Journal of Chromatography B, 883–884 (2012) 68–75

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

LC–MS-based metabolomics in the clinical laboratory☆

Susen Becker[a,b], Linda Kortz[a,b], Christin Helmschrodt[a,b], Joachim Thiery[a,b], Uta Ceglarek[a,b,*]

[a] Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Liebigstraße 27, D-04103 Leipzig, Germany
[b] LIFE – Leipzig Research Center for Civilization Diseases, University Leipzig, Germany

ARTICLE INFO

Article history:
Received 19 July 2011
Accepted 14 October 2011
Available online 22 October 2011

Keywords:
Clinical application
Metabolomics
LC–MS/MS
Tandem mass spectrometry

ABSTRACT

The analysis of metabolites in human body fluids remains a challenge because of their chemical diversity and dynamic concentration range. Liquid chromatography (LC) in combination with tandem mass spectrometry (MS/MS) offers a robust, reliable, and economical methodology for quantitative single metabolite analysis and profiling of complete metabolite classes of a biological specimen over a broad dynamic concentration range. The application of LC–MS/MS based metabolomic approaches in clinical applications aims at both, the improvement of diagnostic sensitivity and specificity by profiling a metabolite class instead of a single metabolite analysis, and the identification of new disease specific biomarkers. In the present paper we discuss recent advances in method development for LC–MS/MS analysis of lipids, carbohydrates, amino acids and biogenic amines, vitamins and organic acids with focus on human body fluids. In this context an overview on recent LC–MS/MS based metabolome studies for cancer, diabetes and coronary heart disease is presented.

Contents

1. Introduction

Metabolomics, the global study of metabolites and their concentration changes, interactions and dynamics in complex biological systems, is a recent discipline in the post genome era. Within the investigation of different functions on transcriptome and proteome levels in biological systems, the analysis of metabolites as

☆ This paper is part of the special issue "LC–MS/MS in Clinical Chemistry", Edited by Michael Vogeser and Christoph Seger.

* Corresponding author at: Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Liebigstraße 27, D-04103 Leipzig, Germany. Tel.: +49 341 97 22460; fax: +49 341 97 22359.
E-mail address: uta.ceglarek@medizin.uni-leipzig.de (U. Ceglarek).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.10.018

Table 1
Analytical principles applied for metabolome investigations.

Physical property	Analytical principle	Disadvantages	Advantages
Separation technique			
Polarity, size	Liquid chromatography	• High solvent consumption and low separation power (normal HPLC)	• Fast high-resolution separation (UHPLC) • Detection of thermolabile molecules
Charge	Capillary electrophoresis	• Buffers not compatible with MS	• High-resolution capacity • Low solvent and sample consumption
Volatility	Gas chromatography	• Analyte volatility, derivatisation • High molecular weight analytes • Retention time inconstant	• High-resolution capacity • Detection of thermostable metabolites
Detection method			
Mass	Mass spectrometry	• No separation of isobaric, isomeric analytes • Ion suppression depending on matrix • Salt-sensitive	• Identification and quantification of low to high molecular weight metabolites • Detection levels ng/mL–pg/mL
Rotation, vibration	Infrared spectroscopy	• Not applicable for analyte identification	• Structural information
$h\nu$ absorption	UV spectroscopy	• Nonspecific	• Salt-insensitive
$h\nu$ emission	Fluorescence spectroscopy	• Nonspecific	• Salt-insensitive
Spin rotation	NMR-, ESR spectroscopy	• Low analytical resolution, insensitivity	• Identification of complex structures of makromolecules (DNA, RNA, proteins) • Structural information

downstream products of cellular processes became more and more important in the last decade [1–3]. Metabolites are low-molecular weight (<1500 Da) components (e.g. carbohydrates, lipids, amino acids, biogenic amines, organic acids). They are influenced by genetic and epigenetic effects, environmental and dietary exposures as well as lifestyle.

Metabolomic investigations have already become an integral part in clinical drug safety assessment, environmental chemistry, plant biochemistry, microbiology, and nutritional studies to gain a more global picture of the molecular networks [4–7]. In the clinical laboratory, metabolome studies are applied for the detection of new biomarkers related to the 'healthy vs. diseased' concept as known from proteomic studies [8–10]. The Human Metabolome Database (HMDB) actually contains about 7900 metabolites found in the human body [4]. The association of metabolites (metabolite classes) in addition to genome data provided new insights into the interplay between genetic variability and metabolic regulations [11]. Moreover, the quantitative analysis of challenging metabolite classes, such as amino acids, steroids, eicosanoids or phospholipids in the clinical laboratory might help to understand the pathophysiology and to assess the individual risk prediction of complex diseases such as diabetes, coronary heart disease and cancer. However, it is still unclear if the investigations of complete metabolite pathways by liquid chromatography tandem mass spectrometry (LC–MS/MS) will improve clinical routine diagnostics as shown for worldwide newborn screening programs. For example, 30 inherited metabolic diseases of the amino acid and fatty acid metabolism are detectable with the newborn screening methodology. It has to be proven in clinical studies if metabolomic investigations result in a significant improvement of the diagnostic sensitivity or specificity compared to established diagnostic assays.

Liquid chromatography coupled to mass spectrometry is an integral part in quantitative metabolome analysis in the clinical laboratory. Particularly current technical developments of new generation quadrupole mass spectrometers enable rapid quantification of multiple metabolites from the ng/L to g/L range in body fluids.

In this paper, recent developments in the field of liquid chromatography tandem mass spectrometry (LC–MS/MS) and their application to metabolome analysis in human body fluids for laboratory diagnostics and clinical research will be reviewed.

2. Analytical concepts for metabolome studies in body fluids

A variety of analytical principles can be used for metabolome analysis due to the diversity of chemical structures including different functional groups, heterocyclic structures, and conjugated double bounds (Table 1). Due to the diversity and broad dynamic range of metabolites in body fluids, it appears to be unreasonably difficult to analyze all metabolite classes with one common method for extraction, chromatography and mass spectrometric analysis. Adapting the overview of the dynamic range of protein biomarkers from Anderson and Anderson [5] and Schiess et al. [6] we have summarized single metabolites or metabolite classes and their concentration levels found in biomarker studies or analyzed in the clinical routine laboratory (Fig. 1). Different analytical approaches have to be applied for metabolome investigations in body fluids, which may provide complementary but also redundant information.

Analysis of metabolites can be performed in two ways: first in 'non-targeted' analyses a large number of metabolites are analyzed in an unbiased approach after minimal sample pretreatment and second in 'targeted' analyses of specific, pre-defined metabolites associated with a specific pathway, enzyme system or complete metabolite class [1,3,7–9].

The development of fast LC concepts offers new possibilities to improve analysis speed and chromatographic resolution of quantitative LC–MS/MS applications [10]. Separation of analytes in body fluids is performed by liquid chromatography, gas chromatography (GC) or capillary electrophoresis coupled with the mass spectrometer to enhance the power of identification [11]. Chromatographic supports based on monolithic material, small porous particles and porous layer beads have been developed to improve throughput and separation efficiency [10,12]. Recently, hydrophilic interaction

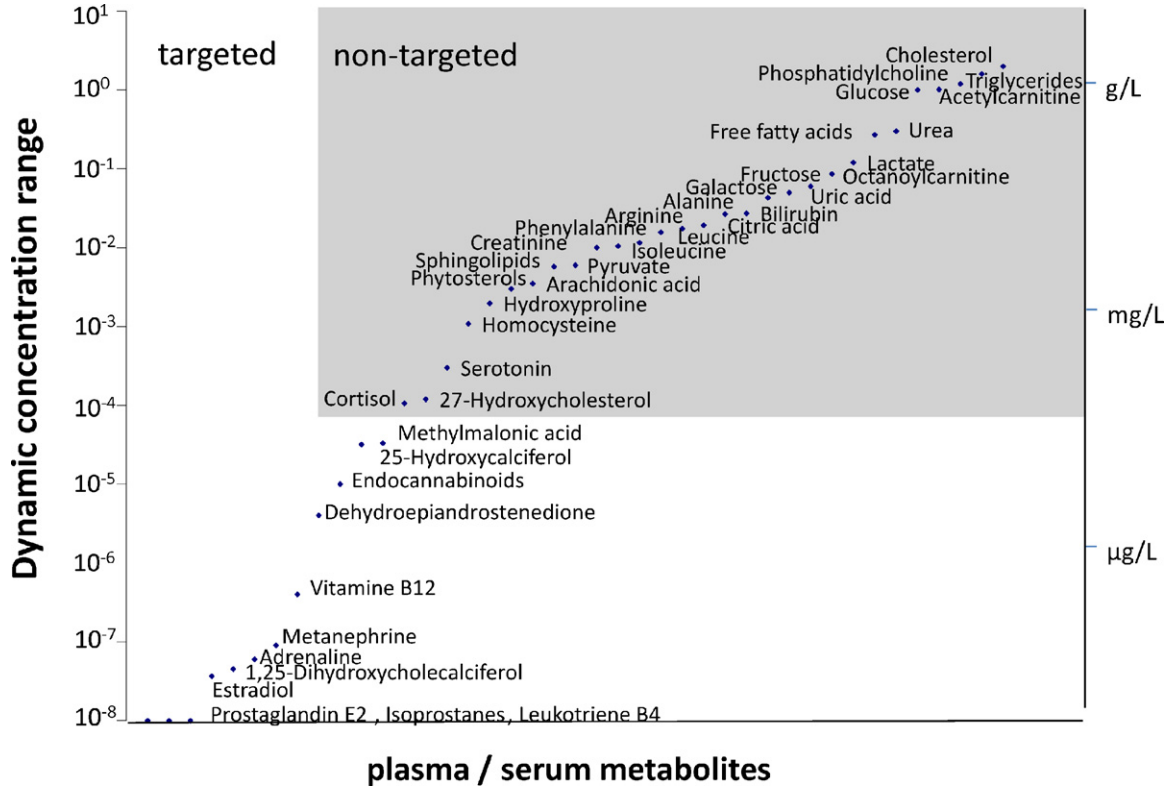

Fig. 1. Endogenous metabolite concentrations in serum/plasma determined by targeted and non-targeted approaches with LC–MS/MS.

chromatography (HILIC) has become a useful tool for the analysis of polar metabolites [24,25]. The development of separation columns containing sub-2 μm particles applied in ultra high performance liquid chromatography (UHPLC) lead to higher chromatographic resolution [10,13,14].

2.1. Non-targeted metabolomics

Non-targeted metabolome approaches for body fluids are mainly based on nuclear magnetic resonance (NMR) spectroscopy or high accuracy mass spectrometry with a mass resolving power between 20,000–1,000,000 and a mass accuracy <3 ppm [2]. Analyses are performed predominantly qualitatively by determining a subset of the whole metabolite profile. Quadrupole/time-of-flight (QTOF), orbitrap and Fourier transform ion cyclotron resonance mass spectrometry systems are applied for the identification of metabolites in global metabolomic approaches [15–20]. Ion mobility time-of-flight mass spectrometry (IM-MS), which separates ions on the basis of their mass/charge ratios as well as their interactions with a buffer gas, becomes a powerful analytical tool for investigating complex samples. Recently, IM-MS with an electrospray ionization source was applied for metabolite studies in whole blood [21]. The main advantage of IM-MS is the potential for separation of metabolite isomers without chromatographic separation [22]. By coupling UHPLC with QTOF mass spectrometry, metabolites (amino acids, organic acids, and phospholipids) which are present in the mg/L range in human plasma were identified [18].

The MS– or LC–MS fingerprints are preprocessed (background suppression, peak picking, deconvolution, scaling, normalization) and processed by use of multivariate analysis [23]. Identification of potential biomarker is performed by the comparison of corresponding fragment mass spectra or the accurate mass with databases. An overview about available MS-databases for the identification of metabolomic signals is given by Werner et al. [2].

Limitations of non-targeted metabolome analyses are the lack of processing software for metabolites with low-abundant signal intensities due to their low concentration ranges in human body fluids or poor ionizability at the given ionization conditions [2].

2.2. Targeted metabolomics

Triple quadrupole mass spectrometry (QqQ) is mainly applied to targeted metabolomics because of its potential in quantitative analysis, the ability to perform multiple reaction monitoring (MRM), neutral loss (NL) and precursor ion (PS) experiments. Analytical strategies include the quantitative study of metabolite classes, networks and targeted analysis of specific metabolites. In the last years, modern QqQs overcame the limitation of low duty cycles with scan times <10 ms/MRM [24]. Furthermore, hybrid instruments, in which the third quadrupole is replaced by a TOF (Q-TOF) or linear ion trap (Q-Trap) are able to perform structural elucidation and quantitative MRM experiments [25–27].

Detection specificity for isobaric and isomeric compounds is indispensable especially in metabolomic approaches. In this case, even high mass resolution spectrometers cannot be applied without a chromatographic separation step prior to mass spectrometric detection. Another limitation of LC–MS/MS analysis is still the lack of detection sensitivity for some analyte groups such as steroid hormones, biogenic amines and cytokines with blood levels in the low pmolar range.

Certified commercial LC–MS/MS based assays or platforms became available during the last years (e.g. Biocrates, Metabolon, Metanomics) which decrease analysis time of metabolome investigations in clinical studies [28]. Limitations of LC–MS/MS such as technical complexity, the need of specially trained technical staff and laborious maintenance procedures could be reduced with the new generation of mass spectrometers.

Table 2
Recently developed analytical methods applying liquid chromatography tandem mass spectrometry for the analysis of different lipid classes in human specimens.

Lipid class	Analytes	Specimen	Volume (μL)	Pre-treatment	Chromatography	Ionization	Analysis (min)	Reference
Glycerophospholipids	Phospholipids	P	n.r.	PP	HPLC	ESI^+, ESI^-	30	[55]
	Lysophospholipids,	P	200	E	Nano LC	ESI^+, ESI^-	110–130	[56]
	Lysophosphatidyl species	P	20	E	–	ESI^+	1.3	[54]
	Sphingosin-1-phosphate	P	1000	E	UHPLC	ESI^-	20	[58]
	Lysophosphatidic acid	P	10	E	HPLC	ESI^+	12	[60]
	Ceramid species	P	50	E	HPLC	ESI^+	21	[59]
Fatty acyls	Eicosanoids	S	100	Online SPE	HPLC	ESI^-	30	[61]
	8-iso-PGF2α	P, U	500	PP, online SPE	HPLC	$APCI^-$	13	[62]
	Endocannabinoids	P	1000	E, SPE	HPLC	ESI^+	21	[64]
	Endocannabinoids	S, P, U	500–2000	SPE	UPLC	ESI^+	4	[65]
	Endocannabinoids	S	1000	PP, E	HPLC	ESI^+	12	[66]
	Endocannabinoids	P	500	LLE	HPLC	ESI^+	9	[67]
Sterol lipids	Sterols, phytosterols	P	10	PP	HPLC	$APPI^+$	10	[70]
	Cholesterol precursor, phytosterols	S	1	D	HPLC	ESI^+	20	[71]
	Steroids	S	100	PP, online SPE	HPLC	$APCI^+$, $APCI^-$	4	[26]
	Steroids	S	200	PP	HPLC	$APPI^+$, $APPI^-$	12	[47]
	Steroids	S	760	PP	HPLC	$APPI^+$	18	[46]
	Bile acids	S, P	100	PP	HPLC	ESI^-	5.5	[48]
	Bile acids	S	100	SPE	HPLC	ESI^-	50	[49]
	Bile acids	P	500	SPE	HPLC	ESI^-	29.3	[50]

APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photo ionization; D, derivatisation; E, extraction; ESI, electrospray ionization; LLE, liquid–liquid extraction; n.r., not reported; P, plasma; PP, protein precipitation; S, serum; SPE, solid-phase extraction.

3. Analysis of metabolites in body fluids by LC–MS/MS

LC–MS/MS became increasingly important for the analysis of complex human body fluids during the last two decades [29]. LC–MS/MS is being adopted in clinical laboratories for the quantitative analysis of a number of analytes from physiological matrices. However, a number of preanalytical guidelines and requirements have to be considered in the validation process [30,31].

3.1. Preanalytical aspects

Definition of preanalytical protocols is a prerequisite for reproducible metabolite analyses. Biologically and technically influencing factors have to be considered depending on the metabolite (class) and body fluid. So far, little is known about the effects of age/gender, body mass, fasting, menstrual cycle/pregnancy etc. on metabolite concentration. Nutrition and physical activity seem to influence amino acid and acylcarnitine concentrations [32]. Differences in lysophosphatidylcholines, branched chain amino acids and acylcarnitines were observed in obese men [33]. Levels of glutamine, glycine, alanine, lysine, serum and creatinine are reduced while levels of acetoacetate and of very low density lipoprotein increase during the luteal phase [34].

In addition, technical factors (sample type, different blood specimens, type of tube for blood withdrawel, stability) have to be considered for metabolome investigations. Serum might be more suited for small molecule analyses because the overall protein content is lower and therefore competing ionization reduced and overall sensitivity increased [35]. However, the comparison of non-targeted analyses in serum and plasma revealed the presence of more peptide/protein fragments in serum, which resulted in additional disturbing effects during ionization [32]. Additionally, the same study showed that the blood collection method itself appears to influence the metabolome profile. Signals attributable to skin sterilization detergents could be identified in mass spectrometric chromatograms. Further, only small differences between plasma specimens with different anticoagulants were identified. In targeted approaches, it was shown that concentrations of metabolites vary between serum, plasma and dried blood specimen [32].

The determination of metabolite stability data consists of the analysis of stability over several freeze–thaw cycles, over sample processing procedures and over post-preparative periods [36]. Such stability data have been recently published for endocannabinoid, phospholipid and oxysterol analysis as well as amino acid and acylcarnitine profiling [37–39]. A systematic review of preanalytical variables was given for the steroid analysis [40]. Sample additives, such as antioxidants can be added to the sample prior to sample storage and further processing to increase analyte stability [41].

Only few data on specimen stability are available for non-targeted metabolome analyses although it is essential to gain non-confounded valid metabolic profiling data sets. It was shown that storage conditions and freeze–thaw cycles lead to relevant changes in the MS urine fingerprint [42].

3.2. Lipids

The mass spectrometric profiling of lipid pathways and networks is challenging due to the diverse chemical structures of the different lipid classes. A huge number of LC–MS/MS applications in the field of lipidomics was published during the last decade [43–45]. Most of them are applied in the field of biomarker search. Only few lipid species (steroids, bile acids) are measured in clinical routine laboratories by LC–MS/MS [26,46–51]. A summary of recently developed LC–MS/MS methods is provided in Table 2.

During the last years, lipidomic studies in bodyfluids were dominated by the analysis of phospholipids with its two main classes, the glycerophospholipids and the sphingomyelins [52–55]. Phosphatidylcholines, lysophosphatidylcholines and sphingomyelins can be monitored by a precursor ion scan of *m/z* 184 (positive ion mode), whereas phosphatidylinositol, phosphatidylglycerol, phosphatidylserin and phosphatidic acid can be profiled by a precursor ion scan of *m/z* 153 (negative ion mode). However, the identification of the fatty acid moieties and the location of double bonds is still challenging. Isobaric phospholipid inter-class and intra-class species separation could be achieved by applying nanoflow LC–MS/MS [56]. Approaches applying flow injection analysis [54], HILIC [57], UHPLC [58] and high performance liquid chromatography (HPLC) [59,60] are available for the analysis of sphingolipid and ceramid species.

Several LC–MS/MS methods have been recently published for the targeted quantification of low abundant eicosanoids, including isoprostanes in human plasma, serum or urine [61–63]. Sample preparation procedures include protein precipitation and solid

phase extraction (on- or offline) steps with further chromatographic separation via HPLC or UHPLC coupled to tandem mass spectrometric detection. However, the clinical application of these methods is still hampered by the very low levels in plasma/serum of healthy subjects (concentration range ng/L). For the analysis of endocannabinoids, LC–MS/MS methods have been recently developed requiring sample volumes of 0.5–2 mL [64–67]. Similarly to the eicosanoid analysis, lipid extraction either via solid-phase or liquid–liquid extraction is performed for endocannabinoid sample preparation.

The endogenous levels of sterol metabolites (either as total or free and esterified sterol levels) are commonly measured via GC–MS [68,69], but can also be determined by HPLC–MS/MS, either directly [70] or after derivatisation [71].

3.3. Carbohydrates

Carbohydrate metabolism includes the pentose, fructose, and galactose pathways, the gluconeogenesis and finally the glycogen storage processes. Up to now, no non-targeted comprehensive metabolomic study investigating the entire carbohydrate metabolome has been published [72].

Several general targeted approaches were published for the quantitative analysis of intermediates of the pentose phosphate pathway [73–75]. These LC–MS methods are based on either Group Specific Internal Standard Technology labeling [73] or the separation on ion-pair-loaded C(18) HPLC columns [74,75]. Reference values for dihydroxyacetone phosphate, D-ribose 5-phosphate, the two sum parameter D-ribulose 5-phosphate/D-xylulose 5-phosphate and D-fructose 6-phosphate/D-glucose 6-phosphate, and D-sedoheptulose 7-phosphate were established for dried blood spots, fibroblasts and lymphoblasts [74]. The analysis of metabolites from the pentose phosphate pathway enables the diagnosis of patients that are characterized by increased concentrations of pentitols (ribitol and D-arabitol), erythritol, and probably sedoheptitol in body fluids [76]. An HPLC–MS method is available for the simultaneous analysis of phosphosugars, phosphocarboxylic acids, nucleotides, and coenzymes, which enables the separation of the structural isomers glucose 6-phosphate and fructose 6-phosphate [77].

3.4. Amino acids and biogenic amines

Amino acids play essential roles in energy metabolism, neurotransmission, and lipid transport. Physiological plasma amino acids are usually analyzed by classical ion-exchange liquid chromatography requiring an analysis time of 2–3 h per sample. Because most amino acids are very polar, analysis without derivatisation by means of classical reversed phase chromatography in combination with mass spectrometry is challenging. By combination of derivatisation (butylation) and ion-pair LC–MS/MS, 22 plasma amino acids could be determined from 10 μL plasma or serum by LC–MS/MS in 11 min [78]. Using UHPLC combined with tandem mass spectrometry, 52 amino acids in plasma or urine can be quantified after a simple dilution step in a run time of 30 min [79,80]. Different methods are published for the analysis of multiple amino acids by coupling ion-pair chromatography to mass spectrometry [81,82]. The analysis of 42 amino acids in urine specimens is enabled by the TRAQ®-LC–MS/MS methodology using different isobaric tags for analyte and internal standard [83]. The simultaneous measurement of homocysteine and related metabolites involved in sulfur amino acid metabolism in biological samples was published by Rafii et al. [84]. A method for the simultaneous analysis of metabolites involved in nitric oxide production (L-arginine, L-citrulline, and dimethylarginine) was recently published [85].

Biogenic amines are neurotransmitters and hormones, synthesized from amino acids. The diagnostic relevant metabolites occur in very low concentrations in human plasma (nmol/L). Therefore high-performing analytical techniques are required for quantification. Different LC–MS/MS approaches for the determination of biogenic amines in plasma and urine including catecholamines, metanephrines, hydroxyindolactic acid, serotonin, tryptophan, kynurenines and homovanillic acid were reviewed by de Jong et al. [86].

3.5. Vitamins, organic acids, intermediary energy metabolites

LC–MS/MS has emerged as a particularly versatile and informative approach for the establishment of methods suitable for the simultaneous analysis of the tricarboxylic acid cycle [87,88]. Two LC–MS/MS methods for the profiling of intermediary energy metabolites are available for haemodialysate [89] or atmospheric aerosols [89,90] but not for serum or plasma. Vitamin B(2) and B(6) species, neopterin, cotinine, tryptophan metabolites, folates and folate catabolites involved in the transfer of one-carbon units, or cofactors for the relevant enzymes were measured by LC–MS/MS [91].

4. LC–MS/MS based metabolomics in clinical applications

In clinical diagnostics quantitative targeted analysis by atmospheric pressure ionization/quadrupole tandem mass spectrometry paved the way of metabolome investigations into clinical routine application [14,20–23]. The first routine application of metabolite screening by tandem mass spectrometry in the clinical laboratory was introduced by Millington and Chace in the 1990s. The screening of amino acids and acylcarnitines for the detection of inborn errors in amino acid and fatty acid metabolism enhanced the panel of screened diseases included in newborn screening programs worldwide [92]. About 30 metabolic parameters of amino acid and fatty acid metabolism were introduced into the screening panel for the detection of inherited metabolic disorders. This was the first multi-parametric metabolic approach, which proved to be a valuable and effective preventive diagnostic strategy [93,94]. During the last decade, numerous targeted and non-targeted metabolome approaches were applied in cross-sectional studies, comparing patients with healthy controls. In this chapter we will highlight recent developments in the clinical routine laboratory and disease-related biomarker search in clinical studies.

4.1. Inherited metabolic diseases

In the field of laboratory diagnostics of inherited metabolic diseases, multiple “second-tier” methods were established during the last year. Overcoming the problem of non-specific first line parameters and a resulting high false positive rate, a second analysis of dried blood from the same newborn screening card is performed for the monitoring of specific metabolites or metabolic pathways [95].

A multiplex enzyme assay for the measurements of the activity of three galactose metabolizing enzymes with UHPLC–MS/MS was developed as second-tier test of galactosemia screening [10]. An alternative diagnostic procedure for the diagnosis of galactosemia applying LC–MS/MS determines hexose monophosphates in dried blood spots with a diagnostic sensitivity and specificity of 100% [96].

For the evaluation of elevated propionylcarnitine concentrations in dried blood, a rapid LC–MS/MS method was developed to identify free methylmalonic, 3-OH propionic acid, methylcitric acid

Table 3
Summary of recent biomarker identifications for several cancer sites applying mass spectrometry.

Cancer type	Material	Approach	Method	Metabolite biomarker	Reference
Renal cell carcinoma	Serum	Non-targeted	LC-QqQ	Phospholipids, phenylalanine, tryptophan acylcarnitines, cholesterol metabolites, arachidonic acid metabolism	[111]
Renal cell carcinoma	Urine	Non-targeted	HILIC-TOF	212 candidate biomarkers, no identifications	[109]
Colorectal cancer	Urine	Targeted	LC-QqQ	Acteylcarnine, phenylacetylglutamin, leucylproline, aspartyllysine	[110]
Colorectal cancer	Serum	Non-targeted	FIA-FTICR FIA-QqQ	Hydroxylated ultra long chain fatty acids	[112]
Ovarian cancer	Serum	Non-targeted	LC-QqQ	27-nor-5β-cholestande-3,7,12,24,25-pentolglucoronide	[106]
Prostate cancer	Tissue/urine	Targeted	LC/GC Q	Sarcosine	[113]
Kidney cancer	Urine	Targeted	LC-QqQ	quinolinate, 4-hydroxybenzoate, and gentisate	[110]
Kidney cancer	Urine	Targeted	LC-QqQ	Acylcarnitines	[108]

Abbreviations: FIA, flow injection analysis; FTICR, Fourier transform ion cyclotron resonance; GC, gas chromatography; HILIC, hydrophilic interaction chromatography; LC, liquid chromatography; Q, quadrupole; QqQ, triple quadrupole.

and total homocysteine (tHCY) [97,98]. A second-tier test applying LC–MS/MS was developed for the newborn screening of maple syrup urine disease including the simultaneous quantification of leucine, isoleucine, *allo*-isoleucine, valine and hydroxyproline [99].

A novel concept for analysis of metabolic pathways by LC–MS/MS was introduced for the newborn screening of lysosomal storage diseases. Here, metabolic rates of specific enzyme substrates were analyzed simultaneously, which enables the newborn screening of Morbus Gaucher, Morbus Pompe, Morbus Fabry, Morbus Niemann-Pick A/B and Morbus Krabbe [100–102].

4.2. Cancer studies

Cancer cells are known to produce a unique metabolic fingerprint. Therefore, the identification of cancer specific biomarkers might lead to earlier detection of cancer development [103]. Numerous NMR-based studies were performed to profile metabolites by comparing cancer patients with healthy controls [104,105]. Tumors, in general, seem to be characterized by elevated phospholipids (e.g. choline-containing phospholipids) [19].

Particularly cross-sectional metabolome studies based on LC–MS/MS were performed for the following cancer types: prostate, breast, ovarian, colorectal, renal cell and kidney cancer [106–114]. A potential role for sarcosine in prostate cancer progression was shown [113]. A summary of metabolite biomarkers which have been identified in cross-sectional LC–MS/MS based cancer studies are summarized in Table 3.

4.3. Diabetes and coronary heart disease

Identification of high-risk individuals for type 2 diabetes (T2D) and cardiovascular disease (CVD) is currently assessed by global risk factors like age, sex, metabolic and lifestyle variables. In the last decade, numerous genomic, transcriptomic, proteomic and metabolomic studies have been performed in T2D and CVD patients searching for biomarkers with higher predictive values. However, most studies described cross-sectional differences in metabolite levels between cases and controls. In serum and plasma, differences in carbohydrates (glucose, mannose, desoxyhexose), ketone bodies, phosphatidylcholines and free fatty acids have been shown to be associated with insulin resistance [115]. Especially the branched chain amino acid metabolism was identified to be associated with severe insulin resistance in obese people [115]. The analysis of branched chain and aromatic amino acids in 201 serum samples from individuals, developing T2D during the last 12 years of follow-up in the Framingham offspring study, identified leucine, isoleucine, valine, phenylalanine, and tyrosine as biomarkers for T2D risk prediction [116,117].

Important cardiovascular risk factors such as smoking, hypertension, obesity, hyper-and dyslipidemia, T2D have been identified in epidemiological studies. However, for a further risk prediction a better understanding of pathophysiological mechanisms is needed [118]. In the CATHGEN study, hydroxylated acylcarnitines were identified as marker for the prediction of myocardial infarction [119]. Additionally, the same group showed a high heritability of metabolite profiles in families burdened with premature cardiovascular disease [120]. During surgical ischemia/reperfusion, acetylcarnitine and 3-hydroxybutyryl-carnitine were shown to be elevated in serum of patients with impaired ventricular function [121].

5. Summary

Global metabolome analysis or metabolite profiling may help to identify new biomarkers or spotlight pathways of common diseases for dietary or drug modulation. Additionally, multivariate biomarkers, including fingerprint profiles or metabolite signatures may improve individual prognostic or predictive interpretation. However, the analysis of metabolites remains challenging because of the structural diversity and broad dynamic concentration range. Despite ample possibilities of LC–MS/MS for metabolite analysis and encouraging results of clinical metabolome studies, only limited clinical applications are available today. Prospective studies have to follow for the validation of metabolites as valuable biomarkers for cancer, diabetes and cardiovascular diseases. Definition of preanalytical protocols, quality management, processing of large and complex data sets, and the simplification of result evaluation has to be addressed before method implementation in the daily clinical routine analyses.

Acknowledgements

This publication is supported by LIFE – Leipzig Research Center for Civilization Diseases, University Leipzig. LIFE is funded by means of the European Union, by the European Regional Development Fund (ERDF) and by means of the Free State of Saxony within the framework of the excellence initiative.

References

[1] R. Goodacre, S. Vaidyanathan, W.B. Dunn, G.G. Harrigan, D.B. Kell, Trends Biotechnol. 22 (2004) 245.
[2] E. Werner, J.F. Heilier, C. Ducruix, E. Ezan, C. Junot, J.C. Tabet, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 871 (2008) 143.
[3] K. Dettmer, P.A. Aronov, B.D. Hammock, Mass Spectrom. Rev. 26 (2007) 51.
[4] D.S. Wishart, D. Tzur, C. Knox, R. Eisner, A.C. Guo, N. Young, D. Cheng, K. Jewell, D. Arndt, S. Sawhney, C. Fung, L. Nikolai, M. Lewis, M.A. Coutouly, I. Forsythe, P. Tang, S. Shrivastava, K. Jeroncic, P. Stothard, G. Amegbey, D. Block, D.D. Hau, J. Wagner, J. Miniaci, M. Clements, M. Gebremedhin, N. Guo, Y. Zhang, G.E. Duggan, G.D. Macinnis, A.M. Weljie, R. Dowlatabadi, F. Bamforth, D. Clive, R. Greiner, L. Li, T. Marrie, B.D. Sykes, H.J. Vogel, L. Querengesser, Nucleic Acids Res. 35 (2007) D521.
[5] N.L. Anderson, N.G. Anderson, Mol. Cell. Proteomics 1 (2002) 845.
[6] R. Schiess, B. Wollscheid, R. Aebersold, Mol. Oncol. 3 (2009) 33.
[7] U. Ceglarek, A. Leichtle, M. Brugel, L. Kortz, R. Brauer, K. Bresler, J. Thiery, G.M. Fiedler, Mol. Cell. Endocrinol. 301 (2009) 266.
[8] P. Kiefer, J.C. Portais, J.A. Vorholt, Anal. Biochem. 382 (2008) 94.
[9] W.B. Dunn, N.J. Bailey, H.E. Johnson, Analyst 130 (2005) 606.
[10] L. Kortz, C. Helmschrodt, U. Ceglarek, Anal. Bioanal. Chem. 399 (2011) 2635.
[11] W.B. Dunn, Phys. Biol. 5 (2008) 11001.
[12] L. Novakova, H. Vlckova, Anal. Chim. Acta 656 (2009) 8.
[13] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, J. Pharm. Biomed. Anal. 44 (2007) 342.
[14] K.C. Saunders, A. Ghanem, W. Boon Hon, E.F. Hilder, P.R. Haddad, Anal. Chim. Acta 652 (2009) 22.
[15] T.C. Walther, M. Mann, J. Cell Biol. 190 (2010) 491.
[16] E. Dudley, M. Yousef, Y. Wang, W.J. Griffiths, Adv. Protein Chem. Struct. Biol. 80 (2010) 45.
[17] G.A. Gowda, S. Zhang, H. Gu, V. Asiago, N. Shanaiah, D. Raftery, Expert Rev. Mol. Diagn. 8 (2008) 617.
[18] S.J. Bruce, P. Jonsson, H. Antti, O. Cloarec, J. Trygg, S.L. Marklund, T. Moritz, Anal. Biochem. 372 (2008) 237.
[19] E. Ackerstaff, K. Glunde, Z.M. Bhujwalla, J. Cell. Biochem. 90 (2003) 525.
[20] F. Michopoulos, G. Theodoridis, C.J. Smith, I.D. Wilson, J. Proteome Res. (2010).
[21] P. Dwivedi, A.J. Schultz, H.H. Hill, Int. J. Mass Spectrom. 298 (2010) 78.
[22] A.B. Kanu, P. Dwivedi, M. Tam, L. Matz, H.H. Hill Jr., J. Mass Spectrom. 43 (2008) 1.
[23] J. Boccard, J.L. Veuthey, S. Rudaz, J. Sep. Sci. 33 (2010) 290.
[24] J.W. Allwood, R. Goodacre, Phytochem. Anal. 21 (2010) 33.
[25] L. Kortz, R. Geyer, U. Ludwig, M. Planert, M. Bruegel, M. Leichtle, G. Fiedler, J. Thiery, U. Ceglarek, J. Lab. Med. 10 (2009) 341.
[26] U. Ceglarek, L. Kortz, A. Leichtle, G.M. Fiedler, J. Kratzsch, J. Thiery, Clin. Chim. Acta 401 (2009) 114.
[27] K. Sandra, S. Pereira Ados, G. Vanhoenacker, F. David, P. Sandra, J. Chromatogr. A 1217 (2010) 4087.
[28] K. Suhre, C. Meisinger, A. Doring, E. Altmaier, P. Belcredi, C. Gieger, D. Chang, M.V. Milburn, W.E. Gall, K.M. Weinberger, H.W. Mewes, M. Hrabe de Angelis, H.E. Wichmann, F. Kronenberg, J. Adamski, T. Illig, PLoS One 5 (2010) e13953.
[29] B. Shushan, Mass Spectrom. Rev. 29 (2010) 930.
[30] J.W. Honour, Ann. Clin. Biochem. 48 (2011) 97.
[31] M. Vogeser, C. Seger, Clin. Chem. 56 (2010) 1234.
[32] R. Brauer, Metabolomics (2011).
[33] J.Y. Kim, J.Y. Park, O.Y. Kim, B.M. Ham, H.J. Kim, D.Y. Kwon, Y. Jang, J.H. Lee, J. Proteome Res. (2010).
[34] M. Wallace, Y.Z. Hashim, M. Wingfield, M. Culliton, F. McAuliffe, M.J. Gibney, L. Brennan, Hum. Reprod. 25 (2010) 949.
[35] J.R. Denery, A.A. Nunes, T.J. Dickerson, Anal. Chem. 83 (2011) 1040.
[36] F.T. Peters, Anal. Bioanal. Chem. 388 (2007) 1505.
[37] A. Floegel, D. Drogan, R. Wang-Sattler, C. Prehn, T. Illig, J. Adamski, H.G. Joost, H. Boeing, T. Pischon, PLoS One 6 (2011) e21103.
[38] X. Jiang, R. Sidhu, F.D. Porter, N.M. Yanjanin, A.O. Speak, D.T. Te Vruchte, F.M. Platt, H. Fujiwara, D.E. Scherrer, J. Zhang, D.J. Dietzen, J.E. Schaffer, D.S. Ory, J. Lipid Res. 52 (2011) 1435.
[39] J.T. Wood, J.S. Williams, L. Pandarinathan, A. Courville, M.R. Keplinger, D.R. Janero, P. Vouros, A. Makriyannis, C.J. Lammi-Keefe, Clin. Chem. Lab. Med. 46 (2008) 1289.
[40] U. Ceglarek, M. Werner, L. Kortz, A. Korner, W. Kiess, J. Thiery, J. Kratzsch, J. Steroid Biochem. Mol. Biol. 121 (2010) 505.
[41] I. Unterwurzacher, T. Koal, G.K. Bonn, K.M. Weinberger, S.L. Ramsay, Clin. Chem. Lab. Med. 46 (2008) 1589.
[42] H.G. Gika, G.A. Theodoridis, I.D. Wilson, J. Chromatogr. A 1189 (2008) 314.
[43] M. Bou Khalil, W. Hou, H. Zhou, F. Elisma, L.A. Swayne, A.P. Blanchard, Z. Yao, S.A. Bennett, D. Figeys, Mass Spectrom. Rev. 29 (2010) 877.
[44] X. Guo, E. Lankmayr, Bioanalysis 2 (2010) 1109.
[45] D. Tsikas, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 878 (2010) 133.
[46] T. Guo, M. Chan, S.J. Soldin, Arch. Pathol. Lab. Med. 128 (2004) 469.
[47] T. Guo, R.L. Taylor, R.J. Singh, S.J. Soldin, Clin. Chim. Acta 372 (2006) 76.
[48] M. Scherer, C. Gnewuch, G. Schmitz, G. Liebisch, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 877 (2009) 3920.
[49] C. Steiner, A. von Eckardstein, K.M. Rentsch, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 878 (2010) 2870.
[50] X. Xiang, Y. Han, M. Neuvonen, J. Laitila, P.J. Neuvonen, M. Niemi, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 878 (2010) 51.
[51] M.M. Kushnir, A.L. Rockwood, W.L. Roberts, B. Yue, J. Bergquist, A.W. Meikle, Clin. Biochem. 44 (2011) 77.
[52] J. Bielawski, J.S. Pierce, J. Snider, B. Rembiesa, Z.M. Szulc, A. Bielawska, Adv. Exp. Med. Biol. 688 (2010) 46.
[53] C.A. Haynes, J.C. Allegood, H. Park, M.C. Sullards, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 877 (2009) 2696.
[54] G. Liebisch, G. Schmitz, Methods Mol. Biol. 580 (2009) 29.
[55] Y.Q. Xia, M. Jemal, Rapid Commun. Mass Spectrom. 23 (2009) 2125.
[56] J.Y. Lee, H.K. Min, M.H. Moon, Anal. Bioanal. Chem. 400 (2011) 2953.
[57] M. Scherer, A. Bottcher, G. Schmitz, G. Liebisch, Biochim. Biophys. Acta 1811 (2011) 68.
[58] A. Cutignano, U. Chiuminatto, F. Petruzziello, F.M. Vella, A. Fontana, Prostaglandins Other Lipid Mediat. 93 (2010) 25.
[59] T. Kasumov, H. Huang, Y.M. Chung, R. Zhang, A.J. McCullough, J.P. Kirwan, Anal. Biochem. 401 (2010) 154.
[60] Z. Zhao, Y. Xu, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 877 (2009) 3739.
[61] C. Ferreiro-Vera, J.M. Mata-Granados, F. Priego-Capote, J.M. Quesada-Gomez, M.D. Luque de Castro, Anal. Bioanal. Chem. 399 (2011) 1093.
[62] M. Haschke, Y.L. Zhang, C. Kahle, J. Klawitter, M. Korecka, L.M. Shaw, U. Christians, Clin. Chem. 53 (2007) 489.
[63] C. Mesaros, S.H. Lee, I.A. Blair, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 877 (2009) 2736.
[64] M.G. Balvers, K.C. Verhoeckx, R.F. Witkamp, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 877 (2009) 1583.
[65] P.M. Lam, T.H. Marczylo, J.C. Konje, Anal. Bioanal. Chem. (2010).
[66] D. Schreiber, S. Harlfinger, B.M. Nolden, C.W. Gerth, U. Jaehde, E. Schomig, J. Klosterkotter, A. Giuffrida, G. Astarita, D. Piomelli, F. Markus Leweke, Anal. Biochem. 361 (2007) 162.
[67] A. Thomas, G. Hopfgartner, C. Giroud, C. Staub, Rapid Commun. Mass Spectrom. 23 (2009) 629.
[68] K.M. Phillips, D.M. Ruggio, J.A. Bailey, J. Chromatogr. B: Biomed. Sci. Appl. 732 (1999) 17.
[69] A. Kuksis, J. Chromatogr. A 935 (2001) 203.
[70] J. Lembcke, U. Ceglarek, G.M. Fiedler, S. Baumann, A. Leichtle, J. Thiery, J. Lipid Res. 46 (2005) 21.
[71] A. Honda, K. Yamashita, H. Miyazaki, M. Shirai, T. Ikegami, G. Xu, M. Numazawa, T. Hara, Y. Matsuzaki, J. Lipid Res. 49 (2008) 2063.
[72] J.M. Buscher, D. Czernik, J.C. Ewald, U. Sauer, N. Zamboni, Anal. Chem. 81 (2009) 2135.
[73] A. Jannasch, M. Sedlak, J. Adamec, Methods Mol. Biol. 708 (2011) 159.
[74] M.M. Wamelink, E.A. Struys, J.H. Huck, B. Roos, M.S. van der Knaap, C. Jakobs, N.M. Verhoeven, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 823 (2005) 18.
[75] J.H. Huck, E.A. Struys, N.M. Verhoeven, C. Jakobs, M.S. van der Knaap, Clin. Chem. 49 (2003) 1375.
[76] M.M. Wamelink, D.E. Smith, E.E. Jansen, N.M. Verhoeven, E.A. Struys, C. Jakobs, J. Inherit. Metab. Dis. 30 (2007) 735.
[77] W.C. Yang, M. Sedlak, F.E. Regnier, N. Mosier, N. Ho, J. Adamec, Anal. Chem. 80 (2008) 9508.
[78] U. Harder, B. Koletzko, W. Peissner, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 879 (2011) 495.
[79] W.A. Waterval, J.L. Scheijen, M.M. Ortmans-Ploemen, C.D. Habets-van der Poel, J. Bierau, Clin. Chim. Acta 407 (2009) 36.
[80] M. Piraud, S. Ruet, S. Boyer, C. Acquaviva, P. Clerc-Renaud, D. Cheillan, C. Vianey-Saban, Methods Mol. Biol. 708 (2011) 25.
[81] M. Piraud, C. Vianey-Saban, K. Petritis, C. Elfakir, J.P. Steghens, D. Bouchu, Rapid Commun. Mass Spectrom. 19 (2005) 1587.
[82] M. Armstrong, K. Jonscher, N.A. Reisdorph, Rapid Commun. Mass Spectrom. 21 (2007) 2717.
[83] H. Kaspar, K. Dettmer, Q. Chan, S. Daniels, S. Nimkar, M.L. Daviglus, J. Stamler, P. Elliott, P.J. Oefner, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 877 (2009) 1838.
[84] M. Rafii, R. Elango, J.D. House, G. Courtney-Martin, P. Darling, L. Fisher, P.B. Pencharz, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 877 (2009) 3282.
[85] S. Shin, S.M. Fung, S. Mohan, H.L. Fung, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 879 (2011) 467.
[86] W.H. de Jong, E.G. de Vries, I.P. Kema, Clin. Biochem. 44 (2011) 95.
[87] B. Luo, K. Groenke, R. Takors, C. Wandrey, M. Oldiges, J. Chromatogr. A 1147 (2007) 153.
[88] G.U. Balcke, S.N. Kolle, H. Kamp, B. Bethan, R. Looser, S. Wagner, R. Landsiedel, B. van Ravenzwaay, Toxicol. Lett. 203 (2011) 200.
[89] R.I. Birkler, N.B. Stottrup, S. Hermannson, T.T. Nielsen, N. Gregersen, H.E. Botker, M.F. Andreasen, M. Johannsen, J. Pharm. Biomed. Anal. (2010).
[90] Z. Kitanovski, I. Grgic, M. Veber, J. Chromatogr. A 1218 (2011) 4417.
[91] P.M. Ueland, O. Midttun, A. Windelberg, A. Svardal, R. Skalevik, S. Hustad, Clin. Chem. Lab. Med. 45 (2007) 1737.
[92] B.A. Tarini, Arch. Pediatr. Adolesc. Med. 161 (2007) 767.
[93] A. Schulze, M. Lindner, D. Kohlmuller, K. Olgemoller, E. Mayatepek, G.F. Hoffmann, Pediatrics 111 (2003) 1399.
[94] P.H. Arn, Health Aff. (Millwood) 26 (2007) 559.
[95] D.C. Lehotay, P. Hall, J. Lepage, J.C. Eichhorst, M.L. Etter, C.R. Greenberg, Clin. Biochem. 44 (2011) 21.
[96] U.G. Jensen, N.J. Brandt, E. Christensen, F. Skovby, B. Norgaard-Pedersen, H. Simonsen, Clin. Chem. 47 (2001) 1364.

[97] G. la Marca, S. Malvagia, E. Pasquini, M. Innocenti, M.A. Donati, E. Zammarchi, Clin. Chem. 53 (2007) 1364.
[98] C.T. Turgeon, M.J. Magera, C.D. Cuthbert, P.R. Loken, D.K. Gavrilov, S. Tortorelli, K.M. Raymond, D. Oglesbee, P. Rinaldo, D. Matern, Clin. Chem. 56 (2010) 1686.
[99] D. Matern, S. Tortorelli, D. Oglesbee, D. Gavrilov, P. Rinaldo, J. Inherit. Metab. Dis. 30 (2007) 585.
[100] D. Matern, Acta Paediatr. Suppl. 97 (2008) 33.
[101] D. Marsden, H. Levy, Clin. Chem. 56 (2010) 1071.
[102] D.C. Kasper, J. Herman, V.R. De Jesus, T.P. Mechtler, T.F. Metz, B. Shushan, Rapid Commun. Mass Spectrom. 24 (2010) 986.
[103] J.L. Spratlin, N.J. Serkova, S.G. Eckhardt, Clin. Cancer Res. 15 (2009) 431.
[104] Q.N. Van, T.D. Veenstra, Genome Med. 1 (2009) 5.
[105] N.J. Serkova, J.L. Spratlin, S.G. Eckhardt, Curr. Opin. Mol. Ther. 9 (2007) 572.
[106] J. Chen, X. Zhang, R. Cao, X. Lu, S. Zhao, A. Fekete, Q. Huang, P. Schmitt-Kopplin, Y. Wang, Z. Xu, X. Wan, X. Wu, N. Zhao, C. Xu, G. Xu, J. Proteome Res. 10 (2011) 2625.
[107] V.W. Davis, O.F. Bathe, D.E. Schiller, C.M. Slupsky, M.B. Sawyer, J. Surg. Oncol. 103 (2011) 451.
[108] S. Ganti, S.L. Taylor, K. Kim, C.L. Hoppel, L. Guo, J. Yang, C. Evans, R.H. Weiss, Int. J. Cancer (2011).
[109] K. Kim, P. Aronov, S.O. Zakharkin, D. Anderson, B. Perroud, I.M. Thompson, R.H. Weiss, Mol. Cell. Proteomics 8 (2009) 558.
[110] K. Kim, S.L. Taylor, S. Ganti, L. Guo, M.V. Osier, R.H. Weiss, OMICS 15 (2011) 293.
[111] G. Liu, H.M. Snapp, Q.C. Ji, M.E. Arnold, Anal. Chem. 81 (2009) 9225.
[112] S.A. Ritchie, P.W. Ahiahonu, D. Jayasinghe, D. Heath, J. Liu, Y. Lu, W. Jin, A. Kavianpour, Y. Yamazaki, A.M. Khan, M. Hossain, K.K. Su-Myat, P.L. Wood, K. Krenitsky, I. Takemasa, M. Miyake, M. Sekimoto, M. Monden, H. Matsubara, F. Nomura, D.B. Goodenowe, BMC Med. 8 (2010) 13.
[113] A. Sreekumar, L.M. Poisson, T.M. Rajendiran, A.P. Khan, Q. Cao, J. Yu, B. Laxman, R. Mehra, R.J. Lonigro, Y. Li, M.K. Nyati, A. Ahsan, S. Kalyana-Sundaram, B. Han, X. Cao, J. Byun, G.S. Omenn, D. Ghosh, S. Pennathur, D.C. Alexander, A. Berger, J.R. Shuster, J.T. Wei, S. Varambally, C. Beecher, A.M. Chinnaiyan, Nature 457 (2009) 910.
[114] W. Wang, B. Feng, X. Li, P. Yin, P. Gao, X. Zhao, X. Lu, M. Zheng, G. Xu, Mol. Biosyst. (2010).
[115] C.B. Newgard, J. An, J.R. Bain, M.J. Muehlbauer, R.D. Stevens, L.F. Lien, A.M. Haqq, S.H. Shah, M. Arlotto, C.A. Slentz, J. Rochon, D. Gallup, O. Ilkayeva, B.R. Wenner, W.S. Yancy Jr., H. Eisenson, G. Musante, R.S. Surwit, D.S. Millington, M.D. Butler, L.P. Svetkey, Cell Metab. 9 (2009) 311.
[116] T.J. Wang, M.G. Larson, R.S. Vasan, S. Cheng, E.P. Rhee, E. McCabe, G.D. Lewis, C.S. Fox, P.F. Jacques, C. Fernandez, C.J. O'Donnell, S.A. Carr, V.K. Mootha, J.C. Florez, A. Souza, O. Melander, C.B. Clish, R.E. Gerszten, Nat. Med. 17 (2011) 448.
[117] C. Langenberg, D.B. Savage, Nat. Med. 17 (2011) 418.
[118] G.D. Lewis, R.E. Gerszten, Circ. Cardiovasc. Genet. 3 (2010) 119.
[119] S.H. Shah, J.R. Bain, M.J. Muehlbauer, R.D. Stevens, D.R. Crosslin, C. Haynes, J. Dungan, L.K. Newby, E.R. Hauser, G.S. Ginsburg, C.B. Newgard, W.E. Kraus, Circ. Cardiovasc. Genet. 3 (2010) 207.
[120] S.H. Shah, E.R. Hauser, J.R. Bain, M.J. Muehlbauer, C. Haynes, R.D. Stevens, B.R. Wenner, Z.E. Dowdy, C.B. Granger, G.S. Ginsburg, C.B. Newgard, W.E. Kraus, Mol. Syst. Biol. 5 (2009) 258.
[121] A.T. Turer, R.D. Stevens, J.R. Bain, M.J. Muehlbauer, J. van der Westhuizen, J.P. Mathew, D.A. Schwinn, D.D. Glower, C.B. Newgard, M.V. Podgoreanu, Circulation 119 (2009) 1736.