# Metabolomics: the way to grow old healthily

Dies lecture given by


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*Esteemed Rector Magnificus, distinguished audience,*

Which of you would like to know whether you're healthy or not? And which of you would like to know what to do in order to stay healthy? How can we make sure that *you* know what *you* need to do to stay healthy?

Over the last hundred years we've learned a great deal about health. We now know some major risk factors, like smoking and high blood pressure, and the result is a considerably higher life expectancy. But make no mistake: there's still a lot that we don't know! We know very little about several complex diseases, such as dementia, and no treatment is available. We also have very little insight into why some people develop diseases and others don't, even though they have the same risk factors.

Today I would like you to join me in the search for answers to urgent health questions, and would also like to show you the tremendous potential of research on metabolism.

**Metabolites give information about health**

Most of you know that genes determine what traits an individual can have, in principle. Abnormalities in someone's genetic make-up partly determine the *risk* of their developing certain diseases. But the genes don't tell us whether we will *actually* become sick and, if so, when that will be. Genetics also gives no information about whether or not we are already in the early stages of a disease. What actually happens with our health is determined by a *combination* of environmental factors and our genes[1–3].

Many different processes and chemical reactions take place within cells and between cells. Metabolites are the intermediate and end products of these processes and reactions. They are small chemical substances, like glucose, adrenalin, fatty acids and fats, which have key functions in the body. We call the sum total of these processes and reactions the 'metabolism'. Within a cell, the metabolites and other biomolecules are in contact with each other via complex networks of reactions. For a particular individual, these networks together form his or her 'metabolic map'[4]. You could compare this to a detailed road map. The networks of one cell are linked to those of other cells. Interactions of cellular networks usually take place in specific types of tissue, such as brain tissue or liver tissue, so there are then many connecting lines within tissues and organs. Similarly, numerous interactions can also be seen between the various organs.

In the healthy condition, also known as homeostasis, all the metabolites are in dynamic equilibrium with all the other biomolecules. You could say: all the cars can keep moving, there are no traffic jams or roadworks. However, if processes are disrupted, for instance because of a disease, a change occurs in the metabolic map: certain metabolites will be formed or not formed, or will not be broken down. Somewhere, so to speak, there are roadworks. And as we know all too well: just one disruption can affect a lot of roads!

Many metabolites are secreted by the organs into the blood, and are then taken up by other organs. Blood is thus a reflection of what is going on in all different parts of the body. The metabolites in blood are the result of interaction with the environment, such as lifestyle and nutrition. The presence or absence and the concentration of metabolites can give us information about the level of stress to which someone's metabolism is exposed, for instance stress due to the immune or hormone system or psychological stress[5]. So while genes can only predict *risks* of contracting diseases, metabolites tell us about someone's *current* state of health[6].

**Measuring metabolites**

For centuries, specific metabolites have been used to diagnose diseases. For instance, as early as the seventeenth century, physicians relied on the analysis of one metabolite for their

diagnosis of diabetes mellitus: they detected sugar by testing urine! I'm glad I wasn't responsible for the analysis back then! Glucose in blood or urine is still used today as an indication of diabetes. A metabolite that serves as a disease indicator in this way is called a 'biomarker'.

In the case of relatively simple diseases, it is also relatively easy to find a biomarker. However, for complex diseases, which are usually caused by a concurrence of circumstances, we need a *combination* of biomarkers. For instance, only *the ensemble* of blood sugar concentration, cholesterol concentration and blood pressure can predict the risk of a heart attack. But again I must emphasise: we're still only talking about a RISK, and not about what happens to an individual in practice. Of all the patients who are labelled as 'high risk' on the basis of these three factors and are therefore prescribed statins, for example, more than 80% gain no benefit from them whatsoever. This means they can experience problems with side effects, such as muscle pain, which are completely unnecessary.



This is why, twenty years ago, I started to develop methods aimed at measuring all the metabolites, collectively known as the 'metabolome'. Many of my colleagues thought this would be an impossible mission. Measuring the metabolome sounds easier than it actually is: what's important for you to realise is that many of the metabolites are found in extremely low concentrations in blood, while others occur in extremely high concentrations. Imagine that we dissolve a couple of bags of sugar in the North Sea, and then we want to determine both the salt concentration and the sugar concentration in a single measurement… I'm pleased to report that in the meantime we've overcome many of the problems and are now able to efficiently measure a large proportion of the metabolome, using techniques that we call 'metabolomics'. We can extract metabolites from their environment (such as blood or tissue), and manipulate them in such a way that they can be reliably measured with mass spectrometry.

Now that our latest metabolomics techniques allow us to measure thousands of metabolites, the way is open to achieve many other ambitions. In the rest of my story, it will be my pleasure to explain a few of them for you.

**Still no treatment for many ageing-associated diseases**

There are several complex, chronic diseases for which we still have no treatment. I already mentioned the example of dementia. Alzheimer's disease is the cause of around 70% of all dementia cases. The mechanism involved in developing Alzheimer's is still not completely understood. Many researchers believe that the disease is caused by an excessive amount of a particular protein, beta-amyloid, which clumps together in the brain. Massive investments have therefore been made by researchers and the pharmaceutical industry in developing a drug that can eliminate or reduce these clumps of protein, known as 'amyloid plaques'. For instance, the pharmaceutical company Lilly recently tested the drug Solanezumab as a treatment for Alzheimer's. Despite the fact that this drug can eliminate some of the plaques from the brain, it failed to halt dementia in patients[7]. Various companies, including Lilly itself and also Pfizer, for example, are now giving up: they have stopped trying to develop drugs to treat dementia. Increasingly, people are aware that dementia is more complex than initially assumed: much more goes wrong in the metabolism *before* the amyloid protein clumps together. It seems likely that turning the tide would require intervention even before dementia becomes evident. There are indications that the amyloid plaques have already been formed more than ten years *before* dementia manifests itself.

Further research appears to indicate that the immune system plays an important role in developing dementia, together with other factors in combination with inflammations in small blood vessels in the brain8. As yet, we haven't found any good biomarkers for these processes. If the large pharmaceutical companies are hesitant to invest in new drugs for these diseases, the initiative to pursue different strategies and try

to identify new treatment options must be taken by us, as academics, together with innovative smaller companies and institutions, such as those located within Leiden Bio Science Park. I see this as our challenge!

**A different approach to dementia**

And here – you won't be surprised to learn – metabolomics again enters the picture. Metabolomics can play a crucial role in finding biomarkers of complex diseases, not only for early diagnosis but also for finding alternative treatment options. Measuring metabolites in people **before** they develop dementia will help to unravel the interplay of different processes. To achieve this, we are currently measuring samples from hundreds of people who have been monitored intensively for the last 20 years in the context of the 'Rotterdam ERGO study'[9]. The aim of this large-scale population study is to gain a better understanding of what causes ageing-associated diseases. It allows us to look retrospectively at people who we now know have developed dementia, by measuring their metabolome in samples taken many years ago. We compare their metabolic pattern with that of people who have not developed dementia. In this way, we have already found the first metabolites that seem to be linked with the early stages of developing dementia; that is to say, even before the symptoms of dementia can be seen[10,11].

**From metabolic biomarkers to insights**

We are therefore looking for metabolic biomarkers, but what we mainly want to investigate is which biochemical processes underlie these metabolites, and the genes that predict dementia. Our research methods for this include computer models that can predict the differences in the metabolic map within cells or organs[4]. These models must naturally be fed the right data and contain the right reaction pathways. This is a real challenge.

If we know what disruptions in our metabolic map lead to dementia, for example because a specific compound is either accumulating or decreasing, we can try to resolve or compensate for these disruptions. Thinking back to the road network that I mentioned earlier, you could say that metabolomics works like a TomTom: if the road ahead is blocked, we look for an alternative route so that the traffic can keep moving normally. This will bring us another step closer to a drug that can preferably cure the disease, or otherwise at least slow it down.

**Organs-on-a-chip to develop personalised treatments**

The process of developing new drugs still pays insufficient attention to the fact that every patient is unique. Radically new techniques are needed to develop treatments that take account of the differences between individual patients.

In this context, we have developed microchips at the Leiden Academic Centre for Drug Research[12]. These chips contain tiny fluid channels, where we can grow dozens of small three-dimensional cell cultures a few millimetres in size. These tissues, or 'mini-organs', are of human origin and can mimic human physiology. This makes them ideal for use in conducting all kinds of tests[13].

We distinguish between two kinds of highly innovative research. First, we can investigate differences between individual patients. Together with the LUMC, we have grown human blood vessels on our chip[14], and can cause patients' blood to flow through them. In other collaborations, we have created intestinal tubes[15], brain cells[16] and even the blood-brain barrier. We can use these mini-tissues, for example, to test the effect of metabolites that we've identified as a biomarker of a disease: do these metabolites perhaps cause instability of a blood vessel? Or do they affect the behaviour of neurones in brain cell cultures? Do blood vessels from different patients react in different ways?

The second kind of research where we can use our chip systems is in testing and developing new drugs. If we know

where something is going wrong, we can investigate whether we can compensate for it by making or blocking alternative metabolic pathways.

There is tremendous interest in our chip technology, so four years ago we set up a spin-off company in Leiden: MIMETAS. MIMETAS is the first organ-on-a-chip company in the world, and is so successful that nearly all the large pharmaceutical companies are now working with our technology or have initiated plans to work with it.

**Innovations in metabolomics technology**

The search for biomarkers of complex diseases in clinical or epidemiological studies can easily require data from a couple of thousand patients, and preferably measured at different times. If you factor in the cost of metabolomics, which is often more than 100 euros per sample, you know that extensive metabolomics research is an extremely expensive affair. At the LACDR, we are therefore working on developing new technologies to make metabolomics more affordable. For instance, we can measure metabolites more efficiently by using new analysis methods on a chip[17,18]. We expect that by 2019 we will be able to measure 100,000 samples a year or more, at much lower costs than now! An additional advantage of this analysis chip is that we can also use it effectively to measure super-small samples, which is essential in research with our tissue chips. We recently even succeeded in measuring metabolites in a single cell!

**Life course metabolomics and big data**

The fact that the possibility of measuring the metabolic profile of a large number of people is now in sight in the near future offers enticing prospects. Let's imagine this together! We could measure the metabolic profile of everyone admitted to hospital. This can tell us which metabolites correlate with the ultimately diagnosed disease, which will lead to a better understanding of the cause and progression of diseases, and will help with early diagnosis. This will considerably increase the chance of recovery.

And we can do more than this. We can also monitor *healthy* people over time[19]. We can painlessly take periodic blood samples with micro-needles from thousands of people, which we then monitor in our central high-throughput metabolomics lab in Leiden. In this way, we can discover what changes take place in a metabolic profile before a depression or migraine attack, for instance, becomes manifest. We can also look at how the metabolome changes before or during the onset of an immune disease.

The ultimate aim is that a change in the metabolic profile will already give us a warning, even before someone has symptoms of a disease. This will enable us to intervene at an early stage, on the basis of the person's genetic and metabolic profile, and perhaps prevent them from becoming sick. This might sound quite futuristic to you, but our research shows that these are realistic expectations. We are working within the '*Weten door meten*' ('Knowing by Measuring') programme of the Dutch National Research Agenda to make these applications possible, eventually for every person in the Netherlands!

**Metabolomics for made-to-measure prevention and treatment.**

Ladies and gentlemen, by now my message will be clear. Metabolomics is going to radically change healthcare! If we can predict the development of diseases like dementia, cardiovascular diseases and even depression at an early stage, and can find out which part of the metabolic map is involved, we can modulate and compensate for these disruptions with advice on nutrition and/or lifestyle, or by using drugs[20]. Healthcare will migrate from a generic approach to made-to-measure treatment.

Another important point to mention here is that all these new *technical* possibilities also confront us with new *ethical* challenges: does everyone always want to know everything, not only about their current health, but also their *future* health? And who will have access to these sensitive data? Will we only give people information if they have real possibilities of

actually turning the tide? Personally, I would like to manage my data *myself*, and be able to decide for myself who has access to it, but we can expect opinions on this to be strongly divided.

**Collaboration is crucial in the life sciences!**
Finally, I would like to say that all the developments I've described here today can only be achieved through intensive collaboration: fortunately, you don't have to be able to do everything yourself. In fact, the League of European Research Universities recently said that choices about investments in research infrastructures at universities should be better coordinated with each other, nationally and internationally. In this context, we recently submitted a research proposal that integrates metabolomics, proteomics and genomics technology in one initiative, X-omics! This kind of clustering of initiatives has a clear effect on young researchers: in their PhD phase, they're already working with researchers from other disciplines. This also has a ripple effect on our students!

I am pleased to be able to work in partnership with so many enthusiastic researchers in my group at the LACDR here in Leiden, in the Medical Delta and at many other universities, hospitals and companies in the Netherlands and elsewhere. Your energy and enthusiasm are extremely inspiring and make this research great fun! Together we can ensure better healthcare, give sound advice in the area of nutrition and lifestyle, and develop better drugs for both the treatment and prevention of diseases. Together we are bringing the ideal of growing old healthily into closer reach. And who knows, perhaps we'll still be meeting each other here in the Pieterskerk in fifty years' time to celebrate our Alma Mater!

I have spoken.

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Whereas genes can give us information about the likelihood of contracting a particular disease in the future, metabolites provide information about the current state of a person's health. Metabolites, products of our metabolism, are the result of the4 interplay between genes and environmental factors such as nutrition and lifestyle. Advanced analytical techniques make it possible to measure thousands of metabolites in blood, urine or other human samples. Measuring metabolites reveals information about all kinds of disruptions to vital processes in the human body, such stress in the immune system, disturbances to the hormonal system and energy management.

Metabolic profiling can detect the presence of diseases such as dementia at an early stage, even before the condition manifests itself. This helps us gain a better understanding of the different processes underlying the development of complex illnesses. We are able to study these processes using minute samples of human tissue. Insight into the underlying processes will allow us to develop medicines that can intervene in disease processes, delaying them, or even preventing them. New technologies are currently under development in Leiden that will make it possible to measure the metabolome of many, if not all Dutch people rapidly and efficiently.

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