Nutrigenomics: exploiting systems biology in the nutrition and health arena

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Nutritional sciences are discovering the application of the so-called 'omics' sciences. Propelled by the recent unravelling of the human genome and the coinciding technological developments, genotyping, transcriptomics, proteomics and metabolomics are now available to nutritional research. In the future we are likely to see new screening tools for the selection of bioactive nutrients, new biomarkers for the *in vivo* efficacy of nutrients, and better insight into the influence of genetic polymorphisms on nutrient metabolism. However, are these promises just based on biotechnological hype or is a real fundamental change in human nutritional sciences at hand?

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Abbreviations
CVD cardiovascular disease
HDL-C high-density lipoprotein cholesterol
LDL-C low-density lipoprotein cholesterol

Introduction: the genomic revolution

Genomics, genotyping, transcriptomics, proteomics and metabolomics, together with bioinformatics, constitute the discipline of functional genomics, which is also referred to as systems biology. The integration of systems biology into nutritional research has started. This new research area is termed nutritional genomics or nutrigenomics. From the limited number of studies performed so far, can we predict whether nutrigenomics will really improve molecular insight into nutrient metabolism and consequently revolutionise biomarker development in relation to health promotion and disease prevention?

Over the past few decades, epidemiological, clinical and mechanistic studies have indicated many relations between nutrition and health. Links have been established between dietary habits and degenerative diseases, including cardiovascular diseases, diabetes and cancer. Major research progress has been made in recent years to understand the causes and mechanisms of degenerative diseases from a biomedical perspective, with the focus on disease cure rather than prevention. However, the mechanisms by which dietary components potentially modulate these diseases are only partly known, primarily because of a lack of appropriate research tools to elucidate the complex mechanisms involved. Until now, the mechanistic effects of dietary components on health and disease were mainly assessed either using functional assays or by determining their effects on the expression of single genes/proteins or single physiological end-points, known *a priori*. In addition, the role of genetic polymorphisms in dietary-influenced disease has been studied (e.g. the role of glutathione *S*-transferase and epoxide hydrolase polymorphisms on food-borne aflatoxin B1-induced hepatocarcinogenesis), again mainly at the individual gene response level. On the basis of this mechanistically derived knowledge, biomarkers have been developed and applied in the area of nutrition and DNA damage, antioxidant-related effects, cardiovascular health, and so on.

A new concept for biomarkers based on systems biology is presented here. The approach fully exploits the multiple minor changes in genomic responses (captured in patterns and profiles and complex datasets) related to nutrition and health, instead of the single 'target' gene responses common in drug therapy. It is concluded that systems biology clearly provides new insights into the molecular action of nutrients, without the need for *a priori* knowledge on any mechanisms. Implications for the development of biomarkers to predict human health effects of nutrients are discussed.

New biomarkers are needed

In applying biomarkers in human studies, the relationship between nutritional intervention, the biomarkers and the (supposed) health effect, in many instances, has not been straightforward. There are several reasons for this discrepancy. Firstly, the biomarker may have been based upon mechanisms that were valid in an isolated form (e.g. an *in vitro* system), but in the much more complex *in vivo* situation could not be used because compensatory mechanisms were active or repair systems nihilated the effect. Secondly, the dose levels at which the *in vivo* biomarker was useful may have been non-physiologically high. The *in vivo* regulation of nutritional effects is a well-balanced homeostasis where nutritional compounds are 'accepted' within certain concentration limits. Excessive dose levels of specific (nutritional) compounds may be necessary to produce a biomarker response (as was established from *in vitro* or mechanistic studies). The magnitude of changes induced by the nutritional compounds with currently used biomarkers usually is not in the range of the effects seen with drugs; the changes are more difficult to quantify than those seen with the more robust markers of disease employed as targets for drugs to resolve diseases. Thirdly, biomarkers may have been developed as (intermediary) endpoint of disease states or as protection against a disease. We now begin to realise that prevention or intervention of a disease may be fundamentally different from maintaining or improving good health. For example, a mutation may

ultimately lead to tumour formation, but prevention of a mutation does not just imply the measurement of DNA repair or DNA damage, but also the maintenance of cellular integrity in terms of a proper balance between proliferation, apoptosis, stress response and redox potential (i.e. those conditions that allow a cellular system to prevent and cope with electrophilic challenges). In nutrition and diabetics research, it does not suffice to measure insulin peaks, but instead it is necessary to assess the complete interaction of metabolic processes that are involved in glycemic responses. Insulin peaks represent extremes, ultimately with pathological effects, whereas nutrition aims at a fine-tuned balance between many processes, involving transport mechanisms, interactions between many metabolic pathways, hormonal regulation, and so on.

By contrast, specific health effects have been attributed to compounds that were later demonstrated to produce biological effects. For example, flavonoid compounds were initially described as antioxidants. Later, drug-metabolising enzyme induction capacities were also described. Thereafter, antitumour effects, endothelial effects, platelet function modulation and many other functions were allocated to the same (group of) compounds, certainly not only based on their antioxidant properties. Although not completely unravelled, molecular interactions have been described at many levels of signal transduction. Furthermore, not only positive (efficacious) but also negative effects of these compounds have been described, depending on treatment and dose [1].

The systems biology biomarker concept

In the relation between nutrition and health (unlike the relation between nutrition and disease) it is necessary to develop a new concept for biomarkers. The biomarker needs to reflect subtle changes in homeostasis and the efforts of the body (cellular systems, organs or inter-organ interactions) to maintain this homeostasis. Also, it should preferably notice a wide variety of biological actions. Furthermore, both efficacy and safety aspects should be monitored simultaneously. Single nutrients may have multiple known and unknown biochemical targets and physiological actions, which may not be easily addressed with classical biomarkers (i.e. the 'single-gene, protein or metabolite' approach), usually under non-physiological conditions. In addition, the efficacy assessment of health effects of nutritional components is even further complicated by the fact that single dietary constituents are hardly consumed as separate entities, but are part of a dietary mixture.

An important challenge in the development of functional foods for the prevention of complex (multifactorial) diseases is to obtain a better and improved overview (holistic) picture of early phases of the process. The concept of systems biology relates to the integration of all information at the different levels of genomic expression (mRNA, protein, metabolite). Thus, systems biology generates pathway information and provides the capacity to measure (small) perturbations of the pathway resulting from nutritional influences. The challenge here is not so much on a technological level, as enormous progress is being made in the 'omics' technologies. Instead, it is the bioinformatics side (data preprocessing, clustering, dynamics, integration of the various 'omics' levels, and so on) that will have to produce major breakthroughs in order for systems biology for nutrition to mature.

A clear example showing the link between dietary habits and disease in its full complexity is the effect of dietary fat on the development of cardiovascular disease (CVD). In the first half of the 20th century it was shown that serum cholesterol could be modulated by the composition of dietary fat and that these changes could be predicted. There is evidence that diets rich in *cis*-unsaturated fatty acids and other factors such as potassium, fruits, vegetables and fibres reduce the risk for development of CVD. In recent years, the dietary responses for serum low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) could be predicted. Next to blood pressure measurements, the LDL-C/HDL-C ratio is considered to be a reliable biomarker in predicting the development of CVD [2–6]. However, early biomarkers for the prevention of CVD or for improving health (maintaining homeostasis) are much more complex. Lipid homeostasis is thought to be achieved by the coordinated action of a large number of biomolecules — including nuclear factors, lipid-binding proteins, apolipoproteins, enzymes and receptors — and involving hundreds of genes. Genetic variability has been demonstrated in humans for the majority of these proteins (e.g. apolipoprotein E), which can explain the inter-individual variability observed in response to LDL-C levels upon a low-fat dietary intervention. Some genotypes predispose an individual for abnormal lipid metabolism and lipoprotein profiles [7], in turn determining the risk for development of atherosclerosis. Therefore, the simple determination of one single biomolecule (e.g. total cholesterol) as a biomarker may be insufficient to fully estimate the risk of developing the disease at an early stage. The relative advantage is that for the role of lipoproteins in the development of CVD, at least mechanistic information has accumulated over the past few decades. The intricate way in which fatty acid metabolism is regulated involving several organs and pathways demonstrates the need for a systems biology type approach. Apart from the obvious relation with sugar metabolism through the insulin/glucagon regulatory mechanism, relationships exist with cholesterol homeostasis through mechanisms controlled by the sterol regulatory element binding proteins SREBP1c and SREBP2 [8••] and through the ABC transporters for cholesterol in the intestines and the liver cells [9]. Furthermore, retinoids exert a specific action in triglyceride homeostasis through the retinoid receptors RXR and RAR [10]. High concentrations of retinoids may even have opposing effects on triglyceride plasma concentration, depending on other factors. Furthermore, the adipocyte regulates fatty acid

metabolism, partly through peroxisome proliferator-activated receptor (PPAR)-related systems [11,12]. As described above, lipoprotein regulation, like the hepatic expression of the LDL-C receptor, plays its part in lipid homeostasis. Also, satiety aspects need to be taken into account, like the function of leptin and many other hormone-like functions [13]. Although for mechanistic purposes the study of isolated gene expression, protein modifications and metabolite concentrations is extremely useful, in complex situations, as described above, the 'whole system' clearly needs to be taken into account.

For other diseases like colorectal cancer or food allergy, mechanistic information on the effects of nutrients on disease prevention, occurrence or progression is less available. Hence, predictive biomarkers for early biological effects in terms of maintaining or improving health in respect to these conditions are lacking, and the influence of genetic variability is even less well understood. Even more complicated is the search for markers predicting relations between nutrition and, for example, immune function.

A special case can be made for the application of functional genomics to easily accessible tissues from human volunteers enrolled in nutritional studies, in particular using white blood cell populations. The first results indicate that genomic fingerprints can be obtained, which indeed can be used as a biomarker [14•,15]. Further investigation is required to see whether, for example, peripheral blood lymphocyte transcriptome patterns or plasma proteome patterns can serve as a predictor for the safety and efficacy of nutrients, in terms of health promotion or disease prevention. Here, the concept of using a pattern of responses instead of a single response can be exploited by using the complete set of gene expressions as a biomarker.

The first steps in functional genomics-based nutritional research

A limited number of papers related to nutrigenomics have appeared so far, most of them focusing on multiple gene expression analysis (transcriptomics). Mariadason and colleagues [16••] compared transcriptome changes induced by the short-chain fatty acid butyrate and curcumin and two drugs (trichostatin A and sulindac) in the colon cancer cell line SW620. Similarities in transcriptional responses demonstrated that sulindac and curcumin, and butyrate and trichostatin A, respectively, are related in terms of mechanism of action. Comparison of the effects of butyrate and trichostatin A, both known to inhibit histone deacetylase, on gene expression and kinetics of histone acetylation identified subsets of induced and repressed genes that are likely to be coordinately regulated by altered histone acetylation. In another study, butyrate treatment of HT29 colorectal cancer cells affected the expression of proteins involved in the ubiquitin-proteasome system and in apoptosis pathways, as determined by proteome analysis [17•]. This suggests that, in addition to the regulation of gene expression through histone deacetylation, proteolysis could be a means by which butyrate regulates the expression of key proteins in the control of the cell cycle, apoptosis and differentiation. Also, the fatty acid palmitate was found to induce gene expression changes in pancreatic β cells [18]. Thus, functional genomics enabled the comparison of the action of different nutrients and drugs at the molecular level. Furthermore, these studies show that functional genomics provides the tools to generate new hypotheses on the mechanism of action of nutrients.

Age-related gene expression changes were found to be completely, or partially, prevented by caloric restriction [19••]. In a similar study [20], caloric restriction enhanced the transcripts of genes involved in reactive oxygen free radical scavenging functions, tissue development and energy metabolism, while decreasing the expression of genes involved in stress responses, signal transduction and structural proteins. Thus, alterations in the transcripts of genes involved in scavenging of reactive oxygen species may contribute to the increased longevity associated with caloric restriction. The same research group also demonstrated gene expression changes in rat muscle, induced by diets high in fat content. In particular, expression changes were seen in genes involved in stress responses and repair mechanisms and were partially reversed by antioxidant supplementation [21]. These studies indicate that gene expression profiles can be used as mechanistic tools, and possible markers, to better understand health effects of certain diets or physiological factors (e.g. ageing) and the beneficial effects of nutritional intervention (e.g. caloric restriction or antioxidant treatment).

Retinoic acid treatment of the neuroblastoma cell line RTBM1 induced changes in the expression of genes related to neuronal differentiation and growth [22]. Another vitamin, 1,25-dihydroxyvitamin D_3, was found to induce changes in gene expression associated with Ca^{2+} influx [23]. Both studies provided mechanistic information on the effects of these vitamins.

Selenium has possible anticarcinogenic properties. Mice fed on a diet low in selenium showed increased expression of genes involved in DNA damage processing, oxidative stress and cell-cycle control and decreased expression of genes involved in detoxification [24•]. Petrault *et al.* [25] demonstrated the effects of magnesium deficiency on gene expression in rat thymocytes and showed increased expression of genes involved in protection and repair of oxidative stress, possibly as a compensatory mechanism. Blanchard *et al.* [26] used cDNA arrays to demonstrate changes in intestinal gene expression, induced by rodent zinc deficiency. Amongst others, these authors found changes in the expression of metallothionein 1, zinc transporter 2 and uroguanylin, which are known to be zinc-regulated. These studies indicate that gene expression profiling can be used to detect suboptimal intakes of essential micronutrients, at least in target organs in animal models. Also, those genes already known to be associated

with micronutrient intake can be confirmed and new hypotheses generated to predict the biochemical action of the micronutrient. However, it remains to be seen whether gene expression profiling can be used to predict suboptimal micronutrient intake or deficiencies in humans *in vivo*.

Transcriptome analysis performed on the hypothalamus obtained from food-deprived rats revealed upregulation of a novel gene, encoding minoxidil sulfotransferase, not previously known to be associated with fasting [27]. The dietary soy isoflavone genistein has possible anticarcinogenic possibilities. Transcriptomics analysis of bladder tumour cells exposed to genistein showed changes in the expression of genes encoding proteins involved in signal transduction and cell-cycle regulation. In addition, the change in expression of the gene *egr-1*, known to be associated with proliferation and differentiation, was pronounced [9]. These studies represent other examples of hypotheses-generating nutrigenomics studies, opening the way for more mechanistic studies.

Conclusions

It is clear that systems biology is capable of providing new mechanistic insights into the molecular effects of nutrients in isolated systems (e.g. cell culture or in animal models). The most promising examples, in terms of providing new biomarkers, are nutrigenomics studies on the effects of caloric restriction on ageing, at least in animal models. In addition, transcriptomics enabled the detection of suboptimal intakes or deficiencies of micronutrients. New hitherto unknown biological activities could be ascribed to nutrients, illustrating that functional genomics paves the way to development of (e.g. *in vitro*) screening tools, of particular importance for the development of new bioactive ingredients. Genomics-based screening tools for nutrients with possible preventive action on the development of colorectal cancer are being developed [28].

Some final considerations need to be given. Functional genomics technologies are not yet developed to their full potential and, while being implemented in nutritional and biomedical studies, still need to undergo further technological improvement. This is particularly true for the areas of proteomics and metabolomics. Promising novel biomarkers or molecular effects should at present still be confirmed with conventional biochemical or molecular biological tools (e.g. RT-PCR of a single gene). The outcome of nutrigenomics studies should be compared with mechanistic, clinical and/or epidemiological data available for the compound under study. Bioinformatics is needed to analyse functional genomics derived data, although it is itself a scientific discipline in its infancy. For example, discussions on the best data analysis tools to decipher tremendous amounts of transcriptome data have not yet come to completion. Also, the linking of information from the genome, transcriptome, proteome and metabolome is a major challenge, as most biologists and nutritional scientists are trained to perform studies in a hypothesis-driven manner, thus step-by-step. Observed expression changes of unsuspected genes with unknown function in relation to the compound under study may be very hard to interpret. It is clear that current scientists are biased to first explaining nutrigenomics data in light of what is already known.

Long-term human intervention studies aimed at measuring the effect of a nutrient on biological endpoints in relation to health promotion or disease prevention are, of course, the golden standard in nutritional sciences. However, these studies are hardly possible for experimental, economical and ethical reasons. Therefore, nutritional sciences need to embrace systems biology, particularly if new mechanistic insights and 'biomarkers of health' are required in relation to health claims. Using early systems biology derived biological markers and mechanistic knowledge, such predictions may become possible. These extrapolations are expected to be more scientifically sound than predictions relying only on the measurement of a single molecule or a few biomolecules at a time.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. MacGregor JT, Jurd L: Mutagenicity of plant flavonoids: structural requirements for mutagenic activity in ***Salmonella typhimurium***. *Mutat Res* 1978, 54:297-309.
2. Zock PL, Katan MB, Mensink RP: Dietary trans fatty acids and lipoprotein cholesterol. *Am J Clin Nutr* 1995, 61:617.
3. Goldstein JL, Brown MS: The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu Rev Biochem* 1977, 46:897-930.
4. Wierzbicki AS, Mikhailidis DP: Beyond LDL-C — the importance of raising HDL-C. *Curr Med Res Opin* 2002, 18:36-44.
5. Sacks FM, Tonkin AM, Craven T, Pfeffer MA, Shepherd J, Keech A, Furberg CD, Braunwald E: Coronary heart disease in patients with low LDL-cholesterol: benefit of pravastatin in diabetics and enhanced role for HDL-cholesterol and triglycerides as risk factors. *Circulation* 2002, 105:1424-1428.
6. Craig IH, Phillips PJ, Lloyd JV, Watts S, Bracken A, Read R: Effects of modified fat diets on LDL/HDL ratio. *Lancet* 1980, 2:799.
7. Weggemans RM, Zock PL, Ordovas JM, Ramos-Galluzzi J, Katan MB: Genetic polymorphisms and lipid response to dietary changes in humans. *Eur J Clin Invest* 2001, 31:950-957.
8. •• Horton JD, Goldstein JL, Brown MS: SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002, 109:1125-1131.
   Excellent review of molecular regulatory mechanisms in nutritional sciences.
9. Chen CC, Shieh B, Jin YT, Liau YE, Huang CH, Liou JT, Wu LW, Huang W, Young KC, Lai MD *et al.*: Microarray profiling of gene expression patterns in bladder tumor cells treated with genistein. *J Biomed Sci* 2001, 8:214-222.
10. Staels B: Regulation of lipid and lipoprotein metabolism by retinoids. *J Am Acad Dermatol* 2001, 45:S158-S167.
11. Seedorf U, Assmann G: The role of PPAR α in obesity. *Nutr Metab Cardiovasc Dis* 2001, 11:189-194.
12. Debril MB, Renaud JP, Fajas L, Auwerx J: The pleiotropic functions of peroxisome proliferator-activated receptor γ. *J Mol Med* 2001, 79:30-47.
13. Soukas A, Cohen P, Socci ND, Friedman JM: Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* 2000, 14:963-980.

14. Tang Y, Lu A, Aronow BJ, Sharp FR: Blood genomic responses
• differ after stroke, seizures, hypoglycemia, and hypoxia: blood genomic fingerprints of disease. *Ann Neurol* 2001, 50:699-707.
This paper both illustrates the possibilities of the technologies and the limitations of the data interpretation that are challenging us.

15. Walker J, Flower D, Rigley K: Microarrays in hematology. *Curr Opin Hematol* 2002, 9:23-29.

16. Mariadason JM, Corner GA, Augenlicht LH: Genetic reprogramming
•• in pathways of colonic cell maturation induced by short chain fatty acids: comparison with trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer. *Cancer Res* 2000, 60:4561-4572.
A nice paper using transcriptomics to study, at the molecular level, genetic reprogramming induced by dietary compounds. Comparison of structurally related compounds (curcumin and sulindac) or compounds related in terms of biochemical mechanism (butyrate and trichostatin A, both inhibitors of histone deacetylase activity) yields information on clusters of genes from which the expression is either associated with the compound's structure or with a particular biochemical mechanism.

17. Tan S, Seow TK, Liang RC, Koh S, Lee CP, Chung MC, Hooi SC:
• Proteome analysis of butyrate-treated human colon cancer cells (HT-29). *Int J Cancer* 2002, 98:523-531.
The first reports describing a proteome study conducted to understand the effects of short chain fatty acid on colorectal cancer cells. To our knowledge, this is also the first published paper describing the effects of a dietary compound using proteomics. Importantly, proteome data were validated using western blotting.

18. Xiao J, Gregersen S, Kruhoffer M, Pedersen SB, Orntoft TF, Hermansen K: The effect of chronic exposure to fatty acids on gene expression in clonal insulin-producing cells: studies using high density oligonucleotide microarray. *Endocrinology* 2001, 142:4777-4784.

19. Lee C-K, Klopp RG, Weindruch R, Prolla TA: Gene expression
•• profile of aging and its retardation by caloric restriction. *Science* 1999, 285:1390-1393.
Provides the first global assessment of a dietary intervention (caloric restriction) at the molecular level and its effects on physiology (ageing).

20. Sreekumar R, Unnikrishnan J, Fu A, Nygren J, Short KR, Schimke J, Barazzoni R, Nair KS: Effects of caloric restriction on mitochondrial function and gene transcripts in rat muscle. *Am J Physiol Endocrinol Metab* 2002, 283:E38-E43.

21. Sreekumar R, Unnikrishnan J, Fu A, Nygren J, Short KR, Schimke J, Barazzoni R, Nair KS: Impact of high-fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle. *Am J Physiol Endocrinol Metab* 2002, 282:E1055-E1061.

22. Ueda K: Detection of the retinoic acid-regulated genes in a RTBM1 neuroblastoma cell line using cDNA microarray. *Kurume Med J* 2001, 48:159-164.

23. Farach-Carson MC, Xu Y: Microarray detection of gene expression changes induced by 1,25(OH)(2)D(3) and a Ca^{2+} influx-activating analog in osteoblastic ROS 17/2.8 cells. *Steroids* 2002, 67:467-470.

24. Rao L, Puschner B, Prolla TA: Gene expression profiling of low
• selenium status in the mouse intestine: transcriptional activation of genes linked to DNA damage, cell cycle control and oxidative stress. *J Nutr* 2001, 131:3175-3181.
Most often, nutrients are studied at non-physiologically high doses. An alternative is to study the nutrient under conditions of deficiencies or suboptimal intake. This study is an example showing the strength of functional genomics in combination with dietary deficiency of selenium.

25. Petrault I, Zimowska W, Mathieu J, Bayle D, Rock E, Favier A, Rayssiguier Y, Mazur A: Changes in gene expression in rat thymocytes identified by cDNA array support the occurrence of oxidative stress in early magnesium deficiency. *Biochim Biophys Acta* 2002, 1586:92-98.

26. Blanchard RK, Moore JB, Green CL, Cousins RJ: Modulation of intestinal gene expression by dietary zinc status: effectiveness of cDNA arrays for expression profiling of a single nutrient deficiency. *Proc Natl Acad Sci USA* 2001, 98:13507-13513.

27. Li JY, Lescure PA, Misek DE, Lai YM, Chai BX, Kuick R, Thompson RC, Demo RM, Kurnit DM, Michailidis G *et al.*: Food deprivation-induced expression of minoxidil sulfotransferase in the hypothalamus uncovered by microarray analysis. *J Biol Chem* 2002, 277:9069-9076.

28. Stierum R, Burgemeister R, van Helvoort A, Peijnenburg A, Schutze K, Seidelin M, Vang O, van Ommen B: Functional food ingredients against colorectal cancer. An example project integrating functional genomics, nutrition and health. *Nutr Metab Cardiovasc Dis* 2001, 11:94-98.