Chemistry is Driving Discovery in Metabolomics

A survey report on new and established research in metabolomics, the study of small molecules associated with the rapid dynamic responses that live cells make to regulate their environment, keep themselves powered, cycling, and communicating.

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About The Author

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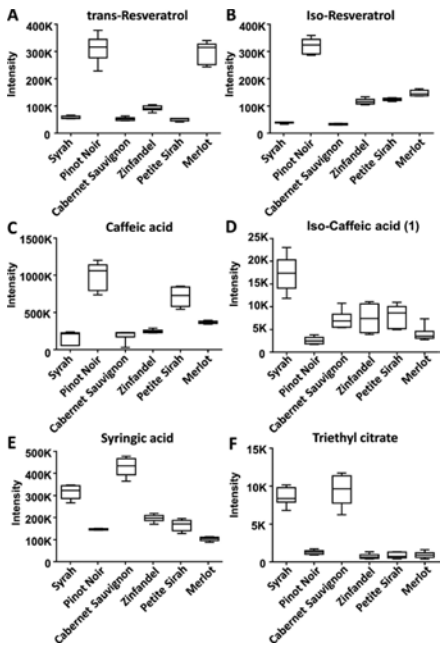
I. AN INTRODUCTION TO METABOLOMICS

Metabolomics is a relatively new area of research focused on studying the chemical reactions involved in maintaining living bodies' cells and organs and the small molecules that circulate in blood, including the amino acids, lipids (fats), nucleotides, and carbohydrates that are involved in metabolism. By almost any measure, the field of metabolomics has grown exponentially over the last decade. The field, which is in many ways driven by analytical chemistry, has generated insights into human diseases including breast cancer, colorectal cancer, prostate cancer, esophageal and gastric cancer, cardiovascular diseases, kidney diseases, and the effects of toxicology and nutrition. It is being used in the life, food and plant sciences, drug development, toxicology, environmental science, and medicine. Because most metabolites are generated by proteins that result from gene expression, and metabolites give organisms their biochemical characteristics, the metabolome links an organism's genes, or genotype, with its expressed characteristics, or phenotype.

Metabolites can reveal much about human health, including how well someone burns fats and how far they can push themselves during physical exertion. Metabolomics also hold promise for investigating health much more comprehensively. Research on the major metabolic killers—diabetes, kidney disease, and heart disease—reveals critical signs of systemic dysfunction at the molecular level years before clinical symptoms appear. Robert Gerszten, a professor of medicine at Harvard Medical School and the director of Clinical and Translational Research for the Massachusetts General Hospital's Heart Center, believes the metabolome is "equally, if not more, important than the human genome" for capturing "the fingerprint of human disease."[1] This is in part because researchers expect that metabolomics will help identify the impacts of exposure to chemicals and other substances in the environment, which scientists estimate are responsible for over 90% of the death and disease in the U.S. and other developed countries.[2]

Metabolomics is also being used in drug discovery and precision medicine,[3] to maximize the benefits of food,[4] to analyze naturally occurring materials in a more holistic way,[5] and in investigations into organisms' physiological development that are shedding light on factors which contribute to the metabolic syndrome that results in obesity, among other things.[6] NASA scientists are employing metabolomics in the study of identical twins, NASA astronaut Scott Kelly, who spent nearly a year living on the International Space Station, and his brother, Mark, who remained on Earth, to investigate how spaceflight affects the cardiovascular system.[7] Researchers regularly realize new ways that the discipline can help inform previously thorny challenges, such as how to detect illegal narcotics designed to evade conventional

chemical tests by screening for chemical structures related to known narcotics[8] and even how to analyze the complex characteristics of a great red wine.[9]



Peak intensities of selected compounds contributing to the difference of the metabolite profiles of six red wines: (A) *trans*-resveratrol; (B) isoresveratrol; (C) caffeic acid, negatively correlated to the tasting score; (D) isocaffeic acid, positively correlated to the tasting score; (E) syringic acid; (F) triethyl citrate, present in only two of the red wines.

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DOI: 10.1021/acs.jafc.5b04890

By identifying novel biomarkers that reveal disease, indicate (or predict) response to therapy, and provide mechanistic understanding of underlying disease processes, metabolomics has generated many important biological and medical insights. A related discipline, metabonomics, investigates how organisms respond to nutrients, drugs, diseases, and other stimuli. In addition to the scientific discoveries themselves, the widespread interest in metabolomics and metabonomics has "resulted in the development and application of numerous advances in analytical science," according to Professor Ian Wilson of Imperial College London.[10]

The burgeoning interest in metabolomics is reflected in predictions for its market growth. The global metabolomics market is estimated to expand between 14.6% and 26% annually from 2016 to 2021. Experts estimate that it will reach $1.4 billion in the U.S. in 2020[11] and $2.39–$3.53 billion globally by 2021[12] due to the increasing need for accurate diagnosis of diseases, rising demand for personalized medicine, increasing pharmaceutical and biotech research and development and the availability of government and private funding. "Being non-invasive in nature and closely linked to phenotype, it is an ideal tool used in agricultural industries, pharmaceutical and preventive healthcare. Rise in the number of clinical trials, awareness about nutritional products, toxicological testing data, rapid growth of metabolomics data analysis software and solutions is expected to propel the growth of this market," according to one report.[13]

One thing that is holding the field back, according to market analysts, is the need for well-qualified researchers. In a recent presentation to the U.S. National Institutes of Health (NIH), the need to "train a new generation of scientists in metabolomics with the skills in technology, biochemistry and physiology needed for metabolomics studies" was raised as being of great importance.[14]

II. A CONCEPT WITH A LONG GESTATION TIME

While metabolomics has been the subject of widespread interest for only a handful of years, the underlying concept can be traced back to ancient China, where ants were used to detect diabetes based on the levels of glucose in urine samples.[15] The scientists and physicians of the middle ages used "urine charts" describing the smell, taste, and color of urine to diagnose various medical conditions that are metabolic in origin.[16] In the 1950s, Linus Pauling proposed that one could study breath condensates in order to capture human physiology.[1] In the 1960s and 1970s, technological advances in gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS) allowed scientists to begin making quantitative metabolic profiling studies.[17] Some of the pioneering studies of using analytical equipment to measure human metabolites were made in 1971 by the husband-and-wife team of Marjorie and Evan Horning.[18,19]

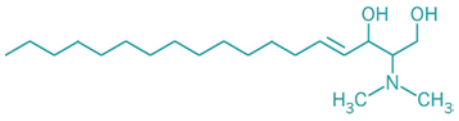
The Hornings and Pauling helped lead the development of techniques for using GC and LC to make metabolic measurements of biological fluids.[20] The term metabolomics was not coined until a quarter of a century later, in 1998.[21] Four years later, in 2002, *Chemical & Engineering News (C&EN)* identified metabolomics as a "new 'ome in town."[22] By 2005, 12 academic research institutions received grants under the NIH's Roadmap initiative to develop metabolomics technology.[23] During this period, the manufacturers of instruments used in metabolomics began collaborating with academic institutions to gain insights into the capabilities they needed to build into their instruments to move the science forward.[24]

The NIH has been supporting metabolomics research for over a dozen years,[25] and the agency has had a metabolomics program in place since 2012 with the goal to expand the capacity of researchers to study metabolomics. The program's current projects include support for a national data repository where researchers can store, or download, metabolomics data.[26] The agency says that its program "strives to create new and exciting ways to diagnose, treat, or potentially prevent many of the common diseases that impact human health."[27] Canada is also funding national metabolomics initiatives and other countries are following suit.[28]

Over the past decade, the ability of metabolomics studies to provide a broad agnostic assessment of compounds that exist within tested samples has revealed a wide array of unexpected findings. One of the most attention-getting was the ground-breaking discovery in 2011 that for the first time linked a common dietary lipid, phosphatidyl choline (or lecithin), a nutrient found in egg yolks, red meat and processed meats, with increased production of intestinal microflora associated with an increased risk of heart disease by a team of Cleveland Clinic researchers.[29]

In 2008, Scripps Research Institute researchers identified metabolites in the cerebrospinal fluid of macaques infected with simian immunodeficiency virus that are associated with developing neurological disorders. Identifying the metabolites revealed important insights into the mechanisms of nervous system diseases.[30]
In 2009, Japanese researchers comprehensively profiled and quantified mouse blood metabolites that oscillate with circadian rhythms and then constructed a molecular timetable for their production. They were able to detect metabolite signatures for conditions that elicit jet lag in people, and their findings opened the door to the development of individualized therapy based on oscillation levels of hormones and metabolites in the human body clock.[31]

In 2012, a team of U.S. researchers from around the country reported new insights into the basis for neuropathic pain, a chronic disorder associated with tissue injury. The team, which included a metabolomics expert from the Scripps Research Institute, identified a previously unknown endogenous metabolite called N,N-dimethylsphingosine (DMS) in injured tissues.



***N,N*-Dimethylsphingosine (DMS)**

Reprinted in part from *C&EN*, 2012, 90 (4): 8

Their findings identify DMS production as a potential novel target to which inhibitors might be directed, and could lead to more effective drugs to treat the condition.[32] Later that year, metabolomics studies revealed important new details about how a 60-year-old tuberculosis drug, p-aminosalicylic acid, attacks the bacteria that causes the disease and may help researchers identify new drug targets.[33]

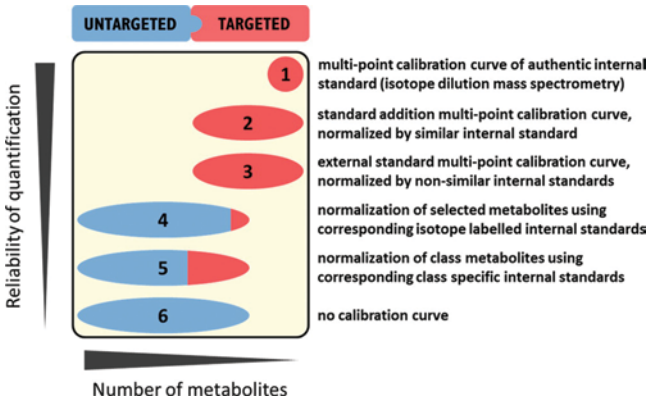
A 2014 discovery resulting from metabolomics studies on thousands of Estonian and Finnish biobank samples shows that four biomarkers, when combined, are significantly associated with increased risk of short-term death from all causes in the studied population.[34]

In 2015, metabolomics helped a team from Johns Hopkins University and the Scripps Research Institute reveal important details about the long-suspected role that bacterial biofilms play in colon cancer.[35] Later that year, researchers from the University of California at San Diego School of Medicine reported on a characteristic "chemical signature" for chronic fatigue syndrome, or myalgic encephalomyelitis, a complex disorder that is difficult to diagnose and treat. Experts believe that the work could lead to a diagnostic test for the syndrome and aid the understanding of its underlying biology and cause.[36]

Achievements propelled by metabolomics in 2016 include insights into why consuming whole grains may boost immune health and research that holds promise for aiding in the development of a blood test for endometriosis, a disease that conventionally can only be definitively diagnosed with surgery.[38]

III. TARGETED VS. UNTARGETED APPROACHES

There are two main approaches that exist for carrying out metabolic profiling: targeted and untargeted. Targeted, hypothesis-driven metabolomics approaches are focused on measuring defined groups of metabolites; this is also known as metabolic profiling. Untargeted metabolomics approaches, also known as metabolic fingerprinting, analyze for all detectable metabolites in a sample to provide a global view that can include identification of tens of thousands of compounds and enable the discovery of previously unknown compounds.[39]



Examples of targeted analyses, which reliably quantify a relatively small number of metabolites, include providing quantitative data on changes, such as in response to stress or disease. Targeted analyses are valuable for validating one or several hypotheses.[40] A recent example is the application

of targeted metabolomics to investigate the optimum growing conditions for enhancing the bioactive content of strawberries.[41]

The untargeted approaches, which quantify orders of magnitude more metabolites with lower reliability, are hypothesis-generating. The intention is to compare patterns or "fingerprints" of metabolites that change in response to disease, toxin exposure, environmental or genetic alterations, rather than to identify each observed metabolite.[42] Many methods used for untargeted analysis were developed by the Human Metabolome Project (HMP), which had the goal of characterizing all of the metabolites in the human body in a range of fluids and tissues, including blood, urine, saliva and cerebrospinal fluid.[2] Examples include the discovery of linking phosphatidyl choline (or lecithin) with an increased risk of heart disease, insights into jet lag, and new insights into neuropathic pain. In the future, experts predict that untargeted and targeted approaches may begin to merge.[40]

Equipment That Makes Metabolomics Possible

Metabolomics relies on analytical chemistry for identifying metabolites, and the reliability of the analytical data is a prerequisite for the correct interpretation of metabolomics analysis.[43] Analytical chemists face unique challenges in analyzing metabolomics data. Because metabolites can occur at a wide range of concentrations, from milli-Molar to femto-Molar, identifying them requires a group of instruments and therefore no single technology is all-encompassing.[2] Compounding the challenge is the reality that many of the compounds revealed by metabolomics were never previously catalogued.

While a variety of equipment can be, and is, used in metabolomic analyses, two main pieces of equipment are MS and nuclear magnetic resonance (NMR). MS is an intrinsically highly sensitive method for detection, quantitation, and structure elucidation of upwards of several hundred metabolites in a single measurement, and is used predominantly.[17,40] NMR is particularly powerful for targeted analysis because it is quantitative, reproducible, and suitable for complex samples such as blood, urine, or tissue extracts with little or no processing.[28] For both targeted and untargeted analyses, numerous techniques within MS and NMR offer multifaceted approaches to detect and identify a variety of metabolites, and measure their concentrations accurately. NMR has the benefit of being a detection technique that does not rely on separation of the analytes, so the sample can be recovered for further analyses. However, NMR detection limits are much higher than those of MS.

While some techniques exist for using only MS for metabolomics analyses, due to the complexity of the biological materials that metabolomics methods are

used to study, it can be necessary to separate metabolites of interest prior to MS acquisition.[17] The main chromatography methods used for separations that are typically coupled with MS for metabolomics studies are high-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE).[42] GC and LC are commonly used methods, while CE is particularly useful for polar metabolites and can be used with samples as small as a single cell.[28] HPLC has lower chromatographic resolution than LC, but it requires no derivatization for polar molecules, has no MW limitations, and separates molecules in the liquid phase. Additionally HPLC has the advantage that a much wider range of analytes can be measured with a higher sensitivity than GC methods. More recently, ultra performance liquid chromatography (UPLC) has also begun to be used for separations in metabolomics.[44,45]

A number of techniques can be used to impart the analytes being analyzed with a charge and transfer them to the gas phase. A common ionization technique applied to GC separations for metabolomics studies is electron ionization (EI) because it is amenable to low pressures. EI fragments the analyte, which can provide structural information but also increases the complexity of the data and may obscure molecular ions. Atmospheric-pressure chemical ionization (APCI) is an atmospheric pressure technique that can be used with HPLC, GC, and CE and is suitable for less polar compounds. Electrospray ionization (ESI) is most commonly used with liquid chromatography coupled with MS, and it works well with polar molecules with ionizable functional groups.[42] Matrix-assisted laser/desorption ionization (MALDI) is also sometimes used.[46]

The matrix-free desorption/ionization approaches that have been applied to the analysis of biofluids and tissues in metabolomics include secondary ion mass spectrometry.[47] Another matrix-free technique for analyzing biological samples is desorption electrospray ionization, which uses a charged solvent spray to desorb ions from a surface. More recently, ambient ionization methods in mass spectrometry have been developed that avoid the use of chromatography separation. Methods used in screening applications because they are fast and involve little or no sample preparation include desorption electrospray atmospheric ionization-mass spectrometry (DESI-MS), direct analysis in real-time-mass spectrometry (DART-MS), and extractive electrospray ionization-mass spectrometry (EESI-MS).[17] Other techniques used in metabolomics that, like NMR, require minimal sample preparation are Fourier transform infrared spectroscopy (FTIR) and direct infusion mass spectrometry (DIMS).[43] The advent of ambient ionization has opened avenues for in situ analysis of tissue specimens, which promises real-time diagnostic information and accurate surgical resection of tumors.[48]

Due to the reality that many compounds discovered through metabolomics studies have not been cataloged, researchers often use multiple platforms and multiple passes to identify samples by generating data that can be compared and contrasted. In a 2015 workshop held by the National Academies Standing Committee on Emerging Science for Environmental Health Decisions, a widely respected researcher reported that popular combinations of equipment include LC/MS coupled with EI, GC coupled with time-of-flight (TOF) detection MS, which is fast and has high resolution over a large mass range, and hydrophilic interaction LC (HILIC) with MS.[2]

Other tools that can help researchers in identifying compounds in metabolomic samples also include databases. The Metabolomics Society includes a webpage (*metabolomicssociety.org*) listing more than 30 databases that may be useful to researchers involved in metabolomics analyses.[49] These include comprehensive databases such as the free Human Metabolome Database (HMDB), containing detailed information including chemical data, clinical data and molecular biology/biochemistry data on small molecule metabolites found in the human body.[50] Compound-specific databases include PubChem, a free database listing chemical structures of small organic molecules and information on their biological activity, including structure, nomenclature and calculated physico-chemical data. Other types of databases listed on the Metabolomics Society web page include metabolic pathway databases, drug databases and disease and physiology databases.

IV. METABOLOMICS RESEARCH

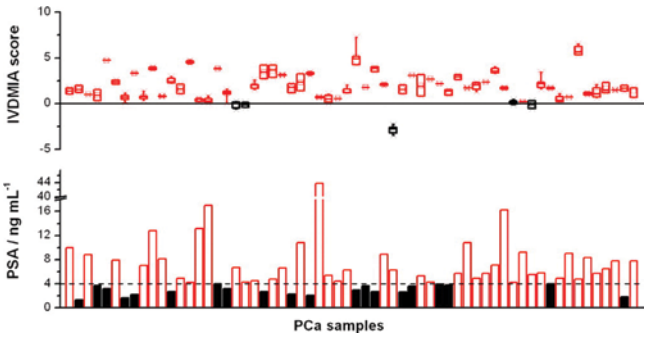
A special issue of *Analytical Chemistry*, published in 2015, for which Ian Wilson of Imperial College London served as editor, identified 30 important articles published on metabolomics and involving human health and disease in *Analytical Chemistry* and the *Journal of Proteome Research*.[51] The selections were inspired by Wilson's "current analytical love affair [with] developments in, and the application of LC–MS (especially where this involves the use of ultra (high) performance methods… [involving] studies in humans)." The highlighted work from *Analytical Chemistry* and highly cited work from other publications shows the breadth of research that is pushing the boundaries of metabolomics and what it can be used to discover.

Insights Into Cancers and Other Diseases

Since 2000, researchers using metabolomics have discovered biomarkers for more than 60 diseases; the 15 cancers in this list include breast, prostate, colorectal, and lung cancers.[52] The insights into cancer generated by metabolomics include

the identification of biomarkers in saliva linked to cancer. A team of Japanese and U.S. researchers conducted a comprehensive metabolite analysis of saliva samples obtained from 215 individuals (69 oral, 18 pancreatic and 30 breast cancer patients, 11 periodontal disease patients and 87 healthy controls) using capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS). They identified 57 metabolites that can be used to accurately predict the probability of being affected by each individual disease. The researchers report in *Metabolomics* that relatively higher concentrations of most of the metabolites were detected in subjects with all three cancers in comparison with those in people with periodontal disease and control subjects. This suggests that cancer-specific signatures are embedded in saliva metabolites, which hold potential for use as biomarkers in medical screening applications.[53]

A publication from the *Journal of Proteome Research* discussed the feasibility of detecting prostate cancer, the second leading cause of cancer-related mortality in men, through metabolomics screening using UPLC-MS. A Georgia Tech research team pointed out that the currently used prostate-specific antigen (PSA) diagnostic test suffers from low specificity, overdiagnosis, and overtreatment. They conducted untargeted metabolomic profiling of age-matched serum samples from prostate cancer patients and healthy individuals and analyzed them with UPLC-MS/MS and machine learning methods. They used what they found to develop a metabolite-based in vitro diagnostic multivariate index assay (IVDMIA) that their tests showed had 92.1% sensitivity, 94.3% specificity, and 93.0% accuracy, higher than the PSA test.[54]



Comparison of IVDMIA vs PSA diagnosis performance for 62 PCa patients. True positive and false negative outputs are highlighted in red and black, respectively. The cutoff point of 4.0 ng mL–1 used in PSA-based diagnosis is indicated with a dotted line. The IVDMIA score output is presented as box plots in the figure, each of which is generated by results obtained for each of the 10 test sets where each sample was selected for validation. No comparison is shown for two of the 64 PCa samples as they were not randomly selected in any of the 10 cross-validation iterations.

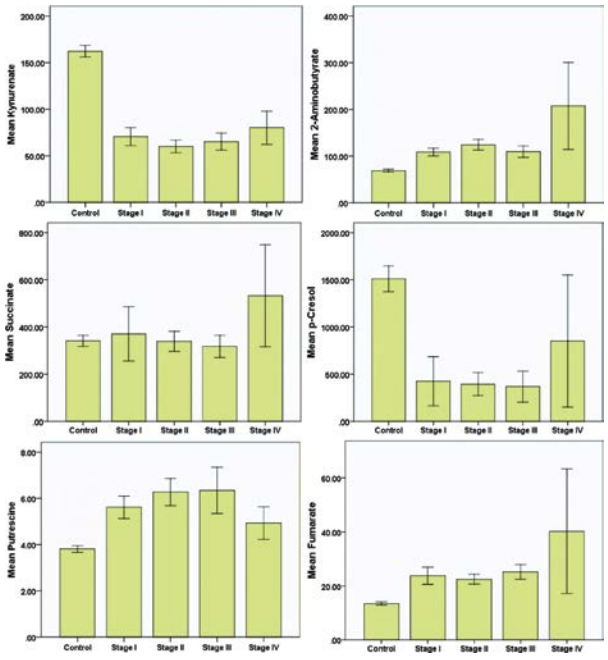
Reprinted in part from: *Proteome Res.*, 2014, 13 (7), pp 3444–3454

DOI: 10.1021/pr500409q

Publication Date (Web): June 12, 2014

To identify metabolites linked to colorectal cancer, a team from China and North Carolina identified urinary metabolite markers from a group of 101 subjects with colorectal cancer and 103 healthy controls, a follow-up to an earlier smaller study. The team used UPLC-qTOF-MS and, as they had previously, identified dysregulated metabolic pathways as well as gut microbial–host co-metabolism in subjects with colorectal cancer to identify a panel of metabolite markers capable of discriminating between subjects with colorectal cancer and their healthy counterparts.[55]

A paper by a team from St. Jude's Children's Research Hospital in Memphis and the Dana-Farber Cancer Institute in Boston reported the optimization of metabolome analysis by nanoflow UPLC coupled to high-resolution orbitrap MS and highlighted the technique's potential for detecting hundreds of small molecule biomarkers involved in cancer drug resistance. Their paper discusses how the technique performed in a case study of drug (bortezomib) resistant and drug-sensitive multiple myeloma cells.[56] Researchers from Thermo Fisher Scientific, Inc and UCLA described a new platform that couples capillary ion chromatography (Cap IC) with a Q Exactive mass spectrometer, which has been developed for metabolic profiling of head and neck squamous cell carcinoma (HNSCC) cells.[57]



Bar charts of mean intensity of six representative metabolites in urine samples of CRC patients with different stages and healthy controls.

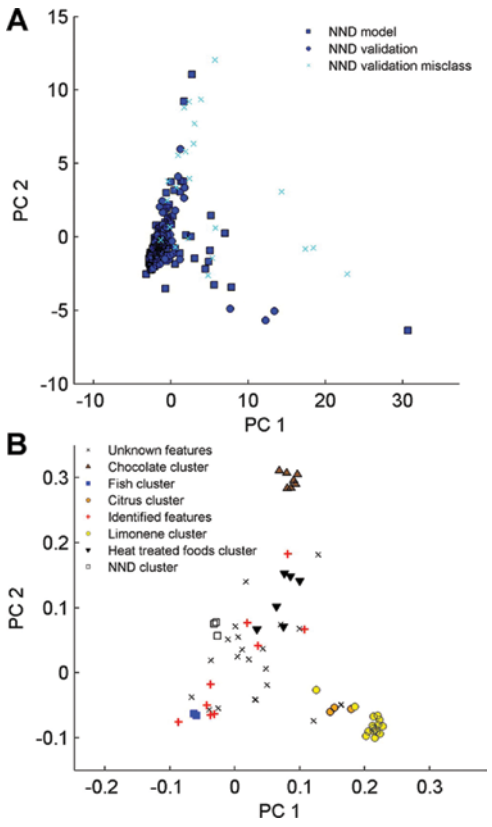
Reprinted in part from: *J. Proteome Res.*, 2012, 11 (2), pp 1354–1363

DOI: 10.1021/pr201001a

Publication Date (Web): December 9, 2011

Food and What We Eat

Thanks to the extremely wide variety of foods people can consume, 25,000 different compounds are known to exist in food. The food metabolome is very complex to analyze because of the differences in how what we eat is digested and biotransformed by our bodies. It comprises much greater chemical diversity than any other part of the metabolome. Food constituents can be metabolized in the body three different ways: digestion in the mouth, stomach and small intestine into simple nutrients that can be absorbed through the gut barrier; these constituents can be further transformed by the liver, kidneys, and other tissues; and can be processed by gut microbes in the large intestine.[58] The Human Metabolome Database tracks constituents in all of these categories.



(A) Score plot and (B) loading plot for PC 1 and 2 in a PCA including all New Nordic Diet (NND) samples (model and validation) and the selected features from partial least-squares discriminant analysis (PLS-DA).

Reprinted in part from: *J. Proteome Res.*, 2014, 13 (3), pp 1405–1418

DOI: 10.1021/pr400964s

Publication Date (Web): January 21, 2014

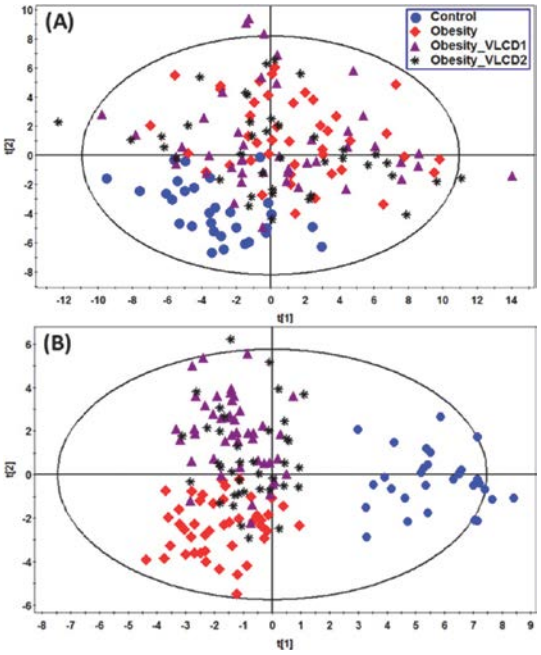
Examples of biomarkers associated with the consumption of foods are caffeic acid sulfate and methylepicatechin sulfate, which are associated with the consumption of raspberries.[59] Proline betaine is a marker of citrus intake, a result confirmed using untargeted metabolomics.[60] Researchers have shown that this marker can be used to identify noncompliant individuals in a dietary study.[61]

Diet and Nutrition

Researchers are also using metabolomics to investigate how what we eat impacts us. A team of researchers from Hawaii and China published insights into how very low carbohydrate diets may protect against the development of obesity. They used a metabonomics approach using UPLC-qTOF MS and GC-TOF MS to identify 113 metabolites that may

serve as biomarkers for evaluating health beneficial effects of dietary interventions involving such diets.[62]

Other recent work includes a method for using stool samples that may be able to identify the presence of Type-2 Diabetes,[63] one showing the metabolite profiles of obese patients to be gender dependent,[64] and a way to analyze urine samples using UPLC-qTOF-MS and a partial least-squares discriminant analysis to monitor compliance with diets in nutrition studies.[65]



(A) PCA and (B) OPLS-DA scores plot constructed with the 113 annotated metabolites.

Reprinted in part from: *J. Proteome Res.*, 2013, 12 (12), pp 5801–5811

DOI: 10.1021/pr4008199

Publication Date (Web): November 14, 2013

Breast Milk and Black Tea

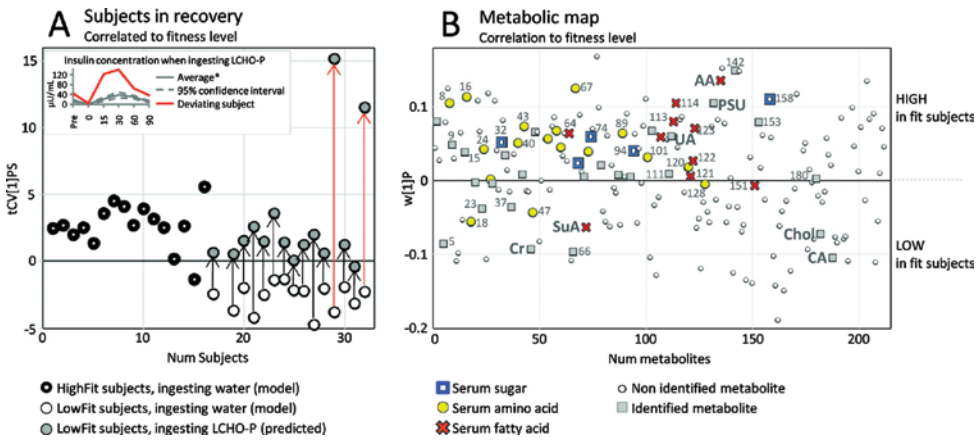
Researchers from London and Madrid described an approach to characterizing the metabolome of breast milk via a single-phase extraction followed by analysis using LC-qTOF-MS and GC-Q-MS for polar and lipidic metabolites.[66] A Dutch team reported their success in identifying, quantifying and assessing the gut microbial catabolites of black tea polyphenols, which have been hypothesized to exert beneficial cardiovascular bioactivity. They used an untargeted LC–MS-based metabolomics approach with a randomized, open, placebo-controlled, crossover study of 12 healthy men who consumed a single bolus of black tea extract (BTE) or a placebo. The relative and, in several cases, absolute concentrations of a wide range of metabolites were determined using UPLC-LTQ-Orbitrap-FTMS of the conjugated and unconjugated catechins and microbial catabolites in the men's plasma afterwards. Their work noted greater inter-individual variation in the gut microbial catabolites and it suggested that the catabolites may be particularly relevant to the proposed health benefits of black tea extracts.[67]

Food scientists are also using metabolomics to investigate qualities of foods, such as the molecular fingerprints of cheeses, wines, and other fermented foods

such as soy. Researchers have found ways to evaluate the quality, traceability and authenticity of some foods.[68] A team from Germany and Switzerland (at the Nestlé Research Center) even found a way to conduct a study which associates consuming dark chocolate with reduced anxiety.[69]

Optimizing Exercise and Oxidative Stress

A longstanding conundrum for exercise enthusiasts is how to best recover from intense bouts of exertion. Research by a Swedish team with a group of 24 men used a predictive metabolomics approach to investigate the impacts of consuming four different kinds of beverages (plain water; low carbohydrate; high carbohydrate; or low carbohydrates with proteins). Their findings suggest that consuming low-carbohydrate beverages with proteins help exercisers build muscle mass, particularly for less-fit subjects.[70]



(A) Cross validated scores from a calculated OPLS-DA model describing the separation of subjects with high and low fitness level in the early recovery phase when ingesting water, that is, samples taken 15, 30, 60, and 90 min after performed exercise. Subjects with high fitness level (HighFit) are plotted as black circles while subjects with low fitness level (LowFit) are plotted as white dots. Corresponding LowFit subjects ingesting low carbohydrate–protein beverage (LCHO-P) following exercise were predicted into existing model and plotted here as gray dots. The corresponding LowFit subjects' are connected with arrows. (Inset) Insulin concentration of LowFit subject with a deviating metabolic pattern when ingesting LCHO-P. (B) Regression coefficient plot, that is, a metabolic map explaining the differences related to subjects fitness level (w1P is the covariance loadings for the predictive component).

Reprinted in part from: *J. Proteome Res.*, 2009, 8 (6), pp 2966–2977

DOI: 10.1021/pr900081q

Publication Date (Web): March 25, 2009

A Canadian team used a differential metabolomics strategy involving capillary electrophoresis–electrospray ionization–mass spectrometry (CE-ESI-MS) to assess the efficacy of nutritional interventions to attenuate oxidative stress induced by strenuous exercise. They recruited a healthy volunteer to perform a submaximal prolonged ergometer cycling trial until they were exhausted, and collected blood from this subject frequently over 6 hours before, during, and after exercising. The subject performed this grueling exercise and testing regime twice, once after a high-dose oral intake of *N*-acetyl-l-cysteine (NAC). The test results showed that the NAC inhibited oxidative stress during the exercise.[71]

Environmental Exposures

Metabolomics is offering a window into the impacts of exposure to chemicals in the environment. An example is cadmium, a common pollutant which scientists have long been able to detect and measure in humans but have known little about the impacts of long-term exposure. A team of researchers from China and Singapore analyzed urine samples from 94 females aged 44–70 who had never smoked and with levels of cadmium ranging from 0.20–.68.[67] µg/L using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-ToF-MS) and gas chromatography–mass spectrometry (GC–MS). Their findings included that metabolites related to the metabolism of amino acids, galactose, and purine as well as the creatine pathway (creatine and creatinine), and steroid hormone biosynthesis were significantly higher among women with a urinary cadmium level higher than 5 µg/L.[72]

Using mice, a team from M.I.T. and the University of North Carolina at Chapel Hill detailed ways that exposure to arsenic can perturb the gut microbiome and its metabolic profile. Arsenic exposure affects large human populations worldwide and has been linked to a number of diseases, including cancer, diabetes, and cardiovascular disorders. The research was inspired by the recognition that the human intestine's enormously complex, diverse, and vast microbial community plays a profound role in metabolic processing, energy production, and more, and the gut's microbial diversity can be readily affected by external factors. The scientists exposed C57BL/6 mice to 10 ppm arsenic for 4 weeks in drinking water and analyzed the changes using 16S rRNA gene sequencing combined with mass spectrometry–based metabolomics profiling. The work showed that arsenic significantly perturbed the gut microbiome composition in the mice and also perturbed a number of gut microflora–related metabolites in multiple biological matrices.

Metabolomics analyses also show potential for helping scientists screen the tens of thousands of chemicals for which very little publicly available data exists, according to a 2011 paper. It reports the use of metabolomics to evaluate how exposure to 11

chemicals of known toxicity, as measured through the U.S. Environmental Protection Agency's ToxCast program, impacts the supernatant of human embryonic stem (hES) cell cultures. The testing initially predicted developmental toxicity from the blinded ToxCast compounds in concordance with animal data with 73% accuracy. Retraining the model with data from the unblinded test compounds at one concentration level increased the predictive accuracy for the remaining concentrations to 83%. The paper's authors say that their results indicate that metabolomics analysis of the hES cultures provides information valuable for predictive modeling and mechanistic understanding of mammalian developmental toxicity.[73]

Alzheimer's Disease

One of the highlighted papers published in *Analytical Chemistry* describes a new isotope labeling LC–MS method for biomarker discovery using mouse urine metabolomics. The paper originally published in the *Journal of Proteome Research* discusses the method's use in a study involving samples from the TgCRND8 mouse model of early onset familial Alzheimer's disease. The researchers from Canada's University of Alberta and its National Institute of Nanotechnology described using the technique to identify distinct metabolomic differences between the Alzheimer's prone mice and the wild type (control) group beginning as early as 15–17 weeks of age, when the mice were pre-symptomatic, to identify candidate biomarkers including ones previously found in metabolomics studies in human cerebrospinal fluid or blood samples.[74]

Identifying Active Compounds in Plant-Based Medicines

In addition to helping discover ways to improve growing conditions for some plants grown as crops, such as strawberries, plant scientists are using metabolomics in a number of other ways. This includes quality control of medicinal plants such as ginseng and St. John's Wort, investigation of the activity of medicinal plants including Echinacea, studies of genetically modified plants like maize and rice, and interrogating the interactions of plants with other organisms.[75]

V. OCCUPATIONAL OUTLOOK

At present, many metabolomics jobs are at academic research centers with government funding, consulting firms, and with chemical equipment manufacturers. The market forecasts for metabolomics research suggest that it is a very hot area with a rapidly growing number of associated professional opportunities. Market researchers' observations that the field is being held back by the need for well-qualified researchers suggests that this is an ideal time to consider entering it. Mass spectrometry is used extensively in metabolomics, and mass spectrometry market forecasts also predict a lack of skilled users as a key market constraint.[11]

The job outlook for analytical chemists with the skills needed to research metabolomics is arguably closer to that for medical and clinical laboratory scientists, technologists and technicians, which the U.S. Bureau of Labor Statistics says is growing at 14% per year,[76] than that of chemists and materials scientists. The U.S. Bureau of Labor Statistics says that the outlook for chemists and materials scientists, which conventionally includes analytical chemistry, is growing at 3% per year.[77] A recent article reported that demand for analytical chemists is so great that some experts worry whether academia can produce enough new researchers to keep pace with industry's growing needs. Analytical chemists with interdisciplinary backgrounds are finding an even greater demand for their skills by employers.[78]

"It's a great time to be an analytical chemist," Dan Shine, president of chromatography and mass spectrometry at Thermo Fisher Scientific, told *C&EN*. The company employs roughly 4,600 scientists and engineers, including analytical chemists. "We typically have hundreds of positions open at any one time," he says. Mary Ellen McNally, Technical Fellow at DuPont, says that the hiring of analytical chemists at DuPont has been on the rise, but finding qualified candidates remains a challenge. "We have closed out searches and started over because we are looking for highly qualified candidates," she says.

One of the reasons behind the growth of analytical chemistry jobs is that industry is recognizing the value that analytical chemists bring to solving challenging problems, says Xiaoli Wang, an R&D manager in the CrossLab Group at Agilent Technologies. Stephen Scypinski, vice president of analytical and bioanalytical development at Bristol-Myers Squibb, says: "Analytical chemistry is [now] an integral part of the development organization."[78]

Another factor explaining why the demand for analytical chemists is growing is the reality that analytical instruments are becoming more powerful. "We're generating tons of information, but how we make sense of that information is critically

important as well," says Thermo Fisher's Shine. "Both of those factors open up more applications, which opens up more jobs."

The NIH's Metabolomics program, which is funded through 2021, is actively aiming to increase the U.S. capacity in metabolomics by supporting the development of next generation technologies to enhance the sensitivity and speed with which specific elements of the cellular metabolome can be identified and quantified. The program also provides training and mentoring opportunities and promotes data sharing and collaboration.[79] The Metabolomics Society points out that many other government bodies are also supporting metabolomics activities internationally.[80]

VI. CONCLUSION

Recent developments in metabolomics show that the field continues to innovate. "Recent technological advancements — especially those made just in the past two years — have revealed that individual cells within the same population may differ dramatically," says Ananda Roy, program leader of the NIH Common Fund working group for single-cell analysis in Bethesda, Maryland. Jonathan Sweedler, an analytical chemist at the University of Illinois at Urbana–Champaign says: "We are getting close to making single-cell metabolomics robust."[81] The ability to detect metabolites located in subcellular compartments while leaving most of the cell intact was recently reported by researchers at George Washington University.[83] Another notable innovation was reported by researchers from Leiden University in the Netherlands, who devised a technique to use microfluidics chips to quickly extract scarce metabolites for metabolomic analysis. Advances in imaging metabolites using NMR, magnetic resonance spectroscopy (MRS), positron emission tomography (PET) can permit non-invasive metabolic imaging for medical diagnosis.

Another reason for optimism is because experts believe that metabolomics may offer a far more cost-effective and productive route to drug discovery, testing and development. This is because many of today's most prominent diseases (such as heart disease, diabetes, obesity, hypertension, depression and inflammatory bowel disease) have a strong metabolic basis or a clear metabolic cause. Additionally, many chronic diseases (including autism, schizophrenia, asthma, cancer and Alzheimer's disease) are being found to have unexpected or unappreciated metabolic causes of associations.[84] An example of a young chemist working in metabolomics is Morgan J. Cichon, who holds a Ph.D. from Ohio State University in food science and technology and was recognized as a SciFinder Future Leader in 2017.[85] She is currently working on the Personalized Food & Nutritional Metabolomics for Health

initiative at Ohio State University. "As I work to define my future career, I know I want to be involved in communicating science on a national and global scale," she says. "I envision a role in which I can utilize my financial economics degree in conjunction with my chemistry background to be a leader in the field and drive important scientific research in an unpredictable economic climate."

Chemists like Cichon are in an ideal position as the discipline moves forward to aid in the development of precision medicine and identifying the metabolic attributes that we associate with health,[52] as well as using it in other fields such as drug discovery, food and plant sciences. Metabolomics pioneer David Wishart of Canada's University of Alberta is one of the experts who believes that metabolomics has the potential to revolutionize drug discovery by dramatically improving the success rate and decreasing the time and cost associated with identifying new therapeutic molecules.[3,86] Caroline H. Johnson of the Yale School of Public Health's Department of Environmental Health Sciences, who uses metabolomics in her research, predicts that a decade from now, "metabolomics will be fully incorporated into most areas of scientific research."[86] Metabolomics is a research area where chemists are truly making a difference.

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