

Metabolomics: new analytical methods for metabolome research

Tobias Demetrowitsch, Karin Schwarz, Kiel

Summary

Metabolome research is based on so-called “omic” techniques, which are particularly used to investigate problems in system biology. The central analytical procedure in metabolomics is mass spectrometry – mostly in combination with chromatographic procedures. The evaluation may either be targeted (hypothesis-supported) or untargeted (hypothesis-free). As these evaluation approaches in combination with mass spectrometry generate high data density, computerised procedures are inevitable. Targets, core statements and the analytical techniques are described.

Keywords: mass spectrometry, metabolomics, data processing

1 Investigation of human metabolism

Fundamental knowledge of human metabolism provides an important foundation for nutritional sciences and modern medicine. Changes in body fluids (e. g. blood plasma and urine) indicate that there have been changes in the conditions of the organism. These are often signs of disease. For a long time, measurements on urine were the central instrument for the diagnosis of diseases without obvious injuries. Even in the fifth century BC, HIPPOKRATES and HERMOGENES diagnosed diseases on the basis of the properties of urine [1].

Citation:

Demetrowitsch T, Schwarz K (2014) Metabolomics: new analytical methods for metabolome research. *Ernahrungs Umschau* 61(7): 102–111

This article is available online DOI: 10.4455/eu.2014.019

In the 1950s, the concept of systematic characterisation of the metabolomic pattern of urine was introduced. The urine of ca. 200,000 individuals was examined by paper and thin layer chromatography [2]. A specific metabolic pattern was found for different diseases.

In the 1960s and 1970s, additional promising results were provided by using gas chromatography and mass spectrometry (see section 3). Thus HORNING and HORNING (1971) listed additional characteristic properties of urine, including increased concentrations of aromatic compounds in various diseases [3]. This approach has been continuously improved by progressive improvements in different analytical techniques, electronic process management and data processing.

2 “Omic” techniques are developed

The introduction of “omic” techniques has led to the adaptation of the experimental design to new

external conditions, such as improvements in the recording and processing of new data. These techniques will now be described and explained using metabolomics as an example.

“Omic” techniques provide the methodological foundation of the new approach of system biology, which aims to investigate the complex interrelationships between physiological processes, whilst considering as many parameters as possible. The objectives of “omic” techniques are to identify and quantify:

- all genes (the genome), together with the corresponding regulatory molecules (genomics);
- all transcripts (e. g. messenger RNA and functional RNA), (transcriptomics) as a map of gene activity;
- all proteins formed (e. g. enzymes and structural and transport proteins) (proteomics);
- all metabolites¹ of an organism (the metabolome) (metabolomics).

◆ Figure 1 gives an overview of the different “omic” areas. On the left, there are various factors, such as age or gender, that influence the different components of the organism (e. g. the genome or endogenous proteins). This influence is evident in the individual phenotype (black arrows).

¹ These include metabolic products up to a molecular weight of 1,500 Daltons.

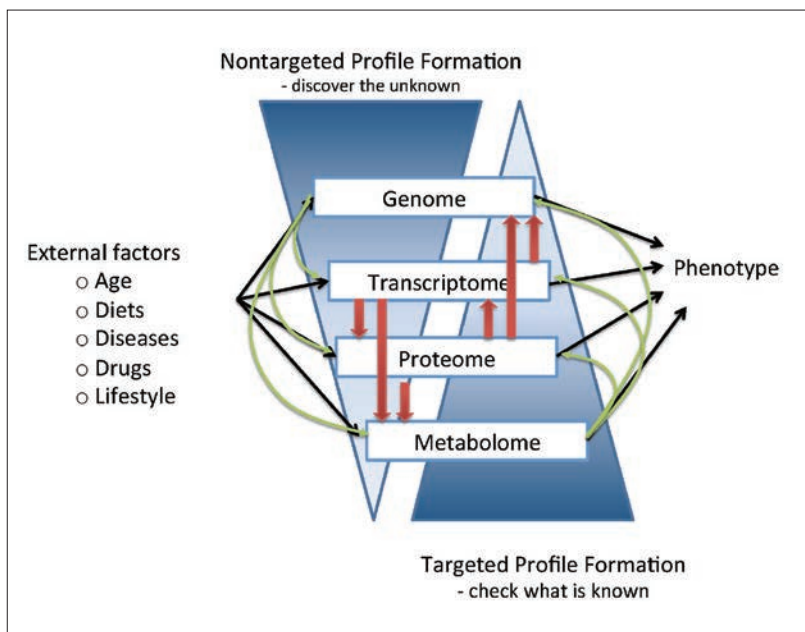


Fig. 1: Sketch of the interactions between the different “omic” areas. On the left are the different external factors acting on the organism, and these directly influence the phenotype (right) (modified from [4])

The metabolome describes all chemical compounds within the individual and is influenced by both external and internal factors. The green arrows show the interrelationships.

The red arrows in the figure illustrate the influence of the transcriptome at the protein and metabolite level and, conversely, the regulatory effect of the proteins on the genome and thus on the transcriptome. The arrows symbolize the complex interactions at the different levels in the organism.

Metabolomics

Metabolomics pursues the goal of identifying and quantifying all potential metabolic products and of studying their interrelationships [5]. The physiological significance of the new information on metabolic products is evaluated with respect to metabolic pathways and functions. Substance flows are quantified and assigned to cell compartments.

Metabolomic research requires effec-

tive and precise analytical systems, as the samples are complex and many compounds must be identified. It is important to remember that complete organisms, organs, individual cells and their organelles each possess their own specific metabolome. This naturally changes, e. g. with age, and is gender-dependent to some extent. Moreover, the metabolome can be influenced by external factors, such as climatic conditions, time of day, season and nutritional habits (♦ Figure 1).

It is particularly interesting to employ a hypothesis-free approach (see section 4) to identify unusual metabolite patterns, which, for example, may occur in individual diseases [5]. This may draw attention to the pathological role of previously overlooked metabolites.

However, this new approach to metabolic research necessitates an expanded experimental design. In the classical experimental design, a single or a few parameters are recorded and analysed. As a consequence, the quantity of data must be minimised

to as great an extent as possible, in order to guarantee simple and clear analysis.

In contrast, in the new approach, as much data as possible must be generated (♦ Box 1), as a complement to the classical approach.

One frequently employed analytical technique is mass spectrometry (vide infra). NMR (nuclear magnetic resonance) spectroscopy is also used, albeit less frequently (cf. ♦ Table 4, Study 4). One advantage of NMR spectroscopy is that it can not only identify metabolites, but also clarify their molecular structures. On the other hand, the use of NMR is greatly restricted by the minimum concentration of $\mu\text{mol/L}$ to mmol/L for individual metabolites [6].

3 Mass spectrometry in metabolomics

After the chromatographic separation, the substance mixture to be analysed is passed continuously into the mobile phase. The chromatography system then serves as autosampler for the mass spectrometer (♦ Box 2). A mass spectrometer exclusively determines the molecular masses of the compounds in a mixture of substances. Many different substances of widely different masses can be analysed in parallel.

Using comparator spectra, the detected masses can then be assigned to specific compounds, e. g. pyruvate, cytosine etc.

There are many different models of mass spectrometer, although all systems consist in principle of the same basic components:

- ioniser,
- analyser and
- detector.

The ioniser (or ion source) converts unionised sample molecules into ions. For example, protons can be attached to the molecule or torn out of the molecule with a strong electric field.

The analyser separates the ionised

Box 1:
Large quantities of data need a novel presentation

Metabolomic techniques provide large quantities of measurement data. To permit rapid comparisons, this may, for example, be presented in the form of the so-called heat map. An essential characteristic of the heat map is that the quantification is colour coded, thus allowing a simple portrayal of a third dimension.

◆ Figure 2 shows two examples of heat maps from urine samples. These show the measurements from two male volunteers (29 and 34 years old, both healthy and sporting).

The left y-axis shows the molecular mass of a substance (the mass to charge ratio “m/z”). On the right side, the colour changes are defined that represent the measured signal intensities (signal strengths). The x-axis shows the time of analysis. This corresponds to the retention time, the time between sample application and the detector signal. This time is influenced by the chromatographic conditions (◆ Box 2: Chromatographic methods).

Each green point in the figures corresponds to a peak to which a mass can be assigned. These are considered for the subsequent analysis.

Heat maps can be used to compare complex samples, so that changes in the pattern are rapidly evident. For example, the red circles within the heat map correspond to four substances that naturally occur in urine. Typical databases include several hundred metabolites (see Section 4 and ◆ Table 3).

Metabolite No. 1 was identified as cytosine. Cytosine has a mass of 111 Daltons and was measured after 1.4 minutes. Cytosine is i. a. a component of DNA.

Metabolite No. 2 is ascorbic acid (vitamin C), with a mass of 176 Daltons and a retention time of 2.2 minutes.

Metabolite No. 3 is isoleucine, with a mass of 131 Daltons and a retention time of 6.1 minutes. Isoleucine is an essential amino acid in man and is a structural element in proteins.

Metabolite No. 4 is creatinine, with a mass of 113 Daltons and a retention time of 7.5 minutes. Creatinine is a natural metabolic product in man and is eliminated in urine.

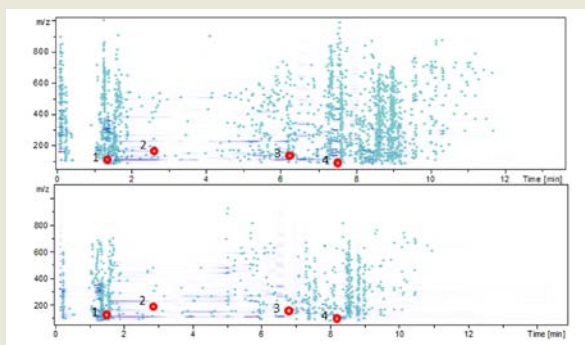


Fig. 2: Presentation of a mass spectrometric measurement of two human urine samples as heat map. The x-axis represents time [min.] and the y-axis mass to charge ratio [m/z]; the concentration (third dimension/z-axis) is portrayed as different colours; the scale ranges from white (absent) to magenta (very high signal intensity); the evaluation was performed with the program DataAnalysis 4.2 from Bruker Daltonik (Bremen).

molecules, so that they can be more easily detected by the following detector. ◆ Table 2 shows an overview of the different analysers.

The detector is the most important component of every mass spectrometer. It determines the molecular mass and thus the composition of the ionised molecules. It produces a digital signal, which can be portrayed by the linked evaluation computer with comparator spectra (◆ Figure 3).

In principle, the ionised sample molecules are accelerated with electrodes of various constructions and “flight chambers”, depending on their mass to charge ratios. They are partially filtered out of the system. Depending on their final velocity, the flight time (in the so-called time of flight (TOF) procedure) and the depth of penetration into the computer, the molecules can be characterised and assigned to possible molecular formulas.

In order to limit the number of possible molecular formulas, only the most common natural atoms (carbon, hydrogen, nitrogen and oxygen) are considered at the start of an analysis. If required (e. g. if expected), any other element in the periodic system can be added. The subsequent identification can be performed with the help of various parameters, such as the deviation between the measured and theoretical mass (e. g. in ppm), the isotopic pattern, or on the basis of known fragments of the expected substance. The probability of successful and correct identification is greater, the smaller the number of possible molecular formulas.

4 Evaluation approaches

Data collection is followed by data evaluation and interpretation. ◆ Figure 3 shows a typical mass spectrometric chromatogram for the measurement of a urine sample. The red chromatogram gives an overview of all measured masses, although only the mass with the greatest intensity per measu-

Box 2:

Chromatographic Methods

To investigate the metabolome, the samples are separated by a chromatographic procedure, before being analysed by e. g. mass spectrometry (see Section 3).

In general, chromatography means the procedure to separate a substance mixture through the interaction of individual substances with a stationary phase. The substances are passed along with the help of a mobile phase, such as water. The substances interact with the stationary phase to various degrees. This means that they are delayed to various extents (and thus separated) during the transport with the mobile phase.

When chromatography was first described in 1907, the system used was thin layer chromatography. With a filter paper as stationary phase, a chlorophyll solution was separated into its components, which were visible as coloured spots on the filter paper. In later years, various different chromatographic techniques were tested, established and refined. The objective has always been continuously to increase the separation efficiency of the chromatographic systems, to shorten the times of analysis and to automate the analytical process. ♦ Table 1 shows an overview of the chromatographic systems, with some advantages and disadvantages.

Work on metabolomics primarily employs gas and high pressure fluid chromatography.

Name	Abbr.	Separation Principle	Sample	Advantages	Disadvantages
Thin layer chromatography	TLC	capillary forces	fluid	simple, quick	quantification difficult
Gas chromatography	GC	chemical interaction	volatile, gas phase	identification and quantification possible	substances must be volatile or gas phase; thermally labile substances decompose
High pressure liquid chromatography	HPLC	chemical interaction	fluid	non-destructive analysis, low sample volume	expensive due to heavy consumption of solvent and materials
Isoelectric focussing	IEF	use of the lowest net charge at the isoelectric point	fluid	multidimensional sample separation	applicable to ionised compounds (amino acids, proteins)
Gel permeation chromatography	GAC	size separation	fluid	quick, simple to handle	separation by molecular size
Supercritical fluid chromatography	SFC	chemical interaction	fluid or gas phase	possible to separate complex mixtures that would not be separable with GC/HPLC	expensive, complex equipment

Tab. 1: Overview of various chromatographic systems

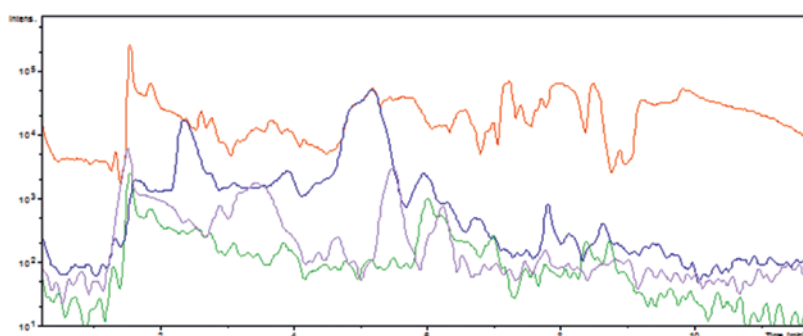


Fig. 3: Portrayal of a urine sample as mass chromatogram (data collected with an LC-QToF/MS); the x-axis represents the retention time in minutes. The y-axis represents the intensity of the peak in logarithmic form ($10^2 = 100$ intensity units); red: base peak chromatogram; green: mass chromatogram for cytosine; blue: chromatogram for ascorbic acid; magenta: mass chromatogram for creatinine.

Analyser	Symbols	Advantages
Quadrupole instruments (e. g. triple quadrupole)	QqQ	<ul style="list-style-type: none"> • suitable for trace analysis (qualitative and quantitative) • very high detector sensitivity
Linear Quadrupol ion traps	LIT	<ul style="list-style-type: none"> • principally suitable for clarifying structure • target molecules can be fragmented several times
Fourier transform ion cyclotron resonance instruments	FT-ICR	<ul style="list-style-type: none"> • extremely high mass resolution and precision • theoretically suitable for all areas of work • high costs of purchasing and demanding structural requirements
Time of flight instruments (e. g. time of flight)	ToF	<ul style="list-style-type: none"> • broad area of application • high mass resolution • very good mass determination
Hybrid instruments (e. g. quadrupole-time of flight)	QToF	<ul style="list-style-type: none"> • combination of different analysers • advantages are effectively combined and exploited

Tab. 2: Different analyser types and their advantages (modified from the German Society for Mass Spectrometry [7])

rement is shown. The other chromatograms shown (green, blue and magenta) each portray the intensity for a specified mass (= substance) (green = cytosine, blue = ascorbic acid and magenta = creatinine).

This type of chromatogram is known as an extracted ion chromatogram (EIC). It is filed for all masses (= substances) thought to be present in a mixture. The EICs can be used to check whether and when a mass is detected.

However, it is very tedious to prepare such chromatograms by manual entry, so that computerised applications are mainly used.

There are various techniques; the two most important for “omic” studies will be described. Hypothesis-supported (targeted) and hypothesis-free (untargeted) approaches are distinguished.

Targeted approach

In the targeted (hypothesis-supported) approach, the measurement results are

analysed for the expected compounds.

♦ Table 3 shows an extract from a database needed for this approach.

The complete database contains more than 750 different compounds [8]. For the process of assignment, not only the substance name, but also the molecular formula must be known. This is used to derive the molecular mass of the substance.

♦ Table 3 also contains quantitative information on some compounds. Quantitative analysis can also be performed with the targeted evaluation approach. The concentration of the substances is then expressed in “mmol creatinine equivalents”, as creatinine is approximately proportional to the dilution of the urine and the metabolites in urine of different concentrations can be compared relative to creatinine.

As there are so many different compounds, it is impossible in practice to set up a calibration line for each one. Relative concentrations can be given with reference to a calibration line for creatinine equivalents.

Various parameters are used as a basis for the evaluation. One of the most

important is the measured intensity (peak height of the substance). Substances with a peak height of less than 100 relative intensity units are generally not considered, as the electronic noise of the detector can cause major interference up to this intensity.

Another parameter for identification is the deviation of the mass and the isotopic pattern (“mSigma” value) of a measured value in comparison to the derived molecular formula.

The measured mass is compared with the theoretical mass. The difference between the two values corresponds to the precision of the measurement. The smaller the value is, the better the measured value fits to the theoretical mass.

As several different isotopes occur in each compound, this gives an isotopic pattern. As the distribution pattern and the probability that isotopes occur in natural compounds are the same, the intensity and number of isotope peaks (isotopic pattern) can be predicted for a molecular formula.

The measured mass is compared with the theoretical mass. The dif-

#	Name	Molecular Formula	Mass (Da)	Concentration $\mu\text{mol}/\text{mmol creatinine}$
1	Pyruvate	$\text{C}_3\text{H}_4\text{O}_3$	88.0155	4.21
2	Uracil	$\text{C}_4\text{H}_4\text{N}_2\text{O}_2$	112.0267	12.00
3	Creatinine	$\text{C}_4\text{H}_7\text{N}_3\text{O}$	113.0584	230.00
4	Nicotinic acid	$\text{C}_6\text{H}_5\text{NO}_2$	123.0315	0.65
5	Norleucin	$\text{C}_6\text{H}_{13}\text{NO}_2$	131.0941	0.0065
6	L-Homocysteine	$\text{C}_4\text{H}_9\text{NO}_2\text{S}$	135.0349	1.78
7	Adipic acid	$\text{C}_6\text{H}_{10}\text{O}_4$	146.0574	5.10
8	Xanthine	$\text{C}_5\text{H}_4\text{N}_4\text{O}_2$	152.0329	2.90
9	1-Methylxanthin	$\text{C}_6\text{H}_6\text{N}_4\text{O}_2$	166.0485	6.00
11	Uric acid	$\text{C}_5\text{H}_4\text{N}_4\text{O}_3$	168.0278	206.00
12	Ascorbic acid	$\text{C}_6\text{H}_8\text{O}_6$	176.0315	32.50
13	Hippuric acid	$\text{C}_9\text{H}_9\text{NO}_3$	179.0577	217.00
18	Caffeine	$\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$	194.0798	1.20

Tab. 3: Extract from the FoCUS database for more than 750 metabolites for the hypothesis-supported evaluation approach; the source data are taken from "The Human Metabolome Database" (www.hmdb.ca) [9–11]

ference between the two values corresponds to the precision of the measurement. The smaller the value is, the better the measured value fits to the theoretical mass.

As several different isotopes occur in each compound, this gives an isotopic pattern. As the distribution pattern and the probability that isotopes occur in natural compounds are the same, the intensity and number of isotope peaks (isotopic pattern) can be predicted for a molecular formula.

A comparison between the measured and theoretical isotope pattern (cf. \blacklozenge Box 3) can be used to derive a value which, in the ideal case, approaches zero. This indicates that there is a very high probability that the compound is present.

By comparing the masses and isotope patterns, large quantities of data can be rapidly evaluated. This methodological approach is therefore particularly suitable for screening analyses, in which many experiments are performed under constant conditions.

It has the disadvantage that it can only be applied for expected substances and that unexpected substances cannot be considered in the automated evaluation.

Nontargeted evaluation approach

The aim of the untargeted evaluation approach is to recognise differences and similarities between different types of volunteers by the simulta-

neous comparison of numerous metabolites. In contrast to the targeted approach, it is initially of no great importance which metabolites are of special interest. It is more important to identify differences in the metabolite profiles in different volunteer types.

Without statistical procedures, the metabolite profiles would have to

Box 3:

Isotope pattern in mass spectroscopy

Isotopes have the same number of protons and electrons, but may have additional neutrons, so that their mass is greater than that derived from the molecular formula. (The upper number before the element symbol corresponds to the sum of the protons and neutrons in the isotope).

The significance of the isotope pattern will be illustrated using the example of pyruvate, which has a molecular formula of $\text{C}_3\text{H}_4\text{O}_3$:

The probability that this compound contains only ^{13}C atoms rather than ^{12}C atoms is ca. 1.11 %. The probability that only deuterium (^2H) rather than hydrogen (^1H) is present is 0.012 %. The probabilities that oxygen is only present as ^{17}O or ^{18}O rather ^{16}O are 0.038 % and 0.025 %, respectively. As a result, different masses are measured for pyruvate. The most frequent mass is 88.0154, corresponding to the main peak. The isotopic forms with masses 89.0189 and 90.0199 correspond to 3.405 % and 0.657 % relative to the main peak, respectively. These masses are the theoretical isotopic pattern for pyruvate.

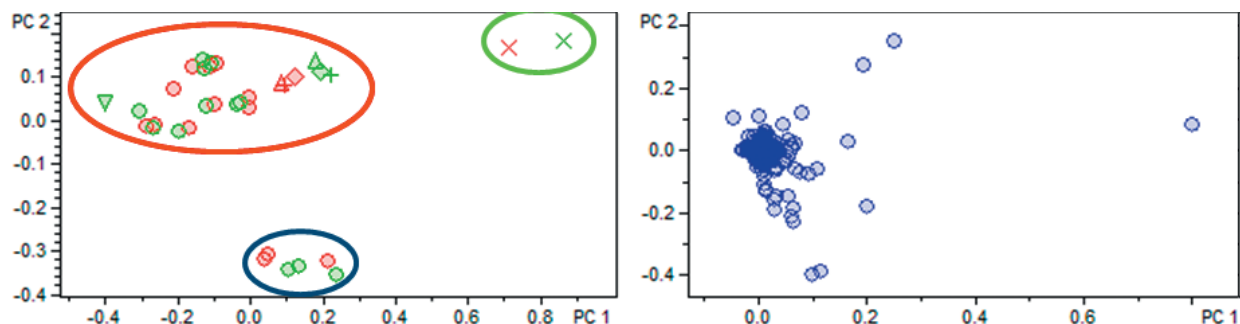


Fig. 4: Result of a PCA of urine samples (QToF/MS) and group formation; left: groups identified for the measured urine samples (Circles and triangles are different samples. The classification does not influence the evaluation algorithm, but serves for graphical classification); right: portrayal of the measured metabolites. Additional explanations in the text.

No. / Author	Subject of Investigation	Volunteers
1. MEDINA et al. (2012) [12]	Study of oxidative stress with biomarkers (e. g. prostaglandins)	15 sportsmen training for a triathlon
2. MAY et al. (2013) [13]	Characterisation of urine in an intervention study; biomarker detection for vegetable consumption	293 healthy study participants; 60 participants for diet-based study; 72 participants for intervention study
3. BAKKER et al. (2010) [14]	Demonstration of the reduction in mild inflammation, metabolic and oxidative stress with food components, such as vitamin C, n3-fatty acids, resveratrol etc.	36 volunteers in a randomised, placebo-controlled double blind study
4. WALSH (2006) [15]	Detection of intra- and inter-individual variation in the metabolites in body fluids, as well as their changes with diet	30 healthy volunteers (23 women, 7 men)
5. REDEUIL et al. (2011) [16]	Detection of polyphenol metabolites after coffee consumption, without sample preparation with enzymes	4 men, 5 women (n = 9); healthy volunteers
6. SCHMERLER et al. (2012) [17]	Research on specific biomarkers to distinguish infection-related systemic inflammation (sepsis) and non-infection-related systemic inflammation	96 volunteers were selected (male/female). 41 were non-infectious, 39 had sepsis and 16 were controls
7. CAI et al. (2011) [18]	Detection of biomarkers for lung cancer patients in comparison to healthy patients	28 healthy volunteers and 66 lung cancer patients (57 men and 37 women)
8. McCLAY et al. (2010) [19]	Comparison of urine and plasma samples in chronic obstructive lung disease (COPD) in comparison with healthy volunteers, with the aim of finding biomarkers	392 volunteers, including 197 with COPD and 195 healthy; no prior diet
9. ISSAQ et al. (2008) [20]	Detection of biomarkers for bladder cancer; these must be collected non-invasively, but still be highly sensitive	48 healthy volunteers and 41 volunteers with urothelial carcinoma (bladder cancer), including 36 men and 5 women

Tab. 4: Examples of studies in metabolite research

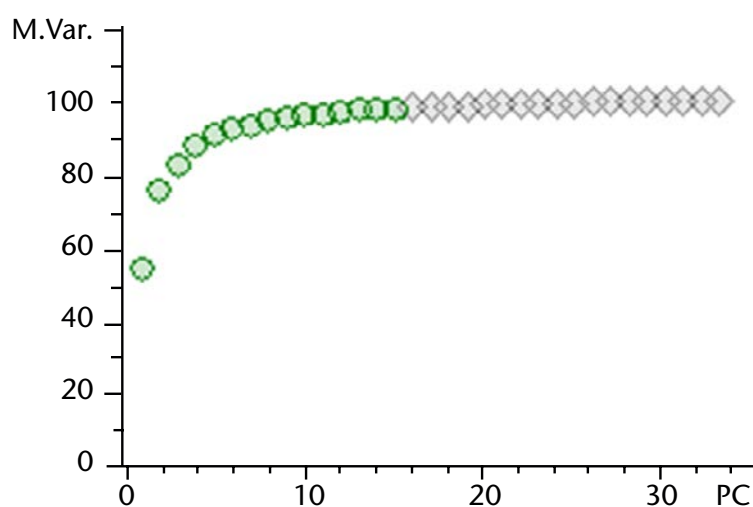


Fig. 5: Comparison of the information content against the number of principle components (principle component analysis). Explanations in text.

Sample Material	Central Conclusion
24h-urine, before and after 2 weeks of training	Significant decrease in 3 stress markers after 2 weeks of training
Urine after at least 8-hour fasting	Proline-betaine, sulphoraphane and isoflavonoids were identified as stable biomarkers for vegetable consumption
Plasma samples after 8-hour fast	The combination of food components influenced the biomarker adiponectin and the biomarkers of oxidative stress, e. g. 8-isoprostaglandin-F2 α
Samples taken in the morning; normal nutrition before visits 1 and 2. Before visit 3, the same diet should be taken as before visit 2. Before visit 3, a standardised diet was taken	Diet had a marked effect in urine, but not in plasma or saliva. The inter- and intra-individual fluctuations in the metabolite spectrum in urine can best be reduced with a standard diet
Before start of study, caffeine-containing foods forbidden. Blood samples taken after defined intervals	34 polyphenol derivatives were identified in blood samples, which were directly linked to coffee consumption. 19 of these were detected in human plasma for the first time
Blood samples were taken from the volunteers within 24 hours of the first symptoms; with sepsis patients, the samples were taken within 24 hours of the first organ failure	Two biomarkers were identified that were significantly raised in plasma after infectious sepsis (acylcarnitine and glycerophosphatidylcholine)
Blood samples taken after fasting; plasma samples were taken without, during or after therapy	19 potential biomarkers were identified; their concentrations changed during therapy. There were differences in the metabolome between healthy and sick volunteers
Urine and blood samples	Three potential biomarkers were identified for lung function, although the corresponding mechanisms are still unknown
All samples were taken before possible cancer therapy	98 % of the sick volunteers and 96 % of the healthy volunteers could be classified to the correct clusters; with PLS statistics, even 100 % were correctly classified

be presented in a multidimensional coordinate system (one metabolite per axis). It would then be practically impossible to recognise correlations or groups by eye. Thus, evaluation without statistical procedures would be extremely complex and tedious.

This approach employs the recognised statistical procedure of principal component analysis (PCA). PCA determines whether there are correlations between individual metabolites, so that – to put it simply – some of the metabolites or their axes can be omitted or replaced by a combination (with one axis for several metabolites). For example, a substance may always occur at a fixed ratio to its degradation product. Finally, a large number of metabolites may be portrayed in two dimensions, using a few combinations of metabolites, so-called “principle components” (♦ Figure 4).

To reduce the dimensions, the distances are repeatedly calculated. These are determined using an axis running through the midpoint of the measured data. The smallest sum of all distances on a line is defined as the first principle component. This distance possesses the greatest variance (the greatest information content) of all metabolites of all samples.

In the next step, an additional axis is identified, that is orthogonal (vertical) to the first. The second principle component is then determined with the same conditions. The data point of the first principle component is then no longer considered. This procedure is repeated until all the points and the corresponding variance (the distance between the axis and the data point) can be explained. ♦ Figure 5 shows that, with the fifth principle component, ca. 90 % of the variance is explained which underlies group formation in ♦ Figure 4.

♦ Figure 4 shows an evaluated PCA and group formation in a serial measurement of different urine samples from different volunteers. The left

side of the graph shows a so-called score plot. This shows group formation of all measured urine samples. The right figure is known as a loading plot and shows the distribution of all measured masses. The masses which are most distant from the point cloud have the greatest influence on the variance and therefore dominate the whole statistical model of principle component analysis leading to group formation (♦ Figure 4, left side).

Masses that influence the whole model to such an extent are of especial importance for further research, as they are not contained in all samples, but greatly influence the statistical model determined.

The advantage of the untargeted approach is that interrelationships can also be established for samples of unknown composition. The evaluation is rapid. Any faulty measurements or outliers can be recognised statistically and removed from the model.

However, the disadvantage is that the calculations are highly complex and require a great deal of computing capacity.

5 Typical applications

Nutritional metabolomic studies with volunteers are performed to identify relevant biomarkers, that allow conclusions about metabolic effects and are relevant to the prevention of chronic diseases. Metabolomic studies also investigate to what extent different nutritional patterns give rise to characteristic metabolite profiles. Studies 1 to 5 in ♦ Table 4 show typical studies performed with healthy volunteers and which were intended to examine the effects of dietetic intervention and physical stress on the metabolome.

The metabolome of volunteers is also measured in the recruitment of large cohorts, in order to achieve additional phenotyping that is complementary to conventional clinical parameters [8]. In addition, metabolomic

studies are used to improve medical diagnostic testing (studies 6 to 9 in ♦ Table 4).

In both healthy and sick volunteers, metabolomic techniques in combination with untargeted statistical evaluation procedures can be used to identify biomarkers that only appear when external factors are varied. Some of these biomarkers were unexpected and therefore could not have been found with the targeted approach alone.

Effects on the metabolome of a diet rich in vegetables and fruit

One example of an untargeted approach is the study of MAY et al. (2013) in ♦ Table 4, in which the urine samples of volunteers were examined with LC/MS [13]. 60 volunteers – 30 with low and 30 with high vegetable consumption – were compared with 72 volunteers who were subject to a controlled nutritional plan for vegetable consumption. The objective of the study was to analyse differences in the composition of the volunteers’ urine. The urine was analysed during a 2-week menu with many different sorts of fruit and vegetables, in comparison with a 2-week diet in which the same food was forbidden.

It was found that, with a diet rich in plants, there were many different and typical metabolites. For example, proline-betaine was detected; this is a metabolite marker that is detected in human metabolism after the consumption of citrus fruits. In addition, sulforaphanes were detected. These are hydrolysis products of the glucosinolate glucoraphanin.

With the untargeted evaluation approach, a total of 46 metabolites could be identified that only occurred in man after consumption of fruit and vegetables.

This study allows the conclusion that metabolomic analyses of urine can be usefully applied in restric-

tion studies – in which one group of foods is omitted – in order to check that dietary instructions are being complied with.

6 Conclusion

Metabolomics offers novel approaches for medical studies and nutritional and food research, particularly in studying very complex interrelationships. The experimental techniques and equipment are designed to collect maximal quantities of data, with a high degree of automation and comprehensive approaches to evaluation.

With the untargeted analysis of the metabolome, it is possible to search for suitable biomarkers in nutritional studies. This can start with screening on a very broad basis and can help to draw attention to metabolites that had previously been overlooked.

As foods are so complex, not only one metabolite, but many, may be found at different concentrations in nutritional studies. The shifts in the resulting metabolite pattern are often only moderate. This problem can be approached by evaluating a very wide range of metabolites, using the specific approaches of metabolomics.

Dr. Tobias Johannes Demetrowitsch
Prof. Dr. Karin Schwarz
Institut für Humanernährung und Lebensmittelkunde
Abteilung Lebensmitteltechnologie
Heinrich-Hecht-Platz 10
24118 Kiel
E-mail: info@foodtech.uni-kiel.de

Conflict of Interest

The authors declare no conflict of interest according to the guidelines of the International Committee of Medical Journal Editors.

References

1. Villas Bôas SG. *Metabolome analysis. An introduction*; Wiley-Interscience: Hoboken, New Jersey [etc.], op. (2007)
2. Williams R. *Individual metabolic patterns and human disease: an exploratory study utilizing predominantly paper chromatographic methods*. The University of Texas Publication. (1951)
3. Horning EC, Horning MG (1971) *Metabolic profiles: gas-phase methods for analysis of metabolites*. *Clinical Chemistry* 17(8): 802–809
4. Preet A (2013) *Metabolomics: approaches and applications to diabetes research*. *J Diabetes Metab* 01 : 1–8
5. Dettmer K, Aronov PA, Hammock BD (2007) *Mass spectrometry-based metabolomics*. *Mass Spectrom Rev* 26: 51–78
6. Beckonert O, Keun HC, Ebbers TM D et al. (2007) *Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts*. *Nat Protoc* 2: 2692–2703
7. Deutsche Gesellschaft für Massenspektrometrie. URL: www.dgms-online.de/ Zugriff 21.03.14
8. FoCUS FoCUS – Food Chain Plus. URL: www.focus.uni-kiel.de Zugriff 21.03.14
9. Wishart DS, Tzur D, Knox C et al. (2007) *HMDB: the Human Metabolome Database*, *Nucleic Acids Research* 35: D521
10. Wishart DS, Knox C, Guo A et al. (2009) *HMDB: a knowledgebase for the human metabolome*, *Nucleic Acids Research* 37: D603
11. Wishart DS, Jewison T, Guo AC et al. (2012) *HMDB 3.0 – The Human Metabolome Database in 2013*, *Nucleic Acids Research* 41: D801–D807
12. Medina S, Domínguez-Perles R, Cejuela-Anta R et al. (2012) *Assessment of oxidative stress markers and prostaglandins after chronic training of triathletes*. *Prostaglandins & Other Lipid Mediators* 99: 79–86
13. May DH, Navarro SL, Ruczinski I et al. (2013) *Metabolomic profiling of urine: response to a randomised, controlled feeding study of select fruits and vegetables, and application to an observational study*. *Br J Nutr*. 110(10): 1760–1770
14. Bakker GCM, van Erk MJ, Pellis L et al. (2010) *An anti-inflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach*. *Am J Clin Nutr* 91: 1044–1059
15. Walsh MC, Brennan L, Malthouse JPG et al. (2006) *Effect of acute dietary standardization on the urinary, plasma, and salivary metabolomic profiles of healthy humans*. *Am J Clin Nutr* 84: 531–539
16. Redeuil K, Smarrito-Menozi C, Guy P et al. (2011) *Identification of novel circulating coffee metabolites in human plasma by liquid chromatography–mass spectrometry*. *J Chromatography* 1218: 4678–4688
17. Schmerler D, Neugebauer S, Ludewig K et al. (2012) *Targeted metabolomics for discrimination of systemic inflammatory disorders in critically ill patients*, *J Lipid Res* 53: 1369–1375
18. Cai X, Dong J, Zou L et al. (2011) *Metabonomic study of lung cancer and the effects of radiotherapy on lung cancer patients: analysis of highly polar metabolites by Ultraperformance HILIC coupled with Q-TOF MS*, *Chromatographia* 74: 391–398
19. McClay JL, Adkins DE, Isern NG et al. (2010) *H Nuclear Magnetic Resonance Metabolomics Analysis identifies novel urinary biomarkers for lung function*. *J Proteome Res* 9: 3083–3090
20. Issaq HJ, Nativ O, Waybright T et al. (2008) *Detection of bladder cancer in human urine by metabolomic profiling using High Performance Liquid Chromatography/Mass Spectrometry*. *J Urol* 179: 2422–2426

DOI: 10.4455/eu.2014.019