Diet is evolving from nourishing populations via providing essential nutrients to improving health of individuals through nutrition. Modern nutritional research focuses on health promotion and disease prevention, on protection against toxicity and stress, and on performance improvement. The concept of developing nutritionally enhanced or functional food requires: (1) the understanding of the mechanisms of prevention and protection; (2) the identification of the biologically active molecules, and (3) the demonstrated efficacy of these molecules.

As a consequence of these ambitious objectives, the disciplines 'nutrigenetics' and 'nutrigenomics' have evolved. Nutrigenetics asks how individual genetic disposition, manifesting as single-nucleotide polymorphisms, copy-number polymorphisms and epigenetic phenomena, affects susceptibility to diet. Nutrigenomics addresses the inverse relationship, i.e. how diet influences gene transcription, protein expression and metabolism. The mid-term objective of nutrigenomics is integrating genomics (gene analysis), transcriptomics (gene expression analysis), proteomics (global protein analysis) and metabolomics (metabolite profiling) to define a ‘healthy’ phenotype. The long-term deliverability of nutrigenomics is personalized nutrition for maintenance of individual health and prevention of disease.

The major challenges for -omics in nutrition and health still lie ahead of us, some of which apply to -omic disciplines in general while others are specific
for -omic discovery in the food context: (1) the integration of gene and protein expression profiles with metabolic fingerprints is still in its infancy as we need to understand how to (a) select relevant sub-sets of information to be merged, and (b) resolve the issue of the different time scales, at which transcripts, proteins and metabolites appear and act; (2) the definition of health and comfort is less a clear-cut case than the one of disease; (3) -omics in nutrition must be particularly sensitive: it has to reveal many weak rather than a few abundant signals to detect early deviations from normality, and (4) in the food context, health cannot be uncoupled from pleasure, that is, food preference and nutritional status are interconnected.

Transcriptomics serves to put proteomic and metabolomic markers into a larger biological perspective and is suitable for a first ‘round of discovery’ in regulatory networks. Metabolomics, the comprehensive analysis of metabolites, is an excellent diagnostic tool for consumer classification. The great asset of this platform is the quantitative, noninvasive analysis of easily accessible human body fluids like urine, blood and saliva. This feature also holds true to some extent for proteomics, with the constraint that proteomics is more complex in terms of absolute number, chemical properties and dynamic range of compounds present. In theory, blood should bear a signature for most biological conditions and urine should reflect the majority of metabolic disorders. The challenge of targeting ‘proteins in blood’ and ‘metabolites in urine’ stems from the complexity regarding compound diversity and range of compound concentrations in which the diluted signals of interest may eventually ‘disappear’.

Proteomics represents an established technology in the pharmaceutical industry mainly for biomarker and drug target discovery. The potential of proteomics for research in the food industry is increasingly being recognized and the employment of proteomic approaches to nutrition and health issues is now emerging. Proteomics in the context of nutrition and health has the potential to: (1) deliver biomarkers for health and comfort; (2) reveal early indicators for disease disposition; (3) assist in differentiating dietary responders from non-responders, and, last but not least, (4) discover bioactive, beneficial food components. Independent of the context of application, proteomics represents the only platform that delivers not only markers for disposition or condition but also targets of intervention: the only way to intervene in a biological condition and to modulate its outcome is interfering with the proteins involved.

It is evident that not only comprehensive analyses with one discovery platform (lateral integration of information) are required but also vertical integration between different -omic levels is indispensable for a deeper understanding of disposition, health, environment and diet [1]. A major ‘vertical integration issue’, to date unresolved, is given by different time scales of transcript production, protein expression and metabolite generation [2]. The transcript machinery usually responds fast to an external stimulus (seconds
to minutes), the proteins may be expressed within minutes to hours (and have a half-life from minutes to even months) and metabolites vary significantly during the day and depend on latest dietary input. This means that data which seem to correlate qualitatively (e.g. reflecting the same pathway) may not necessarily be related time-wise. Rather, they may represent different responses at different time points and, possibly, to different stimuli.

Comprehensive -omic analysis is an essential building block of ‘systems biology’, which can be defined as follows [3]. Systems biology is the comprehensive analysis of the dynamic functioning of a biological system (cell, organ, organism or even ecosystem) at the gene, protein and metabolite (or higher organizational) level, achieved by comparison of two defined biological states of this system, typically before and after perturbation. While a comprehensive list of components (genes, proteins, metabolites) of a given biological system is a prerequisite for this kind of research, the main reasoning for the ‘system view’ is that only information on the interactions between the components gives clues to the function of the entire network. A systems biology approach has recently demonstrated the power of proteomics to dissect immunity and inflammation. Toll-like receptor recognition and signaling was elucidated and showed how bacterial ‘barcodes’ are read and interpreted in order to trigger an adapted immune response [4].

In order to address some of the challenging objectives of -omics-driven nutritional research, we have addressed: (1) the effect of early antibiotic administration on the maturation of intestinal tissues; (2) protein discovery in human milk; (3) the effects of polyunsaturated fatty acids on gene expression and lipid profile in the liver; (4) biomarkers for intestinal stress; (5) biomarkers for allergy disposition and tolerance induction, and (6) inflammation-related gene expression analysis.

(1) Antibiotics and gut maturation: the effects of early administration of antibiotics on intestinal maturation were assessed at the gene expression level in a rat model.

(2) Human milk: rapid enrichment and iterative, consolidated identification of immunologically relevant milk proteins was achieved through the employment of restricted-access media and a tailored proteomic strategy [5–7].

(3) Fatty acids and liver transcriptome/lipidome: epidemiological studies have correlated higher intakes of polyunsaturated fatty acids (PUFAs) with a lower incidence of chronic metabolic disease. The molecular mechanisms regulated by PUFA consumption were examined assaying the liver transcriptome and lipid metabolome of mice fed a control and a PUFA-enriched diet [8].

(4) Gut stress markers: we catalogued protein expression along the jejunum, ileum and colon of the rat intestine and found gut segment-specific proteins [9]. The innovative combination of a neonatal separation model with proteomic analysis allowed us to study whether early life psychological stress may impact the adult gut neuromuscular protein expression and the approach revealed specific protein biomarkers.
Biomarkers for allergy and tolerance: a collaborative effort of combining clinical research and ex vivo/in vitro immunology with proteomic biomarker discovery is undertaken by the Nestlé Research Centre, the Centre Hospitalier Universitaire Vaudois and the Swiss Institute for Allergy and Research. The objective is to identify protein and peptide markers for allergy disposition (clinical samples) and tolerance induction (in vitro stimulation of ex vivo T cells) in defined immune cell populations. A first proteomic survey of human peripheral blood mononuclear cells is presented.

Inflammation-related gene expression: inflammation is implied in a multitude of nutritionally relevant disease conditions. We aim to studying inflammation-related gene expression patterns through the implementation of a custom-array approach [10].

References


Discussion

Dr. Saavedra: Thank you very much, that was a great overview of what is being done, what might be done and hopefully what ideas we can take from all of you to begin and continue doing. I did have to ask that since English seems to be a risk factor, if having a French or a German accent confers some protection to any of the conditions that you talked about. This is open for discussion.

Dr. Sorensen: That was an excellent review. We talked a little bit about this but I am still puzzled by your approach to dealing with individuality, and you showed us experiments in mice that are all of the same genetic background. What is the difference in expression between individuals that are encountered in any human study and that due to disease or stimulation that is artificially introduced when cells are
activated to get them to express certain proteins? How do you know what is due to that fact versus the genetic composition of each individual?

Dr. Kussmann: This is an extremely important point. It is not as though we overlooked this. Referring to this stress study, we believe that inter-individual variability is in that case less than if you look at humans. You can only master this problem by appropriate statistics. If you do mammalian gene expression studies you should at least have 5 or 10 biological replicates, for human gene expression studies you should probably have a lot more. You can only look at this from a statistical point of view because we do not genetically screen the animals beforehand. Statistical processing afterwards tells you something about the biological noise. There is another noise we have to deal with and this is experimental noise, and here we feel quite confident that with the platform we have in place the experimental variability is very much reduced. So wherever we can play on replicates we focus on different subjects and also on experimental repetition.

Dr. Sorensen: How specific are the metabolites? If you have 300,000 proteins you detect 3,000 metabolites, does it mean that the same metabolite may be part of 30 different proteins, and that you would not be able to determine what it really means if you find it in the urine because it could be derived from many different situations?

Dr. Kussmann: Yes, you are right also in this regard. Therefore I suggested in one of the slides that we have to think about what we want to integrate when we look at different -omic levels at the same time. It is simply not good enough to put everything into the same database because things may not be inter-related. The integration of changes in metabolite profiles and gene expression results is combining the most distant parts of the story. I would rather compare data from 'neighboring' platforms, that is to say proteins and metabolites and then proteins and genes and try to close this gap. Another approach to deal with this problem is to introduce causality. Imagine you do a transcript analysis and then you find regulators of transcription factors changed; that is a valuable piece of information because it is way up the cascade; then you would try to use this as a 'hub' and try to cascade down this finding to proteins and metabolites. But a lot of -omic information will not cross-correlate because the complexity levels are different and we may look at very distant effects at the same time. This situation is even a bit more complicated in the sense that metabolites respond in a much shorter time, so we have to think about how we deal with dynamics over time at these 3 -omic levels: your metabolite profile changes over the day significantly, the proteins react much more slowly and with the genes it is again different. You can do time monitoring of metabolites, this is the great advantage of metabolomics. In proteomics you can only take snapshots in which you freeze the biological condition and you get a picture at a given time. When you do cell culture experiments you can do time-course proteomics through metabolic labeling: you culture cells in isotopically enriched media and then you follow many proteins at the same time. I would say that it is fairly advanced, but it is possible.

Dr. Hursting: This is a clear presentation of the 3 -omics together that I never heard, so thanks for that. I am wondering what is your consideration in comparing experiment to experiment, or lab to lab, or platform to platform because sometimes when it comes up we have an opportunity to do things at different platforms beside Affymetrix. But what are the considerations from your experience in trying to compare across these platforms or even within a platform experiment to experiment?

Dr. Kussmann: Are you addressing the reproducibility and comparability of data derived from the same -omic level? It seems that we share the same -omic background. I think we were all blamed for the ‘fact’ that the biological answer you get depends on the platform you use. I think the true story behind this is the challenge of standardizing these studies and this is an issue in nutrition. The same holds for -omic technologies. We have to define the protocols not only on how to perform the laboratory experiment
but also on how to treat the data. There was a recent comparative study published in *Nature Methods* where they looked into different gene expression platforms: for instance the Affymetrix which we have, Agilent and others, and then the so-called ‘gold standard’ reference methods like PCR [1]. They also looked into inter-laboratory comparability: if two laboratories applied the same platform, how do they compare? It is all about standardization, if the entire process from sample preparation down to data processing is defined, you get a nice overlap of regulated genes: typically two thirds correlate. You always find ‘contradictory’ results, but ‘contradiction’ can also mean complementation because any technology gives you only a part of the story. Data that do not overlap are not necessarily false-negatives or false-positives. On the proteomics side the situation depends on the mass spectrometry and the separation techniques we use. You can get two thirds of overlap between two different mass spectrometric platforms. The studies often differ in the sense that the upstream sample preparation is not always standardized: the biological origin of your sample and how you treat it. In proteomics the problem is rather that you rediscover the same proteome in different studies. Studies that had totally different objectives publish similar lists of proteins. So why is that? Because the dynamic range is so challenging. My recommendation is if you want to do something in the protein world you have to enrich for the things you want to look at. You may alter the picture a little bit but if you leave the sample as such, you just scratch the surface.

**Dr. Wang:** Just to follow your idea, to follow now our knowledge, can we go ahead and search for healthomics? In traditional Chinese medicine we pay a lot of attention to the whole body but not the genes, molecules. So we pay attention to health from another direction than in modern Western medicine where you go from molecules, from the organs and to the whole body.

**Dr. Kussmann:** I don't mind the invention of other -omics words, although healthomics is something I haven't thought about yet. It may be a far reach at the moment though. I think you are right in addressing the challenge of defining health at -omic levels which is of course much more difficult than defining disease, because the intrinsic variability is greater. We apply the -omic platforms in order to compare at the global molecular level defined biological conditions. That raises the issue of how defined this condition is. There is some other benefit of these -omic technologies: think about the studies that have been reported throughout the meeting, there are many conflicting results especially in the nutrition business. There is an issue of cohort definition. We may be able to help at an early stage to improve cohort definition; cohort definition at a molecular level and not so much at a phenomenological level; that can help to improve the study outcomes. While the ‘hype’ of -omic discovery is declining in the pharmaceutical world, in nutrition we can take advantage of these learnings and apply these technologies in order to better design studies. Any financial objections, because these technologies are costly, are not justified because there is nothing as expensive as a failed long-term clinical study.

**Dr. Hamburger:** Earlier in your talk you mentioned the desire for personalized nutrition and I wondered if in your studies you encountered an example of any genetic fixed issue that allows you to jump to a personalized nutrition. In other words is there an example that you are able to cite now that leads you to believe that this will ultimately result in a personalized nutrition.

**Dr. Kussmann:** I can only give you the examples of inborn metabolic defects for instance phenylketonuria. This is something which is well established and you can interpret this as an attempt to personalize nutrition because we have to avoid this amino acid. We would like to develop this further in the sense that we would like to have a better understanding of consumer groups; however I cannot give you mature examples at that level yet. There are a few examples where a single genetic defect leads to a metabolic problem and then you simply avoid an ingredient. You can extend
this to the allergy field, the allergens are well characterized but the individual responses are less characterized. One aspect is to avoid allergens, another is how to induce tolerance. We are trying to come up with a more comprehensive set of explanatory markers that allow us to define these consumer groups, but it is not a matured concept you can find in supermarkets, except for these few clinical examples I have given.

Dr. Laron: I would like to contest the example you gave and the structure of your pyramid. I propose to turn it around: the upper wide platform is the genetic background, then you have the ethnic or familial customs and then the individual habits and environmental influences on nutrition. Would that be correct?

Dr. Kussmann: Yes, I think we are quite on the same line. However, I would like to add that genetic disposition is only one aspect. Many individual responses and dispositions can be traced down to SNPs and copy number polymorphisms.

Dr. Laron: But they don't apply to only one person.

Dr. Kussmann: Either using personalized or individualized nutrition you always run into conflicts about how this is interpreted. What we do not want and what we do not envisage is that you enter a restaurant with your gene chip and the menu is then printed out according to your chip. We have to think about how we can get access to sets of biomarkers that are accessible by noninvasive means. I am convinced that the biomarker sets are not comprehensive enough and they often derive only from one analytical level; individualized nutrition means divide the population into groups of people that share the same heritage, the same lifestyle and are exposed to a similar environment.

Dr. Laron: But why not go further, in modern medicine we think we should have a health card from conception on. This health card could also include advice for optimal nutrition for that individual.

Dr. Kussmann: I can see your point and scientifically this may make sense but don't forget that we are in nutrition and not in the pharmaceutical area, so for us all these nutritional recommendations cannot be uncoupled from pleasure, it should still be fun to eat. We don't want to create paranoia and consumers have to have their latest screen before they look at their plate. We can offer means of assessing personal needs, but it is only an offer for choice.

Dr. Saavedra: Then we hope that the array of snacks that await us during the break will actually cover all our potential needs. Thank you very much.

Reference

**Discussion (Refers to the presentation “Mechanistic Research – Future Perspectives” by D.M. Bier)**

*Dr. Bier:* Any specific questions about genomics, proteomics and metabolomics I am going to pass on.

*Dr. Kussmann:* Thank you for this nice wrap up of all these thoughts. I wanted to make a little comment about the amount of information that is already available. We may discuss whether it is necessary to generate all this -omics-derived information and that leads eventually to the term ‘systems biology’, to the understanding of a biological system in a holistic manner. There is already so much information available, it is just a major issue to interrogate this huge data set for particular purposes. There are many groups in the world that are practicing systems biology that there are now major research efforts undertaken to interrogate existing information and try to align it with ongoing studies. The problem we have is that we do global -omics-driven investigations, and then we look at this one study. We are still not capable of integrating it with the information that has already been created.

*Dr. Bier:* I don’t disagree with that by the way. My comments on this are not meant to suggest that we should not go there because I think we should. My concern is what we will eventually be able to get out of it. What I see in the -omics field, in some way including what you said, is that there is promise that if we measure everything we are going to have an answer, and the answer is 42 for those of you who haven’t read the book. It may be hard to get all the information but then when we get it, if we only look at a piece of it, there is going to be a lot of material. One of the issues about choosing what you look at is that you tend to throw away the really unexpected material that puts up those things that we have seen with the knockout mouse experiments. Things have happened that no one expected and that would very likely have been thrown away in a system where it was narrowed down to what you were thinking about. So I don’t disagree with you, I think that there are some problems there.

*Dr. Lucas:* I think probably the greatest problem in pediatric research is not the interpretation of data or the amount of it, it is the ability to be able to do it and all the obstacles that have been put in our way. You discussed that a little. But what can we do as professionals or even as a workshop to make a statement about this? This is really a very serious problem.

*Dr. Bier:* It is a particularly difficult problem because all of us know that even among universities in the same country or even among EU boards in the same university there have to be answers over time. Somehow we have to try to get the pediatric research groups who have contributed to this issue, such as the academy groups that may exist in the EU, to come together to make some generic statements that are going to cross boarders in a very broad way, otherwise we might have difficulty here when we act individually. I think it is a very big problem and the remaining step is having the good scientist, the good question and the tools to carry it out, and in our case the tools are children, and without them we are never going to answer this question.

*Dr. Saavedra:* One of things that you alluded to it in a couple of the slides has to do with ethics. It is tied to what Dr. Lucas was just talking about. On the one hand we have those constraints that we can’t do a lot of things in children because it is not ethical. On the other hand we have some science that is going, we have genetic experiments that are already occurring with no ethical constraints at all. I mean it is not just mature enough to be able to bring in this runaway science that is good, and we have considered it good in many instances, but on the other hand it is going to create problems that we may not be able to get back.

*Dr. Bier:* What kind of experiments do you mean?
Dr. Saavedra: Cloning, for example, is happening right now, and stem cell research. Much of it is happening with not only a lack of constraint with regard to the resources and where they are applied, but also with regard to the ethics and where they are applied. Because we perhaps have too many restrictions, we have done experiments or begun doing fewer experiments in children. We have no control on others or at least we don’t feel there is sufficient control as to what, if any, ethics could or should be applied to this. Could you comment on that?

Dr. Bier: I think in the course of history there has probably never been a time when the human being was ahead of the ethics. The science of the human being was ahead of whatever the prior ethical rules existed for that society, and there is obviously a period of time when it is impossible to match the two. I think in the case of a very large fraction of pediatric research we have 30 or 40 or 50 years of what in fact has a long history of use, meaning safe use, for safe investigation. Those things obviously could be doable. Then there are others which are on the edge which become much more difficult and obviously cannot be dealt with now but maybe dealt with in 10 years. We got to the point where simple things that cause the problem become impediments to doing research or we have the important issues of privacy. By the way I don’t think any of these things have simple answers, but we have privacy things now that are making it impossible to get your partner’s medical records. It is extremely difficult.

Dr. Saavedra: Should resources be allocated and dedicated to the ethics of the research in parallel to the research?

Dr. Bier: Absolutely, in fact many of the ethics questions are being handled by internal review boards where people are spending immense periods of time, have no resources allocated to them, and make decisions without the resources. I think that is a problem.

Dr. Sorensen: I wonder if there is another skeleton in the closet. It may be the way we deliver health care, particularly in this country, even if it would be possible to really gather all the information and put it together. For instance in the case of the asthma guidelines, I think the NIH is the asthma guideline number 10, yet in any community when pediatricians are asked about asthma guidelines, surprisingly few know about them and even fewer ever follow them, and that for a simple thing like asthma. In our present health care system there are ever increasing differences in the kind of care that certain people get versus the care that the majority of our children get, and this is sorry situation for the US. Do you think that no matter how well we solve the issues that you have been discussing, the bigger issue is that we will be totally limited in the possible impact that we can have as health care professionals?

Dr. Bier: I obviously kept my remarks to the research side as that is what I do. I am not an expert in health care delivery, which involves a whole of a set of individuals including the people who pay for it, the American public in our case and congress. I think we have lots of examples where there is a change in health care delivery based on the way research is done. So I will use diabetes control in complication trials as an example: after 10 years and some hundreds of millions of dollars, it was convincingly demonstrated that maintaining normal glycemia decreased retinopathy and people with type-1 diabetes had a number of secondary outcomes. Even when that study was ongoing, we knew that it was impossible for the health care system to deliver that kind of care because these were almost individualized dieticians, social workers, phone access workers, but the demonstration of that effect has lead over the years to slow and steady changes in the health care delivery system: type-1 diabetic payments for more frequent visits, micrometers for measuring blood glucose, and on. So yes, it didn’t cure the problem but it has made a tremendous dent in the average hemoglobin A1 one sees in children with diabetes. So I think there are going to be negative examples too, but there is one in which I think there was positive benefit.