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Sum of the Parts: Mass Spectrometry-Based Metabolomics

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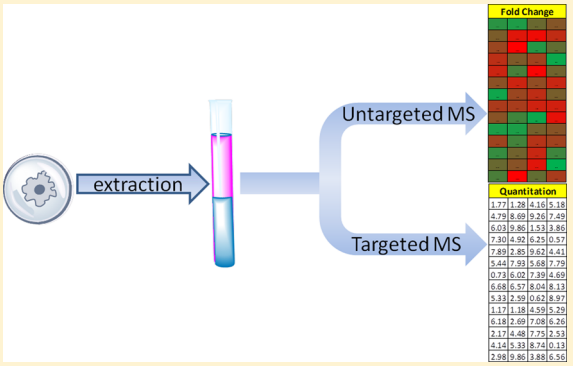
ABSTRACT: Metabolomics is a rapidly growing field of research used in the identification and quantification of the small molecule metabolites within an organism, thereby providing insights into cell metabolism and bioenergetics as well as processes important in clinical medicine, such as disposition of pharmaceutical compounds. It offers comprehensive information about thousands of low-molecular mass compounds (<1500 Da) that represent a wide range of pathways and intermediary metabolism. Because of its vast expansion in the past two decades, mass spectrometry has become an indispensable tool in "omic" analyses. The use of different ionization techniques such as the more traditional electrospray and matrix-assisted laser desorption, as well as recently popular desorption electrospray ionization, has allowed the analysis of a wide range of biomolecules (e.g., peptides, proteins, lipids, and sugars), and their imaging and analysis in the original sample environment in a workup free fashion. An overview of the current state of the methodology is given, as well as examples of application.



During the past decade, metabolomics has become increasingly utilized as a tool in systems biology analyses and has been considered the latest of the "omics" technologies that could provide the most functional information for understanding biological systems. Comprehensive and quantitative study of small molecules (metabolites) as a readout of biological processes is the focus of metabolomics. The metabolome can be thought to encompass the small molecular building blocks (e.g., nucleotides, sugars, and amino acids), metabolic intermediates (e.g., fatty acids), and structural and signaling elements (e.g., lipids) that lie outside of cellular encoding mechanisms (genome, transcriptome, and proteome). Unlike genomics, transcriptomics, and proteomics, where changes are not always associated with a different phenotype, metabolites are small molecules controlling cellular metabolism and represent functional entities that reveal the physiological, pathological, or developmental status of a biological system. More than two decades of experience with the lipidome, a subset of the metabolome that can be extracted from the organic layer in a physical separation, have been devoted to optimizing the resolution of specific classes of lipid molecules. It has become apparent that each class, because of its particular chemistry, requires a unique set of preparatory and chromatographic techniques. This is also the case for metabolite analyses as a whole, as we expect a similar set of chromatographic challenges based on their similar chemical nature. However, the number of analytes associated with cellular metabolism will be substantially greater than for lipids alone, so the field faces an even more daunting challenge.

TARGETED AND UNTARGETED METABOLOMICS

Unlike other omics technologies, metabolomics is presented with the challenge of immense chemical diversity and a number of small molecules in addition to the presence of many experimental artifacts. Some have conjectured that up to 200000 metabolites exist in plants and more than 100000 small molecules exist in humans from the intake of foods and drugs.[1,2] In addition, the wide array of environmental chemicals, microbes, and viruses present in everyone can significantly influence the human metabolome and, thus, considerably increase the total number of metabolites.

Liquid chromatography−electrospray mass spectrometry (LC−ESI-MS) has become a method of choice for metabolomics studies as it allows ionization of a large number of metabolites with minimal fragmentation. The larger portion of scientific publications focuses on "targeted" metabolomics investigation as it involves analysis of metabolites for which there are synthetic standards. Intracellular metabolites are sometimes labile compounds and thus require specific quenching and extractions, which presents a significant challenge given the chemical diversity, instability, and rapid turnover of the cellular metabolites. Metabolite analysis requires tailoring sample preparation, extractions, and analysis methods to afford appropriate analysis in spite of a diverse metabolite concentration range, polarities, and other matrix-dependent variabilities.[3−5]

Targeted metabolomics relies on a pre-established strategy used for metabolite identification [e.g., selected reaction

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monitoring (SRM) by tandem mass spectrometry] and is used for quantitative determination of a set number of metabolites with known and expected chemistry, thus providing information about a specific pathway. Examples of this for multiple classes of metabolites can be found in Table 1. An important step in sample preparation and analysis that has already been implemented in lipidomics analyses is instrument calibration.[24] It is achieved by use of internal (added before extraction) or external (added after extraction) standards that are used to minimize the variability in different samples' preparations and also matrix effects (e.g., tissue, biological fluids, cell preparation, etc.). An excellent review of the application of targeted metabolomics for biomarker identification and qualification has recently been published.[25]

Table 1. LC−MS and GC−MS Protocols for Analysis of Various Metabolite Classes

metabolite class	MS platform	refs
glycerophospholipids	LC−MS	6, 7
eicosanoids	LC−MS	8−11
fatty acids	GC−MS	12
sphingolipids	LC−MS	13, 14
sterols	LC−MS	15
cholesterol esters	LC−MS	16
MAG/DAG/TAG	LC−MS	17, 18
TCA cycle	LC−MS	19
glycolysis cycle	LC−MS	19
nucleotides	LC−MS	19
CoAs	LC−MS	19
sugars	GC−MS	20
pyrophosphates	LC−MS	21−23
amino acids	GC−MS	20

According to Siuzdak, untargeted metabolomic methods "are global in scope and have the aim of simultaneously measuring as many metabolites as possible from biological samples without bias".[26] As opposed to targeted metabolomics that might concentrate on only one pathway with ≤20 analytes, untargeted metabolomics, or metabolite profiling, can involve spectra with thousands of peaks. Experiments of this nature are used to observe global differences between sample types that might lead to potential targets for a pathway of interest, disease states, or the identification of new classes of metabolites.[27−29] Targets of interest can be further analyzed following fraction collection and more extensive structural characterization using methods such as nuclear magnetic resonance (NMR) and MS^n.

In situ labeling of compounds using techniques such a bioorthogonal ligation can also be of utility in compound and pathway elucidation. Azide, alkyne, or biotin tags, to name a few, can be used to identify trace level species, to determine spatial localization within a cell, and to follow compounds through their metabolic pathways.[30−41] In one such application, an alkyne tag has been used to conjugate and visualize cross-linked proteins.[42] Other researchers have used ^{13}C labeling to better distinguish de novo fatty acid synthesis from elongation[43] and to confirm a novel ethylmalonyl-CoA pathway for methanol assimilation in a bacterium by tracking the isotopically labeled intermediates.[44]

SAMPLE ANALYSIS

Optimized Extraction for a Specific Class. Analyses in metabolomics are presented with a number of challenges coming from the nature of the metabolites: diverse chemical properties, rapid turnover, and mixed abundance. These issues determine the necessity of special quenching and extraction methods. A valuable review of methods for quenching and extraction of certain metabolites from a mammalian cell culture was recently published.[45] On the basis of the evaluation of different extraction and quenching methods, it was apparent that the extraction protocol should quantitatively extract as many metabolites as possible without causing chemical or physical degradation. The polarity of the extraction solvents should be suitable for the solubilization of both polar and nonpolar materials, and using highly polar, alkaline, or acidic solvents should be avoided. In this study, it was also determined that to obtain a true "snapshot" of the present metabolites a good quenching method is needed using a solvent that does not damage the cell membrane or cause leakage.[46]

Multiple Species Extractions. To extract metabolites with different polarities in a partially untargeted manner, a three-phase solvent system can be utilized.[47] Solvents such as *n*-

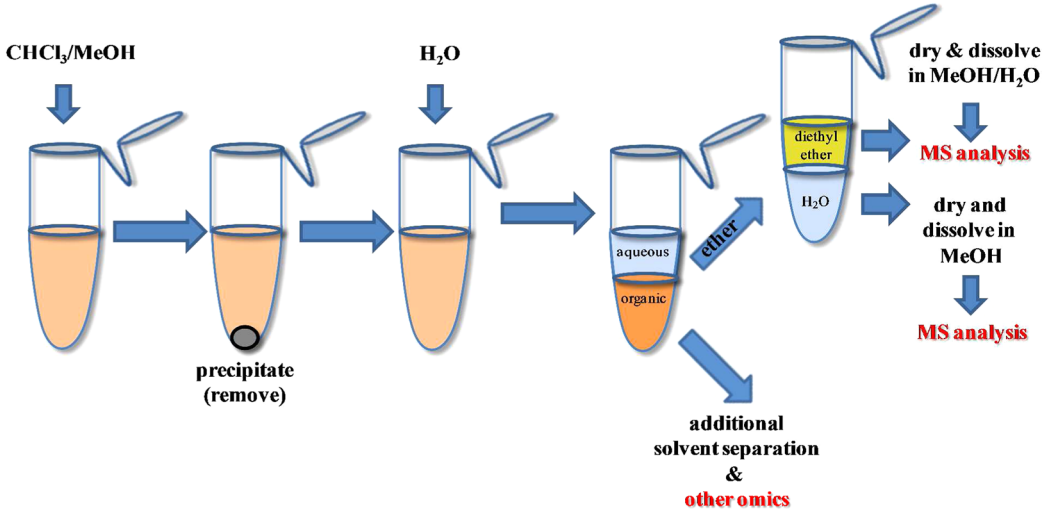

Figure 1. Representation of a "three-phase" extraction technique. Using methodology of this type, a full range of organic and aqueous partitioned metabolites can be recovered.

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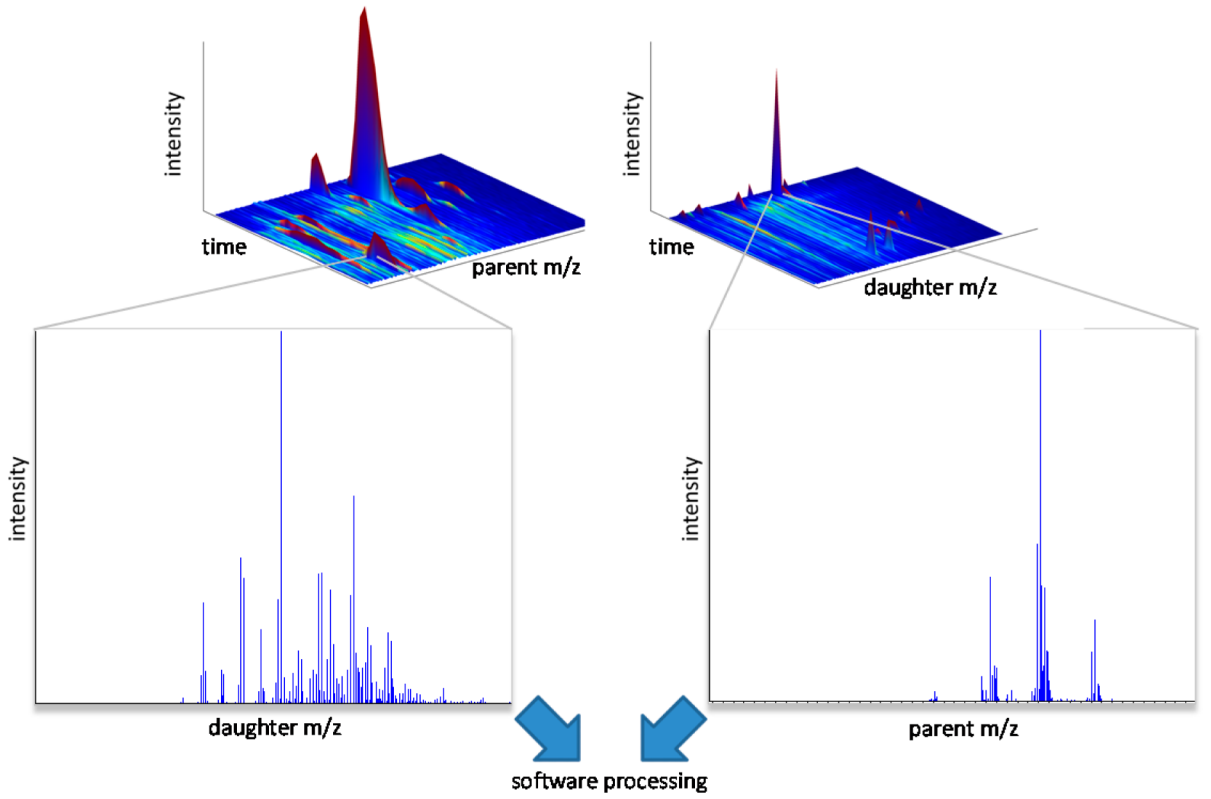

Figure 2. "Fragment-everything" data mining approach. By acquiring MS/MS data for every parent and/or daughter ion, one can, postanalysis, reconstruct precursor ion scan, neutral loss scan, and MRM type experimental data, which greatly enhances the ability of the analyst to identify metabolites within a sample. Rapid scanning technology in advanced commercial mass spectrometers is allowing analyses that can cycle intermittently over (left) all possible parent ion m/z values to construct spectra of daughter ions and (right) scans through all possible daughter m/z values to construct spectra of parent ions.

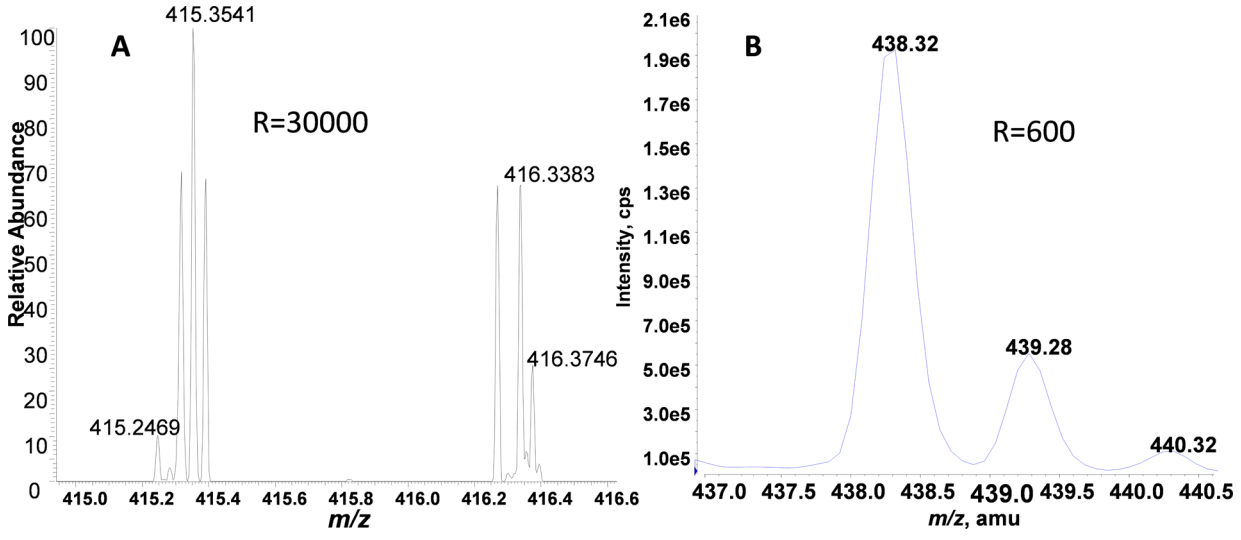

Figure 3. Comparisons of resolution between high- and low-resolution instruments. (A) High-resolution lysolipid spectra obtained on a Thermo Fisher Orbitrap with a resolution of 30000. (B) Low-resolution lysolipid spectra obtained on an AB Sciex 4000 QTrap with a resolution of 600. Orbitrap spectra are courtesy of D. Hachey and the Vanderbilt MSRC Mass Spectrometry Core Lab.

hexane, methyl acetate, acetonitrile, and water in a 4:4:3:4 ratio formed an organic/aqueous solvent mixture with three mutually immiscible phases. It can be used as such for extraction of hydrophobic compounds (upper phase), moderately polar compounds (middle phase), and polar compounds in the aqueous phase (lower phase) (Figure 1). This system can also be utilized in addition to the "traditional" aqueous/organic extraction when a more comprehensive analysis is needed (e.g., lipids, etc.). The lower layer of a traditional Bligh–Dyer extraction[48] can be separated in parts for analysis of lipids, and the rest can be subjected to a three-phase extraction for metabolite analysis.

Another approach for more comprehensive extraction of metabolites that excludes components such as proteins or lipids

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that are not intended for analysis is the use of a biphasic system in which solvent volumes, ratios, and aqueous solvent pH values are carefully considered.[49] This method is based on a traditional organic/aqueous liquid−liquid extraction but includes pH modifications to improve acidic and basic metabolite extractions. Addition of acid to the aqueous phase allows better solubility of the compounds containing basic groups, while a dilute base aids solubilization of acidic metabolites. Formic acid and ammonium hydroxide are often used to adjust the pH of the aqueous phase because of their volatility and compatibility with LC−MS techniques. Utilizing multiple extractions (of the same sample) at different pH values of the aqueous component improves recovery and allows for a more comprehensive analysis of metabolites. In conclusion, depending on the type of analysis and the nature of the metabolites, one may have to extract a sample by multiple methods to obtain a comprehensive representation of its metabolic composition.

■ INSTRUMENT PLATFORMS

The majority of publications in the field of mass spectrometric metabolomics over the past decade have utilized HPLC−MS- or UPLC−MS-based systems because of their wide metabolome coverage using ionization methods such as ESI and MALDI. Recently, more groups have moved to UPLC−MS because of the shorter (90% less) run times and better peak separation inherent with these systems. Similarly, newer-generation mass spectrometry instruments with very high scan rates can detect several-fold more analytes per run than slower detectors present on older triple-quadrupole and ion trap instruments. This advance in technology now makes possible highly data-dependent processes such as SWATH (sequential window acquisition of all theoretical fragment-ion spectra), GPS (global precursor ion scan mode), or MS^{all}.[50−53] These techniques maximize metabolite detection by essentially collecting MS/MS data for every peak that can be used postrun to reconstruct MRM, neutral loss, and precursor ion scan-like spectra (Figure 2). Exponentially more analytes can be detected and quantitated using a high-resolution mass spectrometer compared to a triple-quadrupole or ion trap instrument. As an example, Figure 3 illustrates this point with lysolipid spectra obtained on a low-resolution AB Sciex 4000 Qtrap and a high-resolution Thermo Fisher Orbitrap mass spectrometer. In this case, the Qtrap-generated peak has a resolution of 600, whereas the Orbitrap peaks have a resolution of 30000. Under these conditions, one could easily have ≥10 baseline-separated peaks per m/z unit in an Orbitrap spectrum, whereas only one combined peak (containing multiple species) per m/z unit can be obtained on an ion trap (fwhm of m/z 0.6 compared to fwhm of m/z 0.015). The unambiguous identification of parent metabolites from their MS/MS spectra is greatly enhanced by the use of higher-resolution instruments.

GC−MS Platforms. GC−MS-based metabolomics, a method complementary to LC−MS-based metabolomics, has been pioneered by the Fiehn group, who were among the first to use the term "metabolomics".[54,55] The benefits of using a GC-based platform have been eloquently described by Hankemeier:[20] "As the full scan response in EI mode is approximately proportional to the amount of compound injected, *i.e.* more or less independently of the compound, all compounds suitable for GC analysis are detected non-discriminatively." "Furthermore, problems with ion suppression of co-eluting compounds as observed in LC−MS are virtually absent in GC−EI-MS".[56] GC−MS and especially GC/GC−MS can detect a large number of species. One drawback to this platform is the need to derivatize samples, which can lead to less certainty in compound identification in GC−MS spectra (unknown number of methoximated and trimethylsilated positions) and can also lead to the loss of sample during derivatization. Because the detected GC metabolite set does not totally overlap with that observed by LC−MS, the use of both LC- and GC-based methods, especially for untargeted metabolomics, would greatly increase the coverage of the metabolome.

Other Technology. MALDI-TOF, which does not require extraction, can be used on intact biological samples. Dorrestein has demonstrated the utility of this technique in the monitoring of polymicrobial infections.[57] Using MALDI-TOF, he was able to map the metabolites and signaling molecules between or from different colonies. Additional examples of MALDI-TOF imaging experiments can be found in the recent reviews by Murphy and Wariishi.[58,59] DESI-MS is another useful technique that can be employed to monitor biological samples (such as bacterial colonies).[60] One drawback of DESI is its spatial resolution of 200 μm compared to the resolution of 20 μm for MALDI. Also, whereas abundant molecules in biological samples can easily be detected using DESI, lower-abundance species and low-molecular mass metabolites have not been widely reported using this method.[59] Recent advances in nanoDESI technology have led to substantial improvements in spatial resolution (<50 μm). Once this technology is readily available, this should greatly improve the capabilities of DESI imaging.[61]

LC−MS platforms have difficulty measuring isobaric and structural isomers. Ion mobility coupled to MS (IM−MS) can routinely separate structurally similar molecules and at least double the peak capacity of a MS instrument with MALDI as the ionization source.[62−64] Unlike traditional mass spectrometry, ions in IM−MS travel through a pressurized chamber containing a neutral gas. The mobility of ions through this chamber is governed by the size and structure of the compound. Subtle changes in structure within isobaric pairs, such as leucine and isoleucine, lead to different times for the transition through the ion mobility chamber. Using this technique, small molecules, peptides, and lipids can routinely be separated from the same sample (Figure 4). In addition, it has been shown that using ESI-IM-TOF-MS, peak capacity can be increased 10-fold compared to that of MS alone.[62,63]

■ MS ISSUES (RELATED TO DATA ANALYSIS)

From Spectra to Quantified Results. While there is a wide variety of unique molecules that are commonly encountered in metabolomic analysis, the number of non-natural internal standards available is far smaller. Perhaps the most significant challenge to quantitation of metabolomic data by mass spectral techniques stems from this disparity. In practice, once a particular subset of metabolites within a pathway or class is targeted for quantitative analysis, whether in the absolute or relative to defined benchmarks but without rigorous quantitation, suitable standards that elute and ionize relatively similarly to the molecules of interest must be used. The coverage that one particular standard affords across a swath of metabolomic analytes will necessarily vary. The side chains of larger biomolecules have a tendency to interfere with primary ionization sites for deprotonation, such as for lipids,[6,7,65,66] fatty acids, larger polypeptides, or multiply sugared conglomerates

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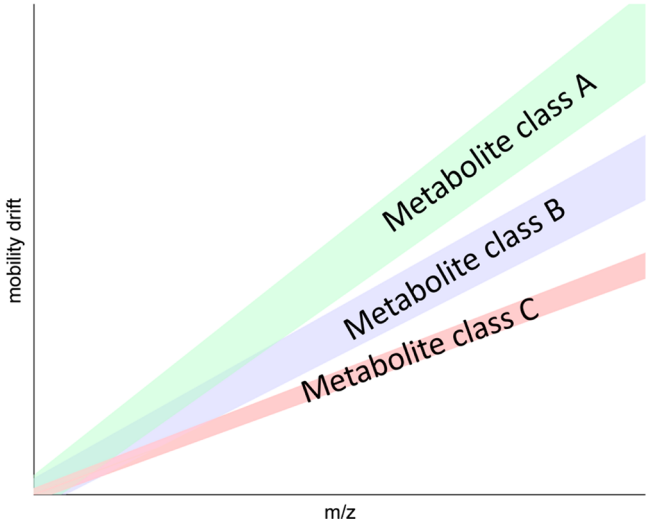

Figure 4. Ion mobility mass spectral analysis of a complex mixture can be used to separate classes of metabolites (class A, class B, and class C) based on ion mobility drift time. Diagram patterned after results obtained by Kliman et al. (Figure 2),[63] based on a methanol extract of whole rat blood, yielding a signal dominated by peptides, lipids, and other metabolites.

containing several backbones. In such cases, considerable care should be applied in determining whether coverage by the selected standards has truly been achieved. Classes that allow for each constituent to be monitored with an accompanying standard exist[12,15,67] but are the exception and not the rule. Pathways with a limited number of common chemical fragments provide appealing targets for adequate coverage by a small number of standards.[68] An example of the problems that can occur when multiple analytes share common MRM pairs can be seen in the analysis of sterols (Figure 5). In this case, four sterols share common MRM pairs but are resolved by HPLC. Without deuterated standards for desmosterol and zymosterol, unambiguous lipid identification during an LC−MS run would be especially challenging for these nearly coeluting isobaric compounds.

Methods for handling peak integration depend on the mode of MS operation (e.g., feature extraction in untargeted[69−72] vs analysis of peak robustness in targeted applications[73,74]) and cannot be ignored. Because of the nature of the types of questions posed, metabolomics is often concerned with quantitative analysis of low-abundance molecules, and assessment of peak quality merits attention.[75] First, full scan information should be retained even for selective reaction monitoring (SRM) data collection, as the level of the background may be substantial for certain extraction protocols and can thereby be monitored more completely, and because the presence of nearby isotopic peaks to analytes of interest may not be as obvious in SRMs alone without examination of the full spectrum. Similarly, high-resolution MS[76] provides the opportunity to retain peak shape information that could indicate interference from different metabolites with nearby/identical mass and/or retention, and thus, capturing the spectra in this format is preferable to data that have already been centroided.[74]

Evolution of Database Approaches. For several years, considerable effort has been spent cataloging information about metabolites in publicly accessible databases with approaches tailored to multiple goals. Searchable indices for unknowns within mass ranges such as HMDB, Metlin, and LipidMAPS[2,77−81] provide one level of interface, while various pathway-oriented solutions can allow researchers to leverage larger-scale patterns among several analytes and/or peaks in their data simultaneously. Examples of such platforms include

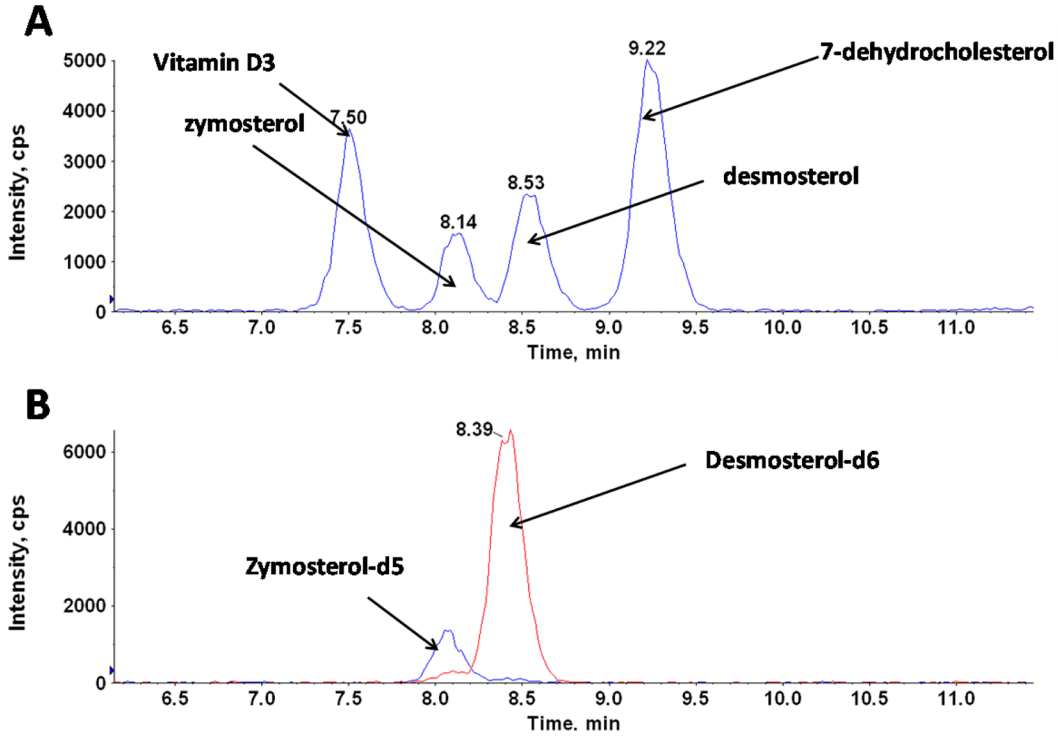

Figure 5. Utility of using internal standards. Four isobaric sterol MRM peaks that are baseline-separated are shown in panel A. Without appropriate internal standards (B), it would be difficult to accurately identify zymosterol and desmosterol by this analysis.

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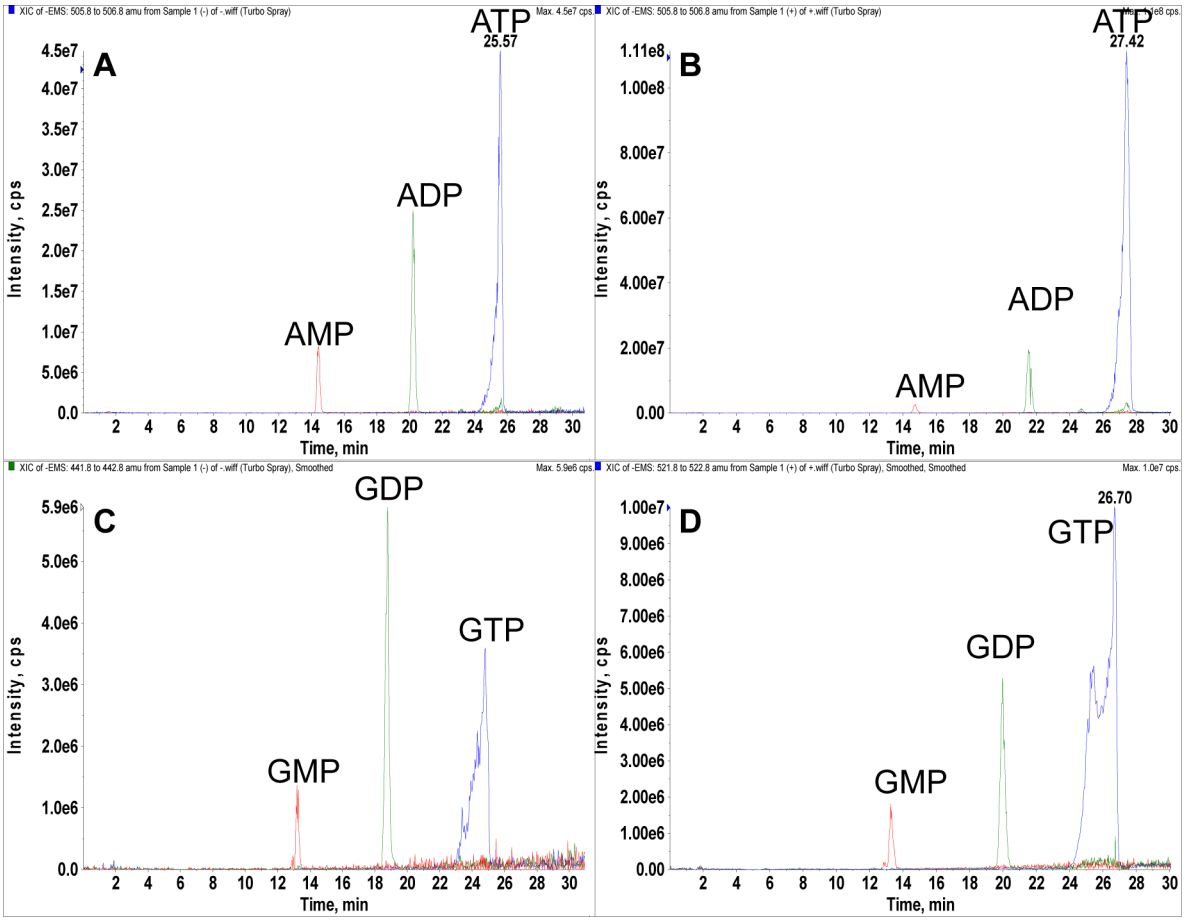

Figure 6. LC−-MS analysis of nucleotides. HEK cells treated with deoxyglucose (A and C) have a dramatically different nucleotide phosphate pattern compared to that of HEK cells treated with glucose (B and D).

KEGG, BioSpider, and MetaCyc.[82−84] While most of the projects mentioned above have typically been scientifically organized as top-down initiatives with editorial control being somewhat centralized, a fully user-driven community library approach to warehousing relevant data and metadata (e.g., MS/MS spectra, pathway involvement, structure drawing, etc.) may be extremely useful.

Further development of tools that facilitate hypothesis-driven research by bringing together the ability to track and quantify trends in both known (and/or quantified) and unknown analytes of interest will be needed to best take advantage of data being collected in the context of interrogating specific metabolic pathways. Algorithms that analyze similarities between unknown peaks in metabolite profiling spectra to assess a correlative[70,85,86] or more heuristic quasi-match with a curated or community-based database of known MS/MS data[87,88] are examples of ways in which the full extent of data sets can be used to place untargeted scanning within a more pathway- or network-oriented context.

■ EXAMPLE OF APPLICATIONS TO PATHWAYS

Technological advances in LC−MS analysis have vastly improved the resolution of small molecule metabolites from complex biological mixtures. While these improvements have only recently allowed for the observation of metabolic flux within a system, the concept of metabolic phenotypes in disease is not new. Perhaps the most well-established metabolic phenotype is the improved "aerobic glycolysis" observed in cancer cells, known widely as the Warburg effect.[89] The increased resolution provided by ultrasensitive mass spectrometers and the decreased run times afforded through the introduction of UPLC have made possible real-time analysis of these complex metabolic phenotypes. The information gathered with these technologies has made the deconvolution of complex and previously unknown metabolic phenotypes readily discernible. Ultimately, understanding these metabolic pathways is essential for the development of future disease treatments.

In particular, altered bioenergetic pathways serve as a potential target to slow or halt the growth of hyperproliferative diseases. Hyperproliferative cell types, such as cancer, rapidly consume ATP relative to the surrounding tissue to meet the energetic requirements of rapid cell division. This metabolic difference is regularly used in the clinic to image tumors with ^{18}F positron imaging.[90] Increased metabolic demands render cells sensitive to slight alterations within a specific bioenergetic pathway, and this has been put forward as an "Achilles heel" of tumorigenesis.[91] HPLC−MS-based quantification of ATP:ADP:AMP ratios can be used to identify differences in cellular bioenergetics (Figure 6).

To understand alterations in metabolic flux and energy production in hyperproliferative cell types, a recently published study[92] melds the evolving metabolomics methodologies discussed in this review to functionally characterize a conserved bioenergetic mutation in glioblastomas. Isocitrate dehydrogenase (IDH) normally functions within the TCA cycle to convert

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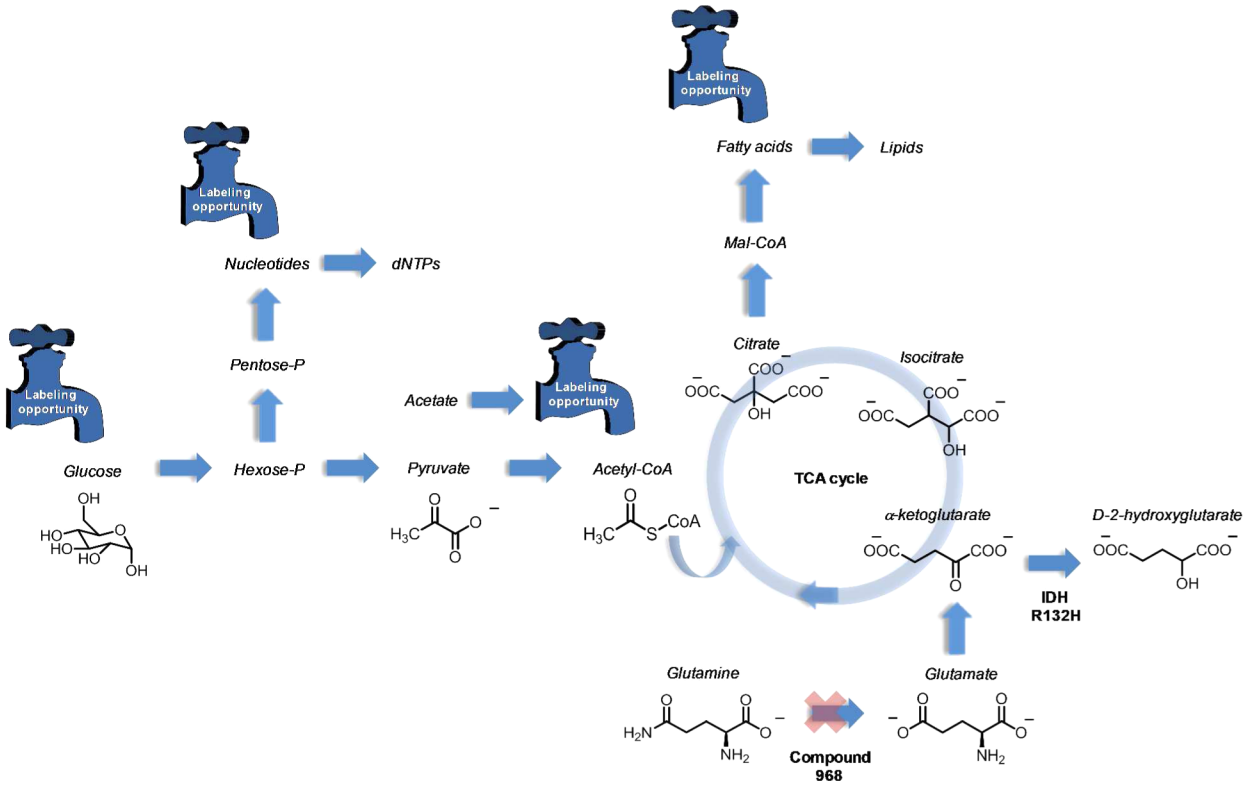

Figure 7. Studying metabolic flux by use of labeled inputs. Glycolytic and TCA cycle analytes are particularly amenable to labeling of initial metabolite pools using stable isotopes (e.g., [^{13}C]glucose or [^{13}C]acetate) and subsequent monitoring of intermediates and bottlenecks in those subsystems. Other similar opportunities exist as well; incorporation of fatty acids into lipids, ^{15}N labeling of nucleotide precursors of deoxynucleotides, and bioorthogonal approaches are all examples. Cells with increased glutaminase activity can be identified by supplementation of isotopically labeled glutamine during introduction into the TCA cycle. Dang et al.[92] monitored TCA cycle metabolites to functionally characterize the R132H mutation in isocitrate dehydrogenase in gliomas. The product, D-2-hydroxyglutarate, has been implicated in affecting oncogenic transformation, although its precise role is undefined.[97,98]

isocitrate to α-ketoglutarate (αKG). This oxidation event yields 1 equiv of NADH, which proceeds to the electron transport chain for ATP production. In humans, IDH is expressed as five distinct isoforms.[93] In a genome-wide study of human gliomas, the IDH1 isoform was found to harbor a specific and conserved point mutation in up to 12% of the gliomas screened.[94] This mutation was tied to a corresponding amino acid replacement (R132H) in the active site of the enzyme. Despite the striking conservation of this mutation, the functional implications are unknown.

Functional characterization of the R132H mutation observed in IDH1 required a multidisciplinary approach. Cells were first transfected with a dominant-negative IDH1 construct harboring an R132H mutation.[92] An upfront nontargeted metabolomic analysis was performed on these cells using a stand-alone Orbitrap mass spectrometer. The increased mass sensitivity afforded by these instruments allows for the calculation of exact chemical masses of metabolites for identification, oftentimes to the fourth of fifth decimal place. Via coupling of this technology with a broad-spectrum extraction and reversed phase HPLC analysis recently reported by Rabinowitz and co-workers,[95] a peak was rapidly identified corresponding to 2-hydroxyglutarate (2HG). As a secondary confirmation step, the flux of 2HG within cells was verified through a targeted and more quantifiable LC–MS method. This analysis was performed on a triple-quadrupole instrument using MRM transitions to verify structural components of the analyte of interest. An upfront HPLC method was developed specifically around 2HG to optimize its elution and concentration before the ESI source is reached. Thus, the exact degree to which 2HG was altered in cells overexpressing the R132H mutated IDH1 protein could be directly assessed.

This approach aptly combines two distinct metabolomic approaches to functionally characterize R132H IDH1 mutations. Unrestricted, exact mass analysis performed with a high-resolution Orbitrap instrument rapidly identified 2HG as the altered metabolite in this system. Once identified, a broad-spectrum mass analysis was insufficient for the exact quantification of flux. Using a more traditional, targeted LC–MS approach, 2HG was reliably quantified with a triple-quadrupole mass spectrometer. Each approach, targeted and untargeted, presents an inverse set of advantages and disadvantages. The marriage of these techniques in this study illustrates the power of an integrated metabolomics platform.

It should be noted that this systematic approach to associating genetic and metabolic phenotypes was confirmed by a concurrent metabolomic study that identified 2HG fluctuations in acute myeloid leukemia. This altered metabolic phenotype was tied to a mutation in the second IDH isoform, IDH2.[96] Since this time, studies have focused on the determination of the molecular mechanisms through which 2HG production leads to oncogenic transformation[97] or a decreased rate of proliferation of human gliomas.[98] In light of the potential importance of this metabolite, a recent paper describes how mice expressing IDH1 mutations known to

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increase 2HG production exhibit an early leukemic histone methylation phenotype.[99]

While the IDH1 report did not identify altered bioenergetics in gliomas per se, it did show the emerging science of metabolomics in a new light, as a platform for discovering novel routes of flux within a system. As we learn more about complex disease states, such as cancer, metabolomics offers a powerful discovery platform. For example, alterations in TCA cycle metabolic flux have recently been demonstrated in Rho GTPase-transformed fibroblasts that are sensitized to glutaminase inhibitor 968 that was developed in the Cerione laboratory.[100] In differentiated tissues, metabolites enter the TCA cycle through glycolysis, yet this process is often dysregulated in hyperproliferative diseases because of functional alterations in glycolytic enzymes.[101] Consistent with this phenotype, Rho GTPase-transformed cells appear to obtain carbon atoms for TCA cycle oxidation from the amino acid glutamine. Glutaminase performs the rate-limiting hydrolysis reaction necessary for αKG production and entrance into the TCA cycle.[102] Thus, treatment with 968 effectively starves these cells of fuel for TCA cycle oxidation, decreasing cell viability and the rate of proliferation (Figure 7).

The commercial availability of isotopically labeled metabolites has had a positive impact on the field of metabolomics as they are ideal tools for documenting how small molecule inhibitors such as 968 alter metabolic flux within a system. Approaches using isotopically labeled metabolites have proven themselves to be excellent tools for investigating TCA cycle metabolic flux. While these techniques have been used previously for ^{13}C NMR detection,[103] their application to a mass spectrometry-based metabolomics study can be equally informative. In particular, this approach has served as a useful paradigm for the study of deoxynucleotides in antiretroviral systems.[68] Figure 7 outlines several labeling opportunities across a number of integrated bioenergetic pathways, including glycolysis, the Krebs cycle, and other pathways (Figure 7). A cellular medium supplemented with isotopically labeled glutamine could serve as an ideal assay condition for cellular glutaminase activity to test the activity of 968. Conversion of glutamine to αKG can be tracked by following isotopically labeled metabolites through the TCA cycle. Because isotopically labeled reagents are available commercially, most metabolic pathways of interest can be examined in this fashion.

Energetically, the TCA cycle is the most efficient mode for hyperproliferative cell types to generate the massive amounts of ATP necessary for growth and replication. By inhibiting conversion of glutamine to glutamate and ultimately αKG, the small molecule glutaminase inhibitor 968 slows the proliferation of malignantly transformed cells. In recent years, we have begun to recognize the importance of cellular energetics in hyperproliferative disease. With the advent of small molecules capable of attenuating metabolic flux, such as 968, the rapid diagnosis of a dysregulated bioenergetic phenotype would have a profound clinical impact. Given that increased metabolic flux often corresponds directly to increased enzymatic activity in diseased cell types, these phenotypes may not be readily identifiable through a genetic screen. Rather, these specific phenotypes require the clinical application of a metabolomics platform. Employing commercially available UPLC systems dramatically decreases run times, allowing one to quickly identify aberrant metabolic phenotypes from primary tissues.

CONCLUDING REMARKS

Over the past 10 years, we have learned a number of valuable lessons in the emerging fields of lipidomics and metabolomics that have advanced biomedical research. First, direct infusion or shotgun approaches can be informative with respect to initial identification of changes in cellular analytes. Some caution must be observed that these changes are not the result of ion suppression and other instrumental artifacts,[24,104] but the combination of profiling analysis using shotgun approaches and subsequent quantitative analysis using appropriate chemically defined internal standards is a powerful advantage. Defects in metabolic pathways are detected by comparisons of analyte profiles and then subsequently quantitated. The effects of remediation can then be interrogated using molecular genetics and small molecule approaches.

In the next 10 years, we predict an increasing level of integration of the multiomic (e.g., genomic, proteomic, and metabolomic) approaches that will transform the way in which we look at biochemical pathways and think about cellular networks. The interconnectedness of pathways that at present seem distinct will increasingly be shown to have feedback loops that allow rapid responses to external stimuli and cellular injury. This kind of approach is represented in a recent, elegant report by Burant and colleagues,[105] in which they show a plethora of changes in intracellular responses that arise during glucose-stimulated insulin secretion. This work provides new insights into chronic insulin resistance associated with obesity and potentially new targets for the treatment of diabetes mellitus. A better understanding of the downstream consequences of various genetic mutations on cell metabolism will advance the ways in which small molecule therapeutics are developed by providing more direct measurement of cellular defects present in a disease at a network level. Metabolomics applications in drug discovery research hold great potential for identification of new drug targets, evaluation of lead compounds and toxicity screening, and tracking drug metabolites, thus contributing to the development of safer and more efficacious drugs. Increasingly, metabolomic analysis will allow monitoring of the downstream consequences of essential cellular metabolites and the consequences of complex feedback loops. This facilitates the ability to better predict where combination therapies might be effectively used to address the multiple cellular defects associated with cellular transformation and tumor growth. This approach will also continue to drive the field of personalized medicine where the responses to specific therapeutic agents can be used to subdivide patients into differential responders based on categories of metabolomic profiles. Mass spectrometry and systems biology analysis are transforming the ways in which the molecular basis of diseases is understood and the approaches used to discover new therapeutics. Over the next decade, metabolomics is likely to evolve into an invaluable screening and diagnostic tool for determining whether patients respond in a beneficial way to specific pharmacological therapies. The prospect of future diagnostics will rely on the ability of researchers to hone the selectivity and specificity of their analyses. Fine-tuning the identification of biomarkers and pathway responses that are predictors of efficacy will make both drug discovery and therapeutics more cost efficient and effective.

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Notes

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ABBREVIATIONS

ESI-MS, electrospray ionization mass spectrometry; GC−MS, gas chromatography with mass spectrometry; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; MALDI-TOF, matrix-assisted laser desorption ionization mass spectrometry time-of-flight; LC, liquid chromatography; HPLC, high-performance liquid chromatography; UPLC, ultraperformance liquid chromatography; DESI-MS, desorption electrospray ionization mass spectrometry; EI, electron ionization; MRM, multiple-reaction monitoring; fwhm, full width at half-maximum.

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