Metabolomics measures and analyses metabolic changes

Metabolomics measures and analyses metabolic changes caused by illness, diet or medication. During metabolism, molecules are created and broken up, and some of these have an effect on health. Their concentrations are measured from blood, urine and tissue samples.

Metabolomics enables the detection of biomarkers that can give an indication of a person's lifestyle, diet, illnesses and the effects of medication and other xenobiotics.

A single measurement yields information about hundreds, possibly thousands of metabolic products (metabolites). The same measurement also reveals external compounds, such as medication, environmental toxins and stimulants.

"Metabolomics enables the comprehensive observation of metabolic phenomena. This gives us an extremely good idea of the body's biochemical state," says Professor **Seppo Auriola**, of the School of Pharmacy of the University of Eastern Finland. Auriola is also the head of the LC-MS Metabolomics Center in Kuopio, which is part of Biocenter Finland's infrastructure network.

One analytical tool used in metabolomics consists of a combination of liquid chromatography and high-resolution mass spectrometry. Liquid chromatography-mass spectrometry (LC-MS) is used to screen and identify compounds in samples. Liquid chromatography separates compounds on the basis of their fat solubility, while a mass spectrometer is used to measure exact molecular weights. The term 'molecular feature' – meaning the signal generated by a compound during ionisation and measurement – is used in metabolomics.

"In metabolomics, we attempt to find the statistically different molecular features between the different groups being studied. These could be 'ill versus healthy', for example. Metabolomics also involves trying to identify such molecular features as molecules, by means of various spectroscopic techniques. Our lab uses mass spectrometry for this," says Laboratory Manager **Marko Lehtonen**.

Metabolomic measurements can be divided into untargeted and targeted methods. The starting point with untargeted analysis is trying to find as many metabolites as possible from a sample. A targeted analysis, on the other hand, focuses on a limited group of known metabolites.

Untargeted measurements can provide a good basis for creating a hypothesis.

"The first screening reveals metabolic products that have changed, for example after the first exposure. Then we start thinking about the theory and try to understand why this occurred," says Auriola, who focuses on analytical chemistry and measurement techniques used on samples.

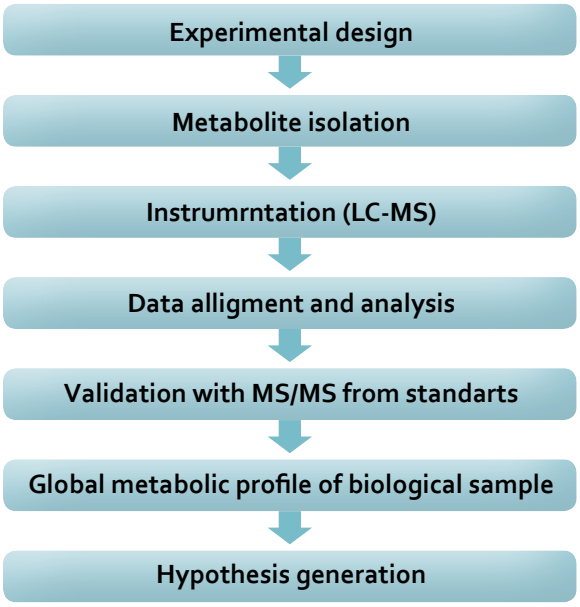
Targeted analysis can be used to verify a theory by observing changes in concentrations of selected metabolites. Such analyses are used to identify measure only compounds that are different from the groups being studied. This may involve differences in samples from people with a certain disease, and healthy controls. Targeted analysis can be used, for example, when looking for biological indicators related to lifestyles or illnesses. Most analyses traditionally involve precisely these types of targeted measurements.

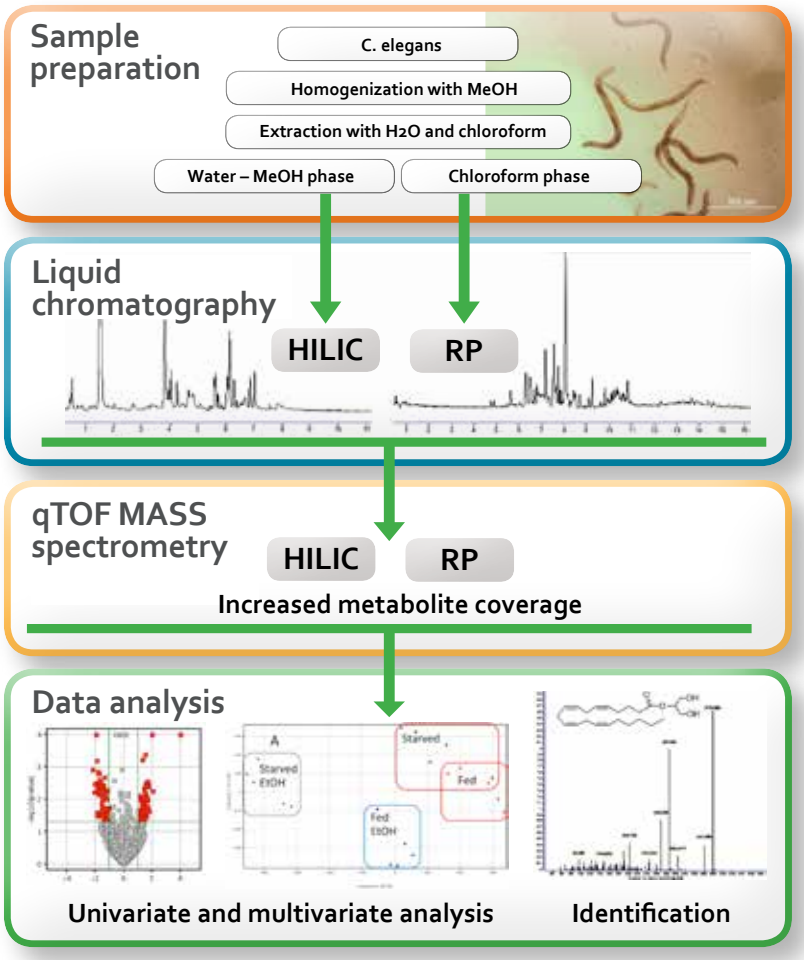
Diet and lifestyle visible in metabolic products

As metabolomics measurement methods become increasingly efficient, more accurate

Workflow

Untargeted metabolite profiling





measurement data will be obtained on the effects of people's lifestyles and environment on their health. Diet is a key external factor affecting a person's metabolism.

"Metabolomics is ideally suited for dietary studies. Analyses provide clear markers on what a person has been eating and how this affects their endogenous compounds," says Auriola.

Endogenous substances comprise all compounds produced by the body, such as hormones and transmitters. These include endocannabinoids, steroids and endorphins.

"We can examine whether a positive lifestyle change also affects metabolite levels. This would be an indication that the body is doing better. Metabolomics can also be used to detect disease biomarkers at an early stage, before diseases actually occur."

Effect of xenobiotics on humans

Another important area suitable for metabolomics analysis is exogenous compounds – that is, compounds from outside the body – such as medication and environmental toxins. This involves looking for biomarkers to show how a medication is affecting the body.

Auriola thinks it is also important to ask why a certain substance affects us negatively. We can also look for such biomarkers in metabolic products that indicate human susceptibility to a xenobiotic, or the effect of a xenobiotic on humans. These include the effect of pesticides on human health.

"We do not understand the mechanisms of all pesticides. As we develop more advanced methods, we will obtain a clearer picture of how humans are affected by exposure to certain substances. We can measure the level of environmental toxins and the corresponding level of endogenous metabolites in human populations."

Studies by the University of Eastern Finland and Karolinska Institute examined the effect of polychlorinated biphenyls (PCBs) on mouse offspring. It has long been known that these substances have most effect in the early stages of development. Animal tests have revealed developmental disturbances in various organs. When the metabolomics profiles of offspring were studied, certain changes were found in males. However, such changes were absent from females. The metabolite changes caused by PCB compounds in males affected the liver and nervous system.

"We will be able to monitor changes in the following generation without knowing in advance what we should be looking for," says Auriola.

"By means of LC-MS equipment and the untargeted metabolomics method, we can find changed molecules among the thousands of molecules we are measuring."

Molecular characteristics are identified by means of algorithms. The study by the University of Helsinki and University of Eastern Finland involved the analysis of compounds sampled from neonatal umbilical cords. Pre-eclampsia (a type of pregnancy disorder) is one of the commonest causes of premature birth and maternal deaths during childbirth. The precise causes of the condi-

Metabolomics can enable the study of the effects of nutrients. Why are certain nutrients essential, in which metabolic reactions are they involved, or which routes do they inhibit?

tion are unknown. It is known to increase the subsequent risk of cardiovascular disease in both mother and child. However, we do not know how the changed metabolism of mothers with pre-eclampsia affects the metabolism of newborns. Metabolites in the umbilical cord tissue of newborns were analysed with the LC-MS equipment in Kuopio, comparing the results between those who had pre-eclampsia, and healthy controls. The study also made use of material by the Finnish Genetics of Pre-eclampsia Consortium. All Finnish university hospitals contributed to the assembly of the FINNPEC cohort.

"Many different research projects use the services of our laboratory," says Marko Lehtonen. For example, research samples related to diabetes and Alzheimer's have been studied in the laboratory. According to Lehtonen, metabolomics will provide more information that can be used to study rare and hereditary diseases.

"Newborns are screened with targeted measurements. This is also an excellent example of an area where metabolomics can be very significant. It will save society money. Based on certain biomarkers found in the body, hereditary diseases among newborns can be identified," says Lehtonen.

Not all molecules yet detected

Not all metabolites can be measured using the current equipment.

"Compounds are present in a sample in such small concentrations that we also need targeted methods. As equipment technologies develop, untargeted methods may become efficient enough to reveal compounds that could not be detected earlier. This will ensure that we do not lose other information from a sample. Targeted methods only track specific compounds and are blind to all other data," says Lehtonen, stressing that the untargeted method provides plenty of data which can be used to investigate new issues.

As equipment becomes more accurate and sensitive, we will be able to observe really small concentrations. We're talking about picograms and nanograms per litre. One picogram is one trillionth of a gram, and one nanogram is one billionth of a gram.

"We can currently see thousands of compounds, but many important molecules remain below our observation horizon," says Seppo Auriola.

"For example, more and more steroids will be identifiable in samples as measurement technology improves. This will enable us to study endogenous steroids and their metabolites."

These include sex hormones, such as testosterone and progesterone, and corticosteroids (e.g. cortisone and cortisol).

"We are involved in a project studying the effect on steroids, and other metabolic characteristics, of exercise and lifestyle choices among children and young people. Other studies involve trying to find compounds that affect steroid metabolism selectively, and may therefore be used as medication."

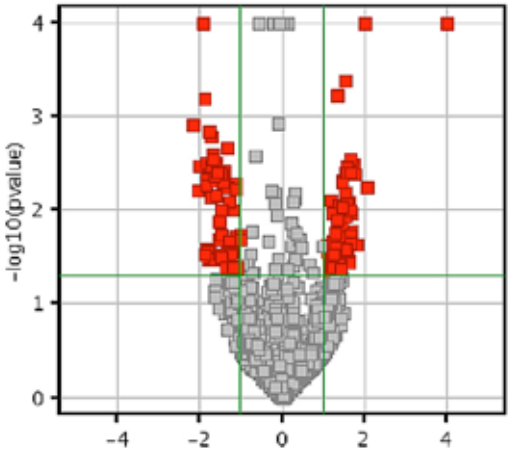
Computing power required to process large amounts of data

Metabolic products studied by means of mass spectrometry are first ionised. These ions are separated from each other on the basis of their mass-to-charge ratio.

"Mass spectra provide a signal of the molecular characteristic of a sample, which is proportional to the presence of a particular molecular characteristic," says Lehtonen.

"Metabolomics compares the signal levels of the possibly thousands of molecular characteristics contained in a sample. We typically study more than one group, each containing several parallel samples. For example, in human studies samples tend to be derived from several hundred subjects. This results in a vast amount of data, requiring not only powerful computers but also software that can process such data volumes. You also need chemometric (univariate and multivariate) methods to detect statistically significant differences."

"The equipment will provide the compound's exact mass and isotope ratio. If the device's resolution is high enough, such data



The endogenous cannabinoid system is related to various physiological and pathological states. To better understand the effect of ethanol and lack of nutrition on roundworms (C. elegans), the research involved a study of how the metabolite profile and certain endocannabinoid levels changed in various exposures. For example, lack of nutrition increases the amount of anandamide, one of the major endocannabinoids.

This is an example of the results obtained with reverse phase chromatography and a high-resolution mass spectrometer. A volcano plot is a type of scatterplot that shows statistical significance (p-value) versus magnitude of change (fold change).

One axis shows the p-values of the t-test, and the other changes in the signal levels between the groups studied. With a single image like this, we can see the results between two different groups that have been studied.

on the mass will provide a pretty good estimate of the compound's potential structure," says Lehtonen.

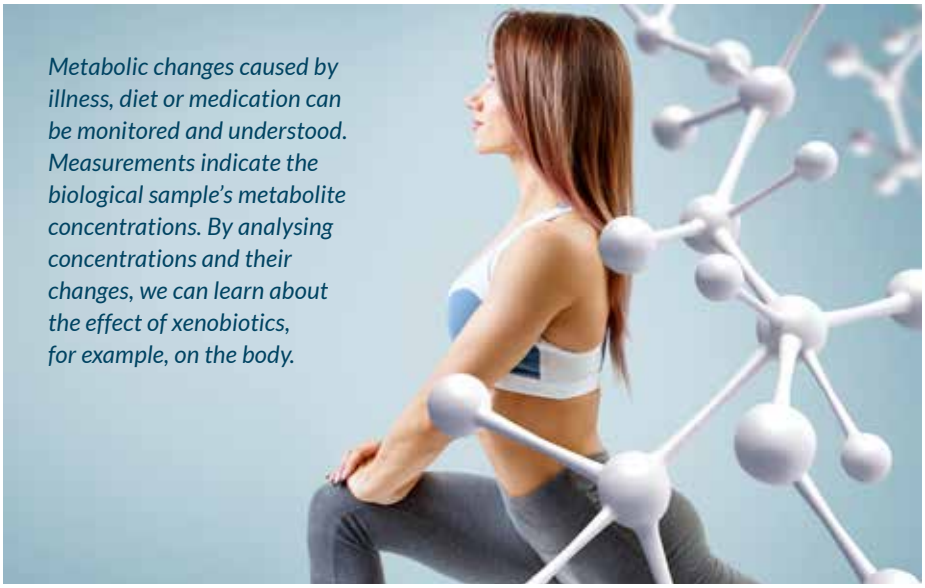
In more advanced compound identification, we measure fragmentation ions and make use of a clean comparison substance's chromatographic retention time. This is the time during which, after injection, the compound travels within columns and is visible to the detector.

"The fragmentation ions are made to collide with an inert gas, causing the mass ion to split into the molecule's component parts. These give their own responses, which are typical of these building blocks. These product ion spectrums can be considered the compound's fingerprint."

According to Lehtonen, the identification of molecular characteristics is the last stage in metabolomics, based on the attempt to clearly identify a statistically different metabolite between two or more groups being studied.

Lehtonen would prefer a model in which laboratory and research data were used as a basis for machine learning.

"Although these spectra can be compared to fragmentation spectra found in mass libraries, the problem is that identification still involves high amounts of manual work. It would be ideal to have a learning algorithm that automatically sought fragmentation spectra and compared them to what was in the library. Such a model could accurately define compounds identified previously in a laboratory. This would be of considerable help to research," says Marko Lehtonen.



Metabolic changes caused by illness, diet or medication can be monitored and understood. Measurements indicate the biological sample's metabolite concentrations. By analysing concentrations and their changes, we can learn about the effect of xenobiotics, for example, on the body.

Unification of tools and data

According to Seppo Auriola, we should make more use of measurement data. The problem lies in the availability and uniformity of data.

"ELIXIR has several processes underway to unify the use of various tools in metabolomics, in order to render them compatible. Measurement data should also be archived."

According to Auriola, in addition to being used for scientific publications, most original measurement data should be made available to other researchers for further analysis.

"The second phase involves adding metadata, determining what kind of data should be available on the samples, how they have been measured and verified, and what kinds of groups have been studied. How will this data be conveyed along with the measurement data? The crucial issue is that data that took a lot of work to obtain could be used for later analyses and comparisons."

Another challenge involves the available tools: how to pick and identify compounds, and what software is required to calculate the results, to identify molecules and compare their numbers in various samples. How are facts presented? How are changes in metabolite levels obtained, how are they found on the metabolite map, where are the compounds located on metabolic routes, and how are their concentrations changed? How can this be described clearly and how should the result be presented? A fair amount of work is required to unify all this. All the related data and tools are currently fragmented between various people's software," says Auriola.

Ari Turunen

MORE INFORMATION:

LC-MS Metabolomics Center
University of Eastern Finland
https://www.uef.fi/en/web/metabolomics-center

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