.

Synovial Fluid

Introduction. Osteoarthritis affects millions of people and contributes substantially to disability. Post traumatic osteoarthritis (PTOA) of the ankle is particularly significant because it often affects younger individuals and can develop years after the initial injury. Despite its prevalence, the early biological processes that drive PTOA remain poorly understood, and the standard of care diagnostic approaches of imaging and clinical exams are insensitive to early disease changes, which limit opportunities for early intervention. Acute joint injury triggers inflammatory responses in synovial fluid, including elevated cytokines, matrix metalloproteinases, and cellular damage, all of which may contribute to cartilage degradation and long-term joint degeneration. Synovial fluid serves as a reservoir of metabolites derived from joint tissues, and prior studies suggest that lipid composition changes with injury, inflammation, and arthritis. However, since earlier work relied on relatively limited analytical techniques, the full metabolic alterations that occur after injury remain poorly understood.

Preliminary Data and Study Goals. The goal of this study was to utilize global metabolomics to identify lipid-related metabolic changes associated with acute injury and early disease processes, with the long-term goal of discovering biomarkers and potential therapeutic targets that could improve understanding and management of PTOA [28].

Methods. The study was a retrospective cohort analysis of patients with unilateral intra-articular ankle fractures requiring surgery. Twenty patients were enrolled, and synovial fluid (SF) was collected from both the injured ankle and the contralateral uninjured ankle at the time of surgery. A subset of seven patients also underwent a second bilateral SF collection 6 months later. Metabolite concentrations were corrected for dilution that occurred during lavage collection by using a urea-based normalization method that compared synovial fluid to serum urea levels. After global metabolomics profiling random forest classification was used to identify key metabolites that differentiated injured ankle SF from control SF. Correlation analyses were performed to identify biological connections between lipid metabolites, inflammatory cytokines, and matrix metalloproteinases.

Results. Long-chain fatty acids, polyunsaturated fatty acids (PUFAs), sphingomyelins, and lysolipids were significantly increased in fractured ankles compared to contralateral controls. Random forest analysis confirmed that fatty acids and lysolipids were the primary drivers distinguishing injured from uninjured joints (**Figure 51**). Over time, many of these lipid abnormalities showed partial resolution, as several fatty acids and sphingomyelins significantly decreased by 6 months post-surgery. Correspondingly, the ability of metabolomic profiles to distinguish injured from control joints diminished at 6 months, suggesting that the acute metabolic response to injury subsides over time. Lipid metabolites showed strong positive correlations with inflammatory cytokines and matrix metalloproteinases, linking lipid dysregulation inflammation to tissue degradation processes (**Figure 52**). The magnitude and breadth of lipid changes were associated with injury severity, with more severe fractures showing greater elevations in fatty acids, lysolipids, and related metabolites.

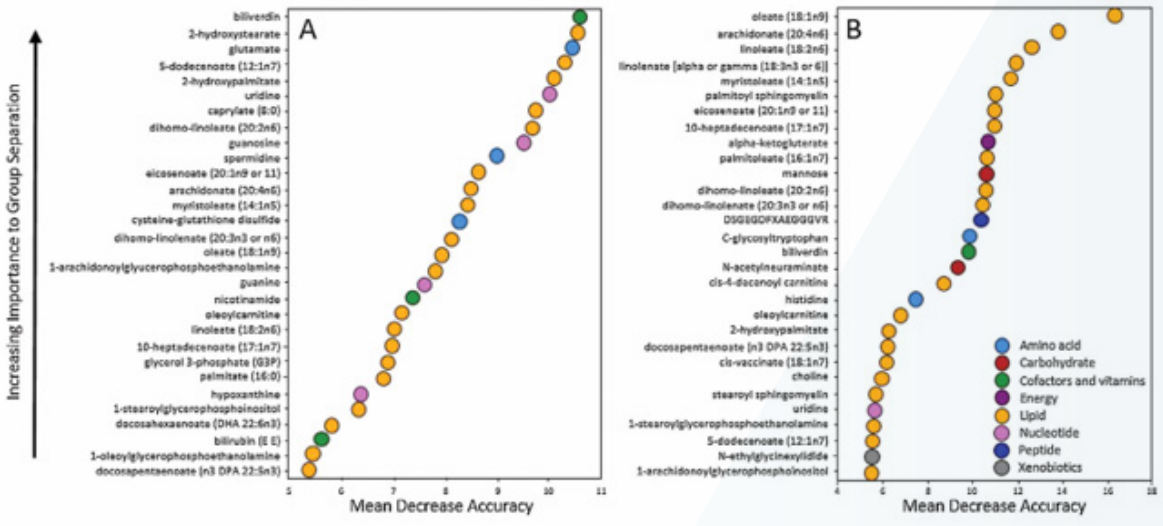


Figure 51. Random Forest classification analysis of Injured versus Contralateral Control at Baseline (**A**), and Injured Baseline versus Injured 6 months (**B**). Image reproduced from Leimer et al., *J Orthop Res*, 2017, licensed under CC BY 4.0.

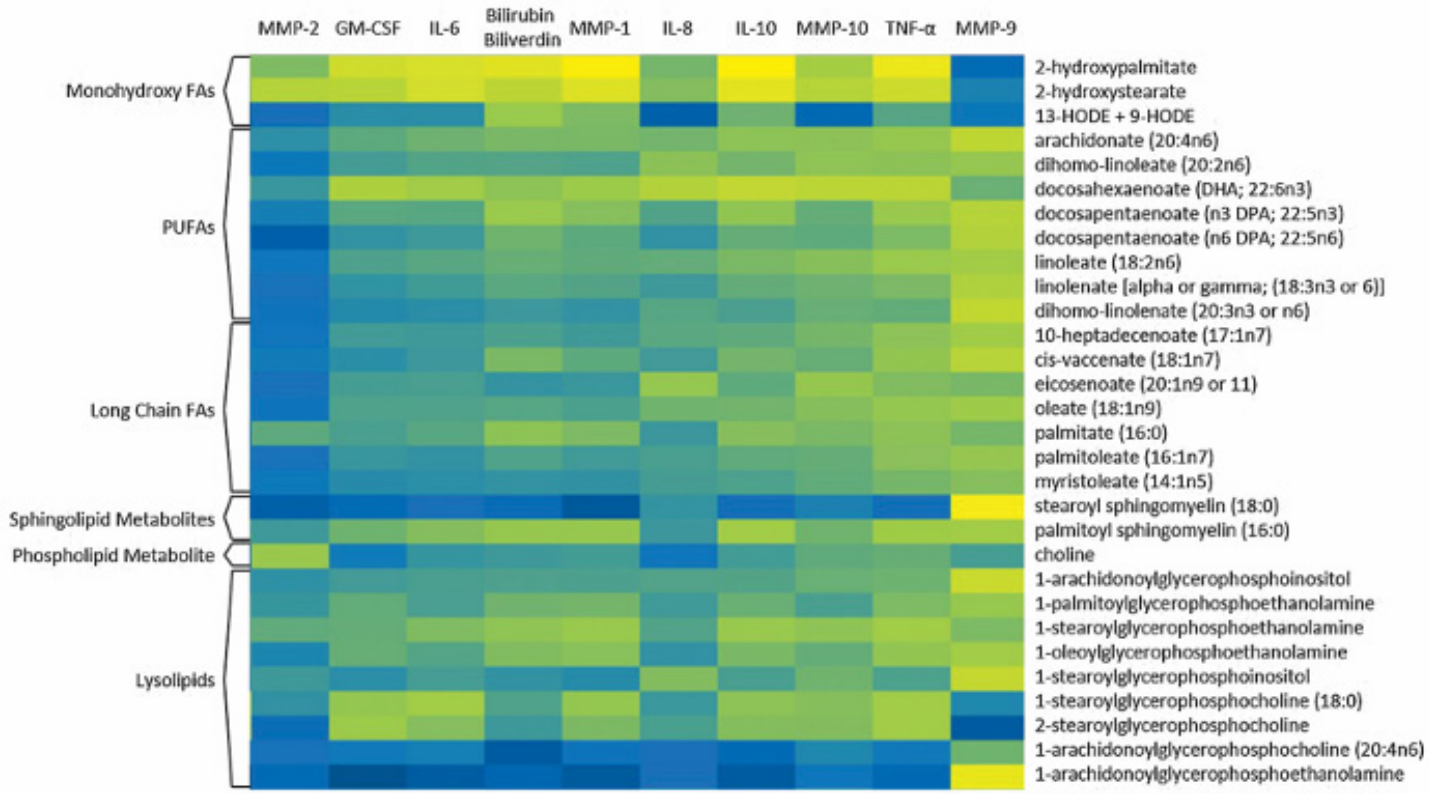


Figure 52. Lipid metabolite correlations with cytokine levels from the same patient cohort. The heat map compartments range from black to blue to yellow, where black represents the weakest correlations and yellow represents the strongest correlations. All significant correlation values were positive. Image reproduced from Leimer et al., *J Orthop Res*, 2017, licensed under CC BY 4.0.

Study Conclusions

- These study findings suggest that acute intra-articular ankle fracture produces a distinct, injury-driven lipid signature in synovial fluid, marked by elevations in free fatty acids, lysolipids, and sphingolipids.
- The strong correlations between lipid metabolites and cytokines or matrix metalloproteinases suggest that these lipid alterations are closely linked to inflammation, tissue damage, and cartilage degradation in the injured joint.
- Since many injury-induced lipid changes were transient, normalizing by 6 months after surgery, the metabolic response may be part of an acute injury and healing process, and early elevations in these metabolites may also trigger signaling pathways that contribute to long-term joint degeneration and progression to PTOA.
- Lipid metabolites may serve as biomarkers of early joint injury and PTOA risk, as well as potential targets for intervention.

REVIEW

Chapter Takeaways

- Metabolomics can be applied to numerous study designs and a diverse array of topics in basic science research.
- In most cases, metabolomics is fundamental to gaining a key insight or making a crucial discovery that propels research forward and elevates it to a higher level of significance.

Chapter 6

Designing a Metabolomics Study

Overview

The three previous chapters discussed instances in which metabolomics provided critical insight or solved a problem that other omics sciences could not have addressed. Some of those studies also showed how using metabolomics alongside other omics sciences can enhance the depth of study findings. In this final chapter we will go over the basic principles of designing a metabolomics study while highlighting Metabolon's experience, products, and tools that have facilitated the successful completion of many thousands of studies and over 2 million samples analyzed.

Establish a Study Goal

The study goal is a highly important factor to consider when designing a metabolomics study because it will determine whether global or targeted profiling should be used. As a general example, studies aimed at discovery of biomarkers, therapeutic targets, or hypothesis generation are best approached with global profiling, while studies that are testing a hypothesis regarding a specific pathway or mechanism, validating prior study findings, or that require absolute quantitation (providing concentration units) would be served best by targeted profiling.

Choose an Appropriate Sample Type

It is crucial to choose a sample type that contains the metabolites of interest in abundance even if the sample matrix is not commonly profiled. Metabolon has developed protocols for many alternative sample types beyond the ones specifically mentioned in this guide including sebum, sweat, dried blood spots, bile, and hair follicles, among others. Metabolon also offers Sample Matrix Validation for sample types that have yet to be validated for LC/MS metabolomics profiling.

For some studies, it is prudent to analyze more than one sample type to ensure full coverage of the appropriate metabolomes. This was demonstrated by a few studies discussed in this guide. In the study by Zgoda-Pols et al. described in Chapter 3, investigators analyzed mouse brain, plasma, and urine to gain the deepest insight into drug-induced toxicity beyond what traditional biomarkers could provide. In the study by Wu et al. discussed in Chapter 4, the research team analyzed both plasma and feces to comprehensively evaluate microbiome-metabolome dynamics as they affect progression of type 2 diabetes. Metabolon is well equipped to advise on which sample matrix or combination of matrices would be appropriate for your study.

It is also important to note that not all sample collection options are amenable to metabolomics analysis. Some buffers and sample treatments can have strong deleterious effects on metabolomics data. Metabolon can assist in these discussions and help guide appropriate sample type selection.

Data Interpretation

Metabolomics data is often interpreted using many types of advanced statistical tests. For example, unique compounds that differ significantly between study groups are often identified using student's t-tests or ANOVAs. The degree of change between metabolic signatures between study groups can further be evaluated using principal component analysis (PCA) and pathway plots. Additionally, the metabolites that contribute the most to an observed change in metabolism can be identified using Random Forest Analysis. Every statistical method is used based on relevance to the study objective. Metabolon uses the aforementioned techniques, along with many others, to ensure accurate interpretation of the data.

We reiterate that interpreting metabolomics data from other providers, especially data from a global profiling study, can feel intimidating due to the sheer number of ion features reported, and low-quality identifications. Metabolon addresses these limitations with its chemo-centric approach, in which extraneous and redundant ion features are omitted from the dataset

prior to statistical analysis. Limiting the data set to biologically relevant ion features that are directly associated with high quality identified compounds makes data interpretation more straightforward and maximizes the likelihood of gaining meaningful insight. Metabolon further supports data interpretation with its bioinformatics platform, which allows rapid pathway analysis and integration of genomics data with metabolomics data. Additionally, project findings are analyzed by Metabolon's PhD level scientists, and their insights are provided in a written report along with all data and statistical analyses, considered a manuscript in hand for those with high publication demands.

Final Conclusions and Metabolon's Commitment

The studies presented in this guide highlight the essential role of metabolomics in providing actionable insights and advancing our understanding of diverse topics, and we hope that these concepts inspire your own research.

Metabolon is committed to supporting investigators through this journey by providing the tools, data, and guidance needed to interpret complex data into meaningful discoveries. Our PhD-trained scientists have decades of experience designing, executing, and supporting the analysis and interpretation of complex metabolomics data. We would be happy to help you design and execute appropriately powered studies to ensure your data delivers accurate scientific insights and propels your research forward.

To learn more about how Metabolon can help your study, speak with an expert today.

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A GUIDE TO
METABOLOMICS

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