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Human metabolomics: strategies to understand biology☆

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Metabolomics provides a direct functional read-out of the physiological status of an organism and is in principle ideally suited to describe someone's health status. Whereas only a limited number of small metabolites are used in the clinics, in inborn errors of metabolism an extensive repertoire of metabolites are used as biomarkers. We discuss that the proper clinical phenotyping is crucial to find biomarkers and obtain biological insights for multifactorial diseases. This requires to study the phenotype dynamics including the concepts of homeostasis and allostasis, that is, the ability to adapt and cope with a challenge. We also elaborate that biology-driven metabolomics platforms (i.e. development of metabolomics technology driven by the need of studying and answering important biomedical questions) addressing clinically relevant pathways and at the same time providing absolute concentrations are key to allow discovery and validation of biomarkers across studies and labs. Following individuals over years will require high throughput metabolomics approaches, which are emerging for nuclear magnetic resonance spectroscopy and direct-infusion mass spectrometry, but should also include the biochemical networks needed for personalized health monitoring.

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Introduction

Metabolomics is concerned with the comprehensive analysis of low-molecular weight compounds in biological samples such as cells, body fluids and tissues [1,2]. Metabolomics provides a direct functional read-out of the physiological status of an organism and is in principle ideally suited to describe someone's health status. Metabolomics has the potential to deliver diagnostic biomarkers for the detection and prognosis of diseases, and the prediction of the efficacy and safety of pharmaceutical interventions [3–5]. Metabolomics can also provide insights into the biochemical mechanisms of diseases and the modulation by drugs. It has become clear that health and disease are optimally studied from a systems perspective [6•,7,8]. Only such an approach will allow a personalized medicine approach, and system fingerprints by metabolomics will play an important role in the future to follow the health state of an individual [9•]. At present, there are still significant challenges in answering biological questions [10,11,12••]. We will discuss these challenges and indicate possible directions of solutions.

Challenges in biomarker discovery with metabolomics: characterization of the clinical phenotype?

Considering the number of metabolites used in a clinical setting as biomarkers of disease onset and/or progression, the picture appears to be rather diverse. In the first place in clinical chemistry a very limited number of small metabolites such as glucose, cholesterol, creatinine, urea, etc., is being used for decades to assess an individual's (pre-) disease condition. Secondly, in the field of inborn errors of metabolism an extensive repertoire of metabolites is used as biomarkers for diagnosis, progression and response to treatment [13]. Finally, in multifactorial disorders like type 2 diabetes, metabolic syndrome or neurodegenerative disorders there is an urgent need for all types of biomarkers. Especially in this area of pathology metabolomics is in principle very well suited to identify and deliver biomarkers for clinical use. In general 'omics' technologies such as proteomics and genomics have hardly contributed to obtain clinically useful and accepted biomarkers, despite the vast number of papers (more than 150 000) published on this subject [12••]. Many of these omics-studies are hampered by the fact that studies were not well designed, findings not validated in independent replica cohorts, but most important no proper clinical phenotyping is available. The latter is in contrast to the field of inborn errors of metabolism, where the cause is always monogenetic and the resulting clinical phenotypes extreme such as is the case in aminoacidopathies, organic acidurias or fatty acid oxidation

Figure 1

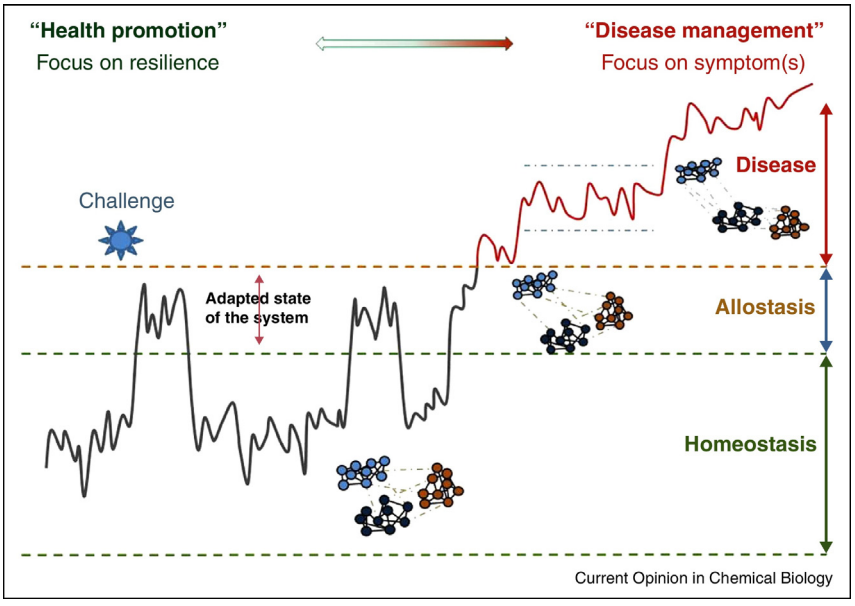


Illustration of dynamic system regulation. A system that is able to respond effectively to a challenge ('allostasis' area in the figure) can be called 'healthy.' When the system is no longer able to respond sufficiently, a disease state develops.
Figure taken from Ref. [9•].

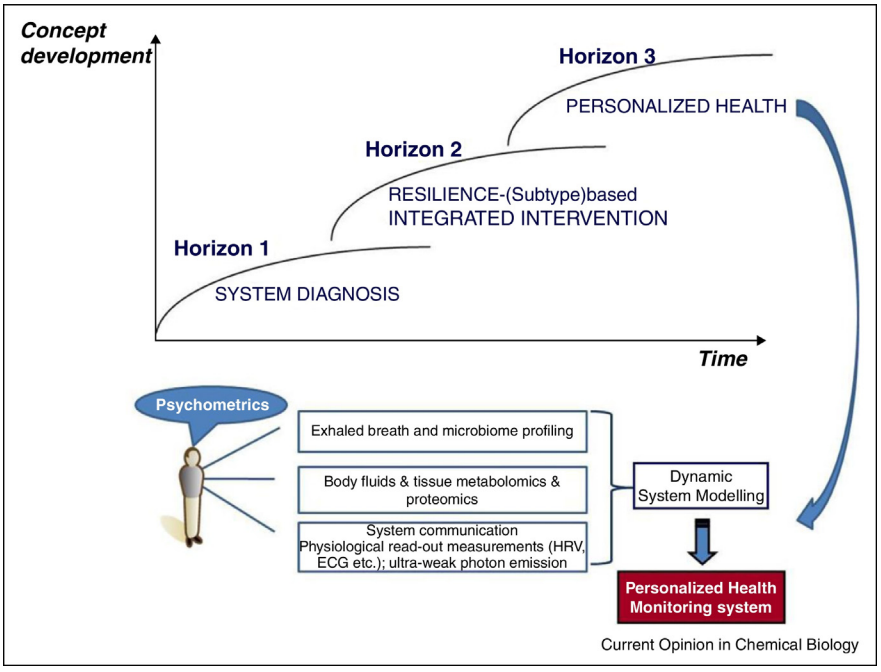
disorders. In multifactorial diseases, the phenotype is more complex, as various genetic and environmental factors are involved, and the phenotype is probably highly dynamic as well. At present the clinical characterization is at a high generic level and consequently inclusion/exclusion criteria will encompass several subtypes. Biomarkers found from those studies can typically not be validated as the subgroup diversity will be different in the next study. This situation is probably better for drug-response biomarkers.

The newer definitions of health and disease emphasizes more a person's ability to adapt and cope in the face of social, physical and emotional challenges than describing it as a state [14]. As a consequence a person's phenotype is considered dynamic and resilience becomes a key parameter. Resilience is best evaluated during a system response, so challenge-tests will become more common in metabolomics research [9•]. A classic example is the oral glucose tolerance test, but also high fat challenges, exercise, stress or mixed diets are used. Phenotype dynamics will differ between individuals, and concepts as homeostasis and allostasis can be considered (Figure 1). However, a precise (dynamic) description of the clinical phenotype is currently missing, which is of utmost importance to guide the discovery of diagnostic and mechanistic biochemical biomarkers. Another challenge is that mostly body fluids such as blood and urine are available, but most studied biochemical networks are at the cellular level and not at the systems regulatory level. Therefore, we need to address cross-compartment communication and system organization more than only the pathways within cells. We expect that with the proper phenotyping/genotyping, metabolomics will play an important role in systems diagnosis, with an emphasis on following the changes over time of an individual [15], and on a somewhat longer term on integrated interventions and personalized wellness (Figure 2). The analytical strategy needed to be developed for achieving this is discussed below.

Size of the human metabolome and what part to address?

In metabolomics the general tendency is to analyze as many low-molecular weight compounds (less than 2000 Da) as possible in a given biological sample at a certain point in time with the aim to obtain maximal biochemical information. The most recent version of the Human Metabolome Database contains 40 335 metabolite entries, of which a major part consists of lipids [16]. This number does not only include endogenous metabolites but also exogenous compounds originating from nutrients, microbiota, drugs and other sources. However, it is our opinion that this number is still an underestimation of the actual size of the human metabolome. Despite the fact that advanced analytical techniques like nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) hyphenated to gas chromatography (GC), liquid chromatography (LC) and/or capillary electrophoresis (CE) have become well-established tools for metabolomics studies [17,18••,19,20,21], they still can only capture a part of the human metabolome and, therefore,

Figure 2



Metabolomics will become an essential part of systems diagnosis strategies, and later in integrated interventions based on identification of subtypes of disease. Ultimately, personalized health strategies will be based on an integrated view of an individual taking different types of biochemical profiling, physiological or systemic read outs and psychological processes into account. Abbreviations: HRV, heart rate variability; ECG, electrocardiography.

provide inherently biased results. We expect that new developments or further refinements of analytical technologies, especially with regards to sensitivity, will significantly increase the coverage of metabolites. For example, the integration of in-capillary preconcentration techniques with a low flow sheathless interface for CE–MS enhances concentration sensitivity by over two orders of magnitude as compared to CE–MS based on a coaxial sheath-liquid interface, resulting in nanomolar detection limits in urine and a threefold increase in detected molecular features [22]. Other improvements emerging are better and innovative fractionation schemes, use of nanoLC approaches, new microfluidic enrichment or separation devices [23] and improvements in mass spectrometry. The majority of peaks observed in a biological sample by global but sensitive mass spectrometry-based analytical platforms are often still unknown as it is a highly challenging and time-consuming procedure to identify them [18••,24]. We expect that recent improvements in metabolite identification/assignment software tools for a more efficient annotation and structure elucidation of the thousands of peaks typically obtained for a complex biological sample will yield many new metabolites [25–29].

Although the general tendency is to analyze as many metabolites as possible in a given biological sample with the aim to obtain maximal biochemical information, this is not necessarily required in order to obtain insights into biological problems. Actually, Christians *et al.* recently suggested that screening for changes in selected metabolic pathways using a set of validated and quantitative analytical platforms would be more suited than a global metabolic profiling approaches, in which many computational and chemometric steps are needed to relate changes in metabolic profiles to biochemical pathways [30]. The available biochemical information for a certain disease is used efficiently in such a biology-driven approach. The global metabolomics strategy and the biology-driven approach are nicely exemplified in the recent work of Hazen and co-workers [31,32]. A global metabolomics analysis of plasma revealed a pathway in both humans and mice linking microbiota metabolism of dietary choline and phosphatidylcholine to cardiovascular disease (CVD) pathogenesis [31]. It was found that plasma levels of three metabolites of dietary phosphatidylcholine — choline, betaine and trimethylamine N-oxide (TMAO) — are associated with increased risk of CVD. In a follow-up study, the gut microbiota-dependent metabolism of L-carnitine to produce TMAO in both rodents and humans was examined using a biology-driven approach [32]. Using stable isotope tracer studies in humans and animal models, the authors demonstrated a role for gut microbiota metabolism of L-carnitine in atherosclerosis pathogenesis.

Strategies to analyze the human metabolome

From the previous section it is clear that the total number of detectable yet identifiable compounds is extensive, indicating that efficient sample pretreatment techniques combined with complementary analytical platforms are minimally required in order to cover a significant fraction of the human metabolome [18••]. The sample pretreatment strategies used in metabolomics prior to separation and detection encompass solvent extraction, ultrafiltration, solid-phase extraction and/or derivatization, which can lead to wide variations in recovery with distinct metabolic profiles measured for the same sample [33•]. Optimization of sample pretreatment for the analytical platforms is key for obtaining reliable and representative metabolic profiles of biological samples [33•,34,35]. Actually, extensive sample preparation is mostly applied due to the limitations of the analysis method such as the limited dynamic range (up to 5 decades) of a mass spectrometer (whereas the concentration range of metabolites is at least nine decades [36,37]) and disturbances of the analysis by matrix components in the samples. Therefore, for each metabolomics study, the sample pretreatment step should be properly evaluated: (stable-isotope) internal standards should be used to evaluate the recovery and analytical performance of metabolites [38•,39,40]. For global metabolic profiling of human serum, Want *et al.* evaluated fourteen procedures commonly used for metabolite extraction and protein removal and found that the most optimal results with regard to metabolic coverage and repeatability were obtained with methanol [41]. In another study, Bruce *et al.* found that two choices of solvent compositions were most optimal for this purpose, that is, methanol/ethanol (1:1, v/v) and methanol/acetonitrile/acetone (1:1:1, v/v/v), which illustrates that there is still no general consensus on the optimal sample pretreatment procedure for human serum/plasma metabolomics [42]. This is even more true for the extraction of intracellular metabolites from human cells/cell lines [34,43]. In biology-driven/targeted metabolomics, sample pretreatment can be directed to the metabolites of interest, and internal standards or isotope-labeled standards can be used for the reliable (absolute) quantification of metabolites [40]. By combining targeted and non-targeted NMR, GC–MS and LC–MS methods to identify and quantify as many metabolites as possible, the group of Dr. Wishart detected 4229 identified metabolites in human serum, of which 1070 were glycerolipids and 2177 phospholipids [36]. In our lab we combine often a global profiling approach using LC–MS, CE–MS and GC–MS covering carbon/energy metabolism, lipids, etc., and more with biology-driven LC–MS/MS platforms for biogenic amines, signaling lipids, hormones, inflammation, oxidative, metabolic stress, etc.

The development of robust, sensitive, high-throughput and low-cost analytical technologies is of pivotal importance for metabolic phenotyping in longitudinal studies with clinically relevant biochemical coverage. At present, NMR-based metabolomics can be performed in a fully automated, reproducible, high-throughput and cost-effective manner [44••]. Although NMR can be considered very robust, the sensitivity and metabolic coverage of MS cannot be matched currently by NMR. Rapid direct flow injection MS analysis is increasingly used for targeted metabolomics or lipidomics based on preselected multiple reaction monitoring (MRM) pairs and isotope labeled internal standards using rapid direct flow injection MS analysis [45]. Recent MS applications demonstrate that progress is being made in this area, indicating that in the near future, MS and NMR will most likely be used as complementary technologies in large-scale epidemiology studies [44••,46••].

When not reporting absolute concentrations but relatively (to internal standards) quantified data of identified/unidentified metabolites, as is often the case in global but also still biology-driven platforms, it is crucial to use pooled samples and/or internal standards as quality controls and for correction of variations and possible biases in the overall analytical procedure during studies [47,48]. However, to accelerate biological interpretation by comparison across studies and labs, and integration with other omics or clinical data (Figure 2), availability of identities and preferably the concentrations of the metabolites is important. As the concentration is influenced by the sample preparation procedure, availability of reference samples is important.

To zoom into biochemical processes and pathways, and/or to validate biochemical mechanisms and to translate findings from cell systems to animals and to humans, and vice versa, stable-isotope based metabolomics is an emerging promising strategy [38•,39,40].

The future of metabolomics for monitoring personal health

For the discovery of biomarkers of disease risk epidemiological studies are typically used. Associations between metabolite profiles and clinical outcome, increasingly also in combination with genetic data, suggest relevant pathways for the onset or progression of a multifactorial disease. However, these biomarkers are not able to predict the disease onset or progression of an individual. For the discovery of metabolic fingerprints to predict disease onset and progression or outcome of interventions at an individual level, longitudinal studies are needed based on monitoring individuals over a year or more. We are convinced that understanding the dynamics during loss of allostasis or (sudden) systemic changes will be crucial to understand the underlying biological processes. As an example the oral glucose tolerance test is the widely expected approach to test for an early onset of diabetes type 2. Whereas under unperturbed conditions no diagnostic conclusion could be obtained, studying the system response revealed differences, and studying the response

from a broader system perspective yielded even more insights [49]. Drugs are an alternative to perturb biological systems to study diseases and their modulation by drugs [3]. These longitudinal studies ask for innovative analytical approaches allowing the analysis of thousands of samples at a low price per sample most likely in the order of tenths of Euro's. Where NMR and direct-infusion mass spectrometry are slowly reaching the desired throughput, they only partially cover the biochemical networks needed for personalized health monitoring. However, we foresee that in the future new MS-based approaches including microfluidic modules will emerge with the desired throughput and biochemical coverage, and measuring absolute concentrations allowing to discover and validate biomarkers within and across the growing metabolomics research community. These metabolic fingerprints will become an important part of a patient-centered personalized, predictive, preventive, and participatory health care system (Figure 2).

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Nicholson JK, Wilson ID: **Opinion: understanding 'global' systems biology: metabonomics and the continuum of metabolism**. *Nature Reviews. Drug Discovery* 2003, **2**:668-676.

2. Patti GJ, Yanes O, Siuzdak G: **Innovation: metabolomics: the apogee of the omics trilogy**. *Nature Reviews. Molecular Cell Biology* 2012, **13**:263-269.

3. Kaddurah-Daouk R, Bogdanov MB, Wikoff WR, Zhu H, Boyle SH, Churchill E, Wang Z, Rush AJ, Krishnan RR, Pickering E *et al.*: **Pharmacometabolomic mapping of early biochemical changes induced by sertraline and placebo**. *Translational Psychiatry* 2013, **3**:e223.

4. Mamas M, Dunn WB, Neyses L, Goodacre R: **The role of metabolites and metabolomics in clinically applicable biomarkers of disease**. *Archives of Toxicology* 2011, **85**:5-17.

5. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL: **Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy**. *Chemical Society Reviews* 2011, **40**:387-426.

6. • Nicholson JK, Holmes E, Kinross JM, Darzi AW, Takats Z, Lindon JC: **Metabolic phenotyping in clinical and surgical environments**. *Nature* 2012, **491**:384-392.
The concept and strategies of using metabolic phenotyping in a clinical environment are outlined in this review.

7. Nicholson JK, Lindon JC: **Systems biology: metabonomics**. *Nature* 2008, **455**:1054-1056.

8. van der Greef J, Stroobant P, van der Heijden R: **The role of analytical sciences in medical systems biology**. *Current Opinion in Chemical Biology* 2004, **8**:559-565.

9. • van der Greef J, van Wietmarschen H, van Ommen B, Verheij E: **Looking back into the future: 30 years of metabolomics at TNO**. *Mass Spectrometry Reviews* 2013.
This review describes the development of system-based metabolomics concepts and applications in the biomedical, pharmaceutical, nutritional, and microbiological fields during the last 30 years, and provides an outlook for the future.

10. Nordstrom A, Lewensohn R: **Metabolomics: moving to the clinic**. *Journal of Neuroimmune Pharmacology: the official journal of the Society on NeuroImmune Pharmacology* 2010, **5**:4-17.

11. Van QN, Veenstra TD: **How close is the bench to the bedside? Metabolic profiling in cancer research**. *Genome Medicine* 2009, **1**:5.

12. •• Poste G: **Bring on the biomarkers**. *Nature* 2011, **469**:156-157.
This paper outlines why 'omics' approaches hardly provided any clinically useful and accepted biomarkers so far. A coordinated 'big science' approach for finding disease-associated biomarkers is advocated.

13. Wikoff WR, Gangoiti JA, Barshop BA, Siuzdak G: **Metabolomics identifies perturbations in human disorders of propionate metabolism**. *Clinical Chemistry* 2007, **53**:2169-2176.

14. Huber M, Knottnerus JA, Green L, van der Horst H, Jadad AR, Kromhout D, Leonard B, Lorig K, Loureiro MI, van der Meer JW *et al.*: **How should we define health?** *BMJ* 2011, **343**:d4163.

15. Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HY, Miriami E, Karczewski KJ, Hariharan M, Dewey FE, Cheng Y *et al.*: **Personal omics profiling reveals dynamic molecular and medical phenotypes**. *Cell* 2012, **148**:1293-1307.

16. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, Djoumbou Y, Mandal R, Aziat F, Dong E *et al.*: **HMDB 3.0 — The Human Metabolome Database in 2013**. *Nucleic Acids Research* 2013, **41**:D801-D807.

17. Ramautar R, Somsen GW, de Jong GJ: **CE–MS for metabolomics: developments and applications in the period 2010–2012**. *Electrophoresis* 2013, **34**:86-98.

18. •• Kuehnbaum NL, Britz-McKibbin P: **New advances in separation science for metabolomics: resolving chemical diversity in a post-genomic era**. *Chemical Reviews* 2013, **113**:2437-2468.
An excellent and comprehensive overview of recent developments in analytical technologies for metabolomics is given.

19. Theodoridis GA, Gika HG, Want EJ, Wilson ID: **Liquid chromatography–mass spectrometry based global metabolite profiling: a review**. *Analytica Chimica Acta* 2012, **711**:7-16.

20. Theodoridis G, Gika HG, Wilson ID: **Mass spectrometry-based holistic analytical approaches for metabolite profiling in systems biology studies**. *Mass Spectrometry Reviews* 2011.

21. Dunn WB: **Current trends and future requirements for the mass spectrometric investigation of microbial, mammalian and plant metabolomes**. *Physical Biology* 2008, **5**:011001.

22. Ramautar R, Busnel JM, Deelder AM, Mayboroda OA: **Enhancing the coverage of the urinary metabolome by sheathless capillary electrophoresis-mass spectrometry**. *Analytical Chemistry* 2012, **84**:885-892.

23. Quist J, Janssen KG, Vulto P, Hankemeier T, van der Linden HJ: **Single-electrolyte isotachophoresis using a nanochannel-induced depletion zone**. *Analytical Chemistry* 2011, **83**:7910-7915.

24. Weber RJ, Southam AD, Sommer U, Viant MR: **Characterization of isotopic abundance measurements in high resolution FT-ICR and Orbitrap mass spectra for improved confidence of metabolite identification**. *Analytical Chemistry* 2011, **83**:3737-3743.

25. Barupal DK, Haldiya PK, Wohlgemuth G, Kind T, Kothari SL, Pinkerton KE, Fiehn O: **MetaMapp: mapping and visualizing metabolomic data by integrating information from biochemical pathways and chemical and mass spectral similarity**. *BMC Bioinformatics* 2012, **13**:99.

26. Peironcely JE, Rojas-Cherto M, Tas A, Vreeken R, Reijmers T, Coulier L, Hankemeier T: **Automated pipeline for de novo metabolite identification using mass-spectrometry-based metabolomics**. *Analytical Chemistry* 2013, **85**:3576-3583.

27. Rojas-Cherto M, Peironcely JE, Kasper PT, van der Hooft JJ, de Vos RC, Vreeken R, Hankemeier T, Reijmers T: **Metabolite identification using automated comparison of high-resolution multistage mass spectral trees**. *Analytical Chemistry* 2012, **84**:5524-5534.

28. Menikarachchi LC, Cawley S, Hill DW, Hall LM, Hall L, Lai S, Wilder J, Grant DF: **MolFind: a software package enabling HPLC/MS-based identification of unknown chemical structures**. *Analytical Chemistry* 2012, **84**:9388-9394.

29. Uppal K, Soltow QA, Strobel FH, Pittard WS, Gernert KM, Yu T, Jones DP: **xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data**. *BMC Bioinformatics* 2013, **14**:15.

30. Christians U, Klawitter J, Hornberger A: **How unbiased is non-targeted metabolomics and is targeted pathway screening the solution?** *Current Pharmaceutical Biotechnology* 2011, **12**:1053-1066.

31. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM *et al.*: **Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease**. *Nature* 2011, **472**:57-63.

32. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L *et al.*: **Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis**. *Nature Medicine* 2013, **19**:576-585.

33. • Simo C, Ibanez C, Gomez-Martinez A, Ferragut JA, Cifuentes A: **Is metabolomics reachable? Different purification strategies of human colon cancer cells provide different CE–MS metabolite profiles**. *Electrophoresis* 2011, **32**:1765-1777.

This paper nicely demonstrates that sample pretreatment strategies used in metabolomics can lead to wide variations in recovery with distinct metabolic profiles measured for the same sample.

34. Leon Z, Garcia-Canaveras JC, Donato MT, Lahoz A: **Mammalian cell metabolomics: experimental design and sample preparation**. *Electrophoresis* 2013.

35. Vuckovic D: **Current trends and challenges in sample preparation for global metabolomics using liquid chromatography–mass spectrometry**. *Analytical and Bioanalytical Chemistry* 2012, **403**:1523-1548.

36. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B *et al.*: **The human serum metabolome**. *PloS ONE* 2011, **6**:e16957.

37. Krug S, Kastenmuller G, Stuckler F, Rist MJ, Skurk T, Sailer M, Raffler J, Romisch-Margl W, Adamski J, Prehn C *et al.*: **The dynamic range of the human metabolome revealed by challenges**. *FASEB Journal: official publication of the Federation of American Societies for Experimental Biology* 2012, **26**:2607-2619.

38. • Bueschl C, Krska R, Kluger B, Schuhmacher R: **Isotopic labeling-assisted metabolomics using LC–MS**. *Analytical and Bioanalytical Chemistry* 2013, **405**:27-33.

Progress and challenges in the important field of isotope labeling approaches in metabolomics are provided.

39. Fan TW, Lorkiewicz PK, Sellers K, Moseley HN, Higashi RM, Lane AN: **Stable isotope-resolved metabolomics and applications for drug development**. *Pharmacol Ther* 2012, **133**:366-391.

40. Lane AN, Fan TW, Bousamra M 2nd, Higashi RM, Yan J, Miller DM: **Stable isotope-resolved metabolomics (SIRM) in cancer research with clinical application to nonsmall cell lung cancer**. *OMICS: A Journal of Integrative Biology* 2011, **15**:173-182.

41. Want EJ, O'Maille G, Smith CA, Brandon TR, Uritboonthai W, Qin C, Trauger SA, Siuzdak G: **Solvent-dependent metabolite distribution, clustering, and protein extraction for serum profiling with mass spectrometry**. *Analytical Chemistry* 2006, **78**:743-752.

42. Bruce SJ, Tavazzi I, Parisod V, Rezzi S, Kochhar S, Guy PA: **Investigation of human blood plasma sample preparation for performing metabolomics using ultrahigh performance liquid chromatography/mass spectrometry**. *Analytical Chemistry* 2009, **81**:3285-3296.

43. Dettmer K, Nurnberger N, Kaspar H, Gruber MA, Almstetter MF, Oefner PJ: **Metabolite extraction from adherently growing mammalian cells for metabolomics studies: optimization of harvesting and extraction protocols**. *Analytical and Bioanalytical Chemistry* 2011, **399**:1127-1139.

44. •• Ala-Korpela M, Kangas AJ, Soininen P: **Quantitative high-throughput metabolomics: a new era in epidemiology and genetics**. *Genome Medicine* 2012, **4**:36.

Developments in mass spectrometry and proton nuclear magnetic resonance spectroscopy as two key technologies for high-throughput metabolic phenotyping are outlined.

45. Suhre K, Meisinger C, Doring A, Altmaier E, Belcredi P, Gieger C, Chang D, Milburn MV, Gall WE, Weinberger KM *et al.*: **Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting**. *PLoS ONE* 2010, **5**:e13953.

46. •• Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wagele B, Altmaier E, Deloukas P, Erdmann J, Grundberg E *et al.*: **Human metabolic individuality in biomedical and pharmaceutical research**. *Nature* 2011, **477**:54-60.

The interaction of genetic predispositions with environmental factors is key to the pathogenesis of complex diseases. A promising approach to understanding this relationship combines a genome-wide association study with the analysis of blood metabolites as functional intermediate phenotypes. The potential of this method is shown in this landmark paper.

47. Lenz EM, Wilson ID: **Analytical strategies in metabonomics**. *Journal of Proteome Research* 2007, **6**:443-458.

48. Koek MM, Jellema RH, van der Greef J, Tas AC, Hankemeier T: **Quantitative metabolomics based on gas chromatography–mass spectrometry: status and perspectives**. *Metabolomics: Official Journal of the Metabolomic Society* 2011, **7**:307-328.

49. Wopereis S, Rubingh CM, van Erk MJ, Verheij ER, van Vliet T, Cnubben NH, Smilde AK, van der Greef J, van Ommen B, Hendriks HF: **Metabolic profiling of the response to an oral glucose tolerance test detects subtle metabolic changes**. *PLoS ONE* 2009, **4**:e4525.