

# Nutrigenomics: goals and strategies

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Nutrigenomics is the application of high-throughput genomics tools in nutrition research. Applied wisely, it will promote an increased understanding of how nutrition influences metabolic pathways and homeostatic control, how this regulation is disturbed in the early phase of a diet-related disease and to what extent individual sensitizing genotypes contribute to such diseases. Ultimately, nutrigenomics will allow effective dietary-intervention strategies to recover normal homeostasis and to prevent diet-related diseases.

In the past decade, nutrition research has undergone an important shift in focus from epidemiology and physiology to molecular biology and genetics. This is mainly a result of three factors that have led to a growing realization that the effects of nutrition on health and disease cannot be understood without a profound understanding of how nutrients act at the molecular level. First, the completion of several large genome projects has markedly altered the research agenda by drawing attention to the importance of genes in human nutrition, and has provided a wealth of new genetic information to be explored<sup>1–3</sup>. Second, there has been a growing recognition that MICRONUTRIENTS and MACRONUTRIENTS can be potent dietary signals that influence the metabolic programming of cells and have an important role in the control of homeostasis<sup>4</sup>. Third, nutrition researchers have increasingly started to recognize that genetic predisposition can be an important contributor to the main causes of mortality that are linked to diet, such as cardiovascular disease, **diabetes type II** and cancers<sup>5</sup>.

New tools that have allowed increasingly detailed molecular studies of nutrition have also helped to change the focus of the field. Subtle changes in gene expression, even at the single-cell level, can now be measured by quantitative techniques such as real-time PCR and high-density microarray analysis<sup>6–10</sup>. The latter allows the entire nutrition-relevant TRANSCRIPTOME to be studied simultaneously. Such studies are one important focus of the new field of nutritional genomics or NUTRIGENOMICS<sup>11–15</sup>. Comparable progress in the analysis of the nutrition-relevant

metabolome (METABOLOMICS)<sup>15,16</sup> and the nutrition-relevant proteome (PROTEOMICS)<sup>17</sup> should soon allow the analysis of the response of whole systems to nutrients, from genes to organisms. In future, studying organismal responses to particular dietary components at the metabolome, proteome and transcriptome levels will hopefully show valuable organ-specific patterns. An ambitious challenge for the next decade is to translate this type of nutrigenomics data into an accurate prediction of the beneficial or adversary health effects of dietary components.

Nutrigenomics is new, it is not yet well defined and there are still relatively few convincing studies in the area. However, high expectations are already being placed on nutrigenomics. We believe that now is the right time for a ‘reality check’: a pragmatic consideration of what realistically can be achieved within the limits of available budgets. We argue that the main goal for the application of genomics in nutrition science should be the prevention of diet-related diseases. Therefore, we do not review all aspects of this new field in great detail here; instead, we discuss the concepts, some recent

promising results and our opinions on the future of the field.

## What is nutrigenomics?

Nutrigenomics attempts to study the genome-wide influences of nutrition. From a nutrigenomics perspective, nutrients are dietary signals that are detected by the cellular sensor systems that influence gene and protein expression and, subsequently, metabolite production. So, patterns of gene expression, protein expression and metabolite production in response to particular nutrients or nutritional regimes can be viewed as ‘dietary signatures’. Nutrigenomics seeks to examine these dietary signatures in specific cells, tissues and organisms, and to understand how nutrition influences homeostasis. Furthermore, nutrigenomics aims to identify the genes that influence the risk of diet-related diseases on a genome-wide scale, and to understand the mechanisms that underlie these genetic predispositions.

Genomics tools can be used in two different, but complementary, strategies in molecular nutrition research. The first strategy is the traditional hypothesis-driven approach: specific genes and proteins, the expression of which is influenced by nutrients (TABLE 1), are identified using genomics tools — such as transcriptomics, proteomics and metabolomics — which subsequently allows the regulatory pathways through which diet influences homeostasis to be identified (BOX 1). Transgenic mouse models (BOX 2) and cellular models are essential tools

Table 1 | **Transcription-factor pathways mediating nutrient-gene interactions**

Nutrient	Compound	Transcription factor
<b>Macronutrients</b>		
Fats	Fatty acids Cholesterol	PPARs, SREBPs, LXR, HNF4, ChREBP SREBPs, LXRs, FXR
Carbohydrates	Glucose	USFs, SREBPs, ChREBP
Proteins	Amino acids	C/EBPs
<b>Micronutrients</b>		
Vitamins	Vitamin A Vitamin D Vitamin E	RAR, RXR VDR PXR
Minerals	Calcium Iron Zinc	Calcineurin/NF-ATs IRP1, IRP2 MTF1
<b>Other food components</b>		
	Flavonoids Xenobiotics	ER, NFκB, AP1 CAR, PXR

AP1, activating protein1; CAR, constitutively active receptor; C/EBP, CAAT/enhancer binding protein; ChREBP, carbohydrate responsive element binding protein; ER, oestrogen receptor; FXR, farnesoid X receptor; HNF, hepatocyte nuclear factor; IRP, iron regulatory protein; LXR, liver X receptor; MTF1, metal-responsive transcription factors; NFκB, nuclear factor κB; NF-AT, nuclear factor of activated T cells; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; SREBP, sterol-responsive-element binding protein; USF, upstream stimulatory factor; VDR, vitamin D receptor.

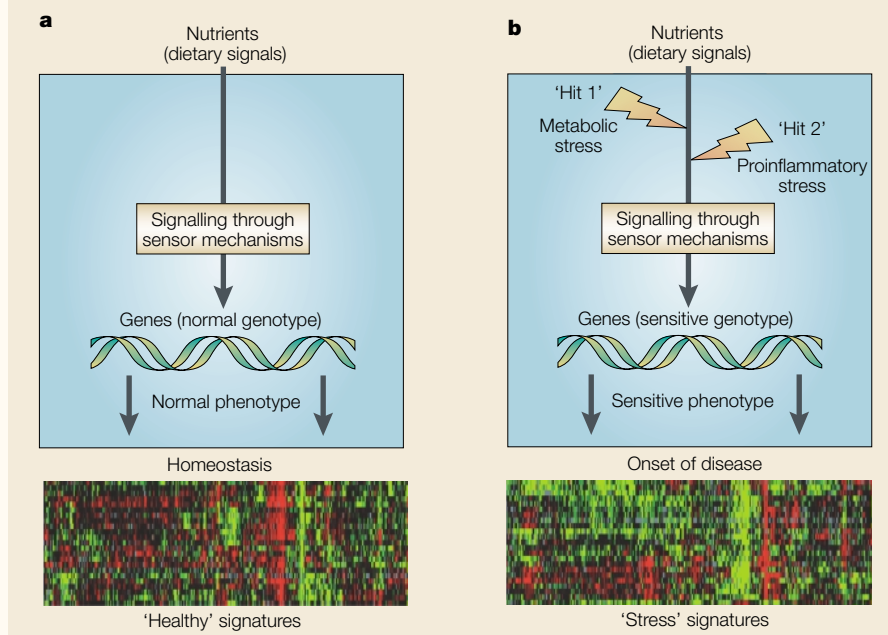
Box 1 | **Detecting the two hits: pro-inflammatory and metabolic stress**

Cells are regularly exposed to stress, which mainly consists of inflammatory stress and metabolic stress. Inflammatory stress is exerted by cytokines that are released in large quantities by immune cells in response to invading microorganisms. Cytokines such as tumour necrosis factor- $\alpha$  (TNF), interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6 induce the hepatic ACUTE-PHASE RESPONSE which consists of local and systemic reactions and is accompanied by upregulated or downregulated synthesis and/or activation of liver-enriched transcription factors<sup>68–71</sup>. Cytokines promote the synthesis of acute-phase proteins, in part by downregulating nuclear receptors, such as peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ), which suppress the expression of genes encoding acute-phase proteins such as serum amyloid protein and C-reactive protein<sup>37,72</sup>. However, this inflammatory response is a double-edged sword, particularly if it is chronic. Pro-inflammatory cytokines can induce cytotoxicity that, in the worse-case scenario, can lead to liver failure<sup>68,73</sup>.

Pro-inflammatory stress is directly linked to an immune response, whereas metabolic stress describes changes in the plasma and/or cellular concentration of nutrients and metabolites, which might lead to the disruption of cellular function. One important group of compounds that cause metabolic stress are lipids, or more specifically fatty acids. In healthy individuals, the negative-feedback system that is mediated by PPARs acting as nutrient sensors (see discussion in the main text) can deal with fluctuations in free fatty-acid levels in the plasma (panel a). However, in individuals with conditions such as diabetes and obesity that cause permanently elevated plasma levels of free fatty acids (metabolic stress; ‘hit one’), who then, as part of an immune response, have cytokine-induced downregulation of PPAR $\alpha$  and other nuclear receptors (pro-inflammatory stress; ‘hit two’), the system is overtaxed (panel b). In this case, fatty acids accumulate as triglycerides and spill over into harmful pathways. If triglycerides accumulate in non-adipose tissues, the individual’s sensitivity to proinflammatory stress will increase further and might lead to significant organ dysfunction. For example, a combination of excess fat storage and inflammatory stress in the liver can ultimately result in cirrhosis<sup>74</sup>.

We are convinced that the interaction between pro-inflammatory stress and metabolic stress is the key to understanding diet-related diseases. Although some might disagree with this view, the role of inflammatory processes in diseases such as atherosclerosis, insulin resistance and cirrhosis is widely recognized<sup>75–78</sup>. So, we believe that understanding how the ‘two hits’ interact is essential for the application of nutrigenomics in disease prevention.

In future, nutrigenomics tools should allow the collection of ‘healthy’ diet-related expression signatures as appropriate baseline data (panel a). By comparing these signatures with ‘stress’ signatures (panel b) that are derived from nutrigenomics experiments, we might be able to identify early molecular biomarkers for individuals with sensitive genotypes under sustained metabolic and pro-inflammatory stress that could lead to serious conditions such as cirrhosis or insulin resistance. With enough early warning, dietary intervention might reverse this process, regain homeostatic control and prevent these conditions in at-risk groups. Microarray panels reproduced with permission from REF. 46 © (2003) National Academy of Sciences.



in this approach, which can allow new genes and pathways to be identified. In future, such models might provide the key to understanding the interactions between metabolic and INFLAMMATORY signalling routes (BOX 1). The second strategy, which is largely theoretical at this stage, is the SYSTEMS BIOLOGY approach: gene, protein and metabolite signatures that are associated with specific nutrients, or nutritional regimes, are catalogued, and might provide ‘early warning’ molecular biomarkers for nutrient-induced changes to homeostasis. The first strategy will provide us with detailed molecular data on the interaction between nutrition and the genome, whereas the second strategy might be more important for human nutrition, given the difficulty of collecting tissue samples from ‘healthy’ individuals.

Keeping in mind these two broad strategies, the following goals of nutrigenomics research can be defined: the identification of transcription factors that function as nutrient sensors (TABLE 1) and the genes they target; the elucidation of the signalling pathways involved, and characterization of the main dietary signals; the measurement and validation of cell- and organ-specific gene-expression signatures of the metabolic consequences of specific micronutrients and macronutrients; the elucidation of the interactions between nutrient-related regulatory pathways and proinflammatory stress pathways, to understand the process of metabolic dysregulation that leads to diet-related diseases; the identification of genotypes that are risk-factors for the development of diet-related human diseases (such as diabetes, hypertension or atherosclerosis) and quantification of their impact; and the use of nutritional systems biology to develop biomarkers of early metabolic dysregulation and susceptibility (stress signatures) that are influenced by diet.

**Dietary signals: from nutrients to genes**

In some ways, the nutrigenomics agenda can be seen as analogous to that of PHARMACOGENOMICS<sup>18,19</sup>. However, an important difference is that pharmacogenomics is concerned with the effects of drugs that are pure compounds — administered in precise (usually small) doses — whereas nutrigenomics must encompass the complexity and variability of nutrition. The body has to process a huge number of different nutrients and other food components. Nutrients can reach high concentrations ( $\mu\text{M}$  to  $\text{mM}$ ) without becoming toxic. Each nutrient can also bind to numerous targets with different affinities and specificities. By contrast, drugs are used

at low concentrations and act with a relatively high affinity and selectivity for a limited number of biological targets. Despite these differences, nutritional research could benefit greatly, as has pharmacology, from detailed information on the effects of compounds at the molecular level.

It is now evident that, as well as their function as fuel and co-factors, micro- and macronutrients can have important effects on gene and protein expression and, accordingly, on metabolism. The molecular structure of a nutrient determines the specific signalling pathways that it activates. Small changes in structure can have a profound influence on which sensor pathways are activated. This fine-tuned molecular specificity explains why closely related nutrients can have different effects on cellular function. One example is how the nutritional effects of fatty acids vary depending on their level of SATURATION. The  $\omega$ -3 polyunsaturated fatty acids have a positive effect on **cardiac arrhythmia**<sup>20</sup>, whereas saturated C16–18 fatty acids (stearic acid and palmitic acid) do not. Furthermore,  $\omega$ -6 unsaturated C18 fatty acids (oleic acid and linoleic acid) decrease plasma levels of low-density lipoprotein (LDL) cholesterol<sup>21</sup>.

The challenge for the next decade is to identify nutrient-influenced molecular pathways and determine the down-stream effects of specific nutrients. Nutrigenomics can assist in this identification because it allows the genome-wide characterization of genes, the expression of which is influenced by nutrients. It is only with a complete understanding of the biochemical links between nutrition and the genome that we will be able to comprehend fully the influence of nutrition on human health.

### Nutrient sensors

Transcription factors are the main agents through which nutrients influence gene expression. The nuclear hormone receptor superfamily of transcription factors, with 48 members in the human genome, is the most important group of nutrient sensors (TABLE 1)<sup>4,22,23</sup>. Numerous receptors in this superfamily bind nutrients and their metabolites. These include retinoic acid (retinoic acid receptor (RAR) and retinoid X receptor (RXR)), fatty acids (peroxisome proliferator-activated receptors (PPARs) and liver X receptor (LXR)), vitamin D (vitamin D receptor (VDR)), oxysterols (LXR), bile salts (farnesoid X receptor (FXR), also known as bile salt receptor) or other hydrophobic food ingredients (constitutively active receptor (CAR) and pregnane X receptor (PXR))<sup>4,22–24</sup>.

### Box 2 | Use of knockout mice for nutrition research

To raise nutrigenomics above the level of purely descriptive data, we must understand how food components regulate gene or protein expression. For this purpose, mutant mice (particularly knockout mice) have become an invaluable tool. Using knockout mice, we can unambiguously establish how a particular transcription factor mediates the effect of a specific nutrient: a goal that is impossible to achieve in human studies. In combination with cell-culture studies, the use of knockout mice will greatly contribute to the generation of detailed molecular pathways showing how nutrients regulate gene and protein expression.

Recent studies investigating how polyunsaturated fatty acids (PUFAs) influence lipid metabolism elegantly show the power of knockout models. PUFAs usually stimulate the expression of several genes that are involved in fatty-acid oxidation. However, peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ )-null mice lack this response<sup>79</sup>. In these mice, PUFAs suppress the expression of genes that are involved in lipogenesis. Studies with the same mice showed that PPAR $\alpha$  is not the nutrient sensor that mediates the lowering of plasma triglyceride levels induced by fish oil<sup>80</sup>.

Similarly, retinoic-acid receptor knockout mice have provided insights into the molecular mechanism of vitamin A action. These mice mimic the symptoms of vitamin A deficiency and are, therefore, important tools for the study of the genomic effects of vitamin A<sup>80,81</sup>. Further examples of the use of knockout mice for nutritional genomics research are cited in TABLE 2. The increasing number of available knockout (or knockdown) mice<sup>82–84</sup> should allow us to investigate many more nutrient signalling pathways.

Nuclear receptors bind with RXR to specific nucleotide sequences (response elements) in the promoter regions of a large number of genes. During ligand binding, nuclear receptors undergo a conformational change that results in the coordinated dissociation of co-repressors and the recruitment of co-activator proteins to enable transcriptional activation. In metabolically active organs, such as the liver, intestine and adipose tissue, these transcription factors act as nutrient sensors by changing the level of DNA transcription of specific genes in response to nutrient changes<sup>4</sup>. Nuclear hormone receptors have important roles in the regulation of numerous processes, including nutrient metabolism, embryonic development, cell proliferation and differentiation. So, it is easy to envision how nutrients, by activating these receptors, are able to influence a wide array of cellular functions.

To briefly illustrate the strategy that cells use to adapt to changes in nutrient and metabolite concentrations through these nutrient-sensing transcription factors, we discuss two examples: bile-salt sensing and fatty-acid sensing during feeding and fasting.

**Bile-salt sensing.** Bile salts are metabolites of cholesterol that are formed in HEPATOCYTES and secreted across the CANALICULAR MEMBRANE by the ATP-binding cassette transporter (ABC) **ABCB11** (REF. 25). Bile salts are important components of bile, and are necessary for lipid digestion in the intestinal tract. However, at elevated concentrations, these potent detergents are cytotoxic. An ingenious

sensor mechanism protects cells from these cytotoxic effects, allowing them to rapidly reduce the free intracellular concentration of bile salts.

The nuclear hormone receptor FXR is the nutrient sensor that mediates this response to elevated levels of bile acids<sup>26</sup>. Through this receptor, bile acids increase the expression of numerous gene products that are involved in lipid metabolism, including ileal bile-acid binding protein, **PPAR $\alpha$** , short heterodimeric partner, phospholipid transfer protein, apolipoprotein E (**APOE**), **APOCII** and the bile-salt export pump (**ABCB11**)<sup>4,26–31</sup>. Overall, the increased expression of these genes inhibits the synthesis of bile acids and stimulates the transport of bile acids out of the cell, through **ABCB11**, into the **BILE CANALICULI**<sup>25,27</sup>.

### Fatty-acid sensing during feeding and fasting.

Fatty acids influence human health in numerous ways. Epidemiological studies show that certain fatty acids are linked to the increased occurrence of certain diseases<sup>32,33</sup>. Nutritional trials, in which the fats are enriched in specific fatty acids, show that fatty acids influence several indicators of health status. Unfortunately, until recently, our understanding of the molecular mechanisms that underlie these results was patchy. Early studies indicated that dietary poly-unsaturated fatty acids potentially repress the hepatic expression of several genes involved in fatty-acid synthesis<sup>34,35</sup>. However, it was not until several nuclear hormone receptors were discovered and characterized that some details of the manner in which fatty acids induce changes in gene expression emerged.



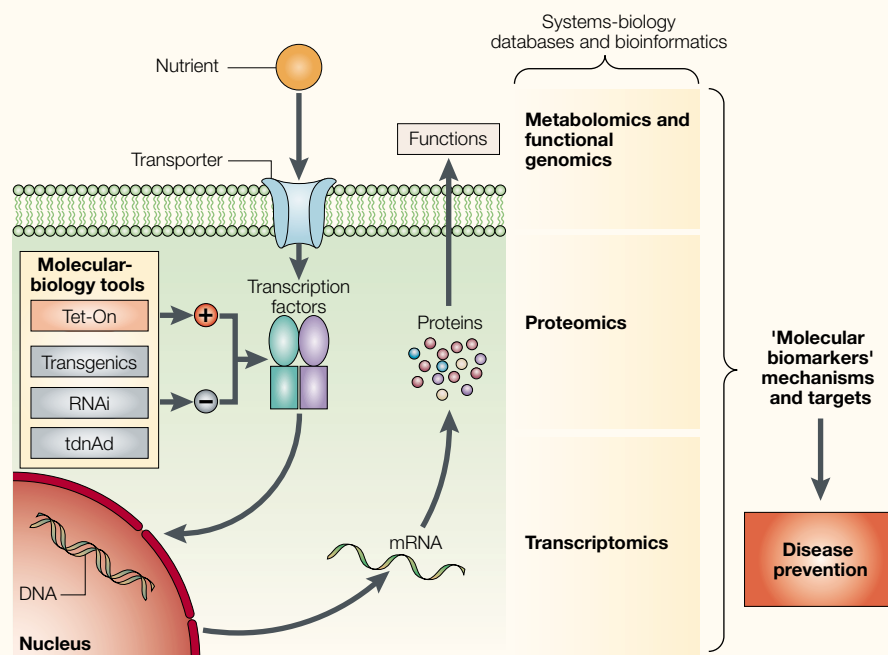


Figure 1 | **The ‘smart’ combination of molecular nutrition and nutrigenomics.** Molecular-biology tools, such as transgenic animal or cell models, RNA interference (RNAi), transdominant negative adenoviral constructs (tdnAd) and inducible gene-expression systems (for example, using tetracycline-inducible expression systems such as Tet-On), will be used to modulate the expression levels and functionality of nutrient-sensor systems. This will allow the discovery of dietary target genes and the characterization of the mechanisms that underlie dietary sensing. Nutritional systems biology will take advantage of the combination of transcriptomics, proteomics and metabolomics, to identify molecular biomarkers. These biomarkers will allow early dietary intervention to reverse the onset of diet-related diseases and to regain homeostasis.

We now know that PPARs — another group of nuclear hormone receptors — act as nutrient sensors for fatty acids and influence the expression of specific genes<sup>4,36–38</sup>. One of the three PPAR isotypes — PPAR $\alpha$  — is present mostly in the liver and is important during food deprivation and fasting. During fasting, free fatty acids are released from the adipose tissue. These fatty acids then travel to the liver, where they undergo partial or complete oxidation. However, these fatty acids also bind PPAR $\alpha$ , which then increases the expression of a suite of genes through binding to specific sequences in their promoter regions. Further, genes can also have their expression increased indirectly, through the genes that are directly affected by PPAR $\alpha$ . The target genes of PPAR $\alpha$  are involved in numerous metabolic processes in the liver, including fatty-acid oxidation and KETOGENESIS, apolipoprotein synthesis, amino-acid metabolism, cellular proliferation and the acute-phase response<sup>39–42</sup>. This is an elegant pathway in which the signal that initiates adaptive changes in liver metabolism during fasting originates from the adipose tissue and acts through a receptor, the expression of which is upregulated by fatty acids during fasting.

### Molecular tools for nutrition research

How can we extend our knowledge of the interaction between nutrition and the genome? Although, ideally, we would like to study the mechanisms in humans, this is often prohibited by the difficulty of collecting tissue samples. Also, the manipulative experiments that are required to show the mechanisms of nutrient signalling are not possible in humans. Therefore, transgenic and knockout mouse models (BOX 2), as well as *in vitro* experiments using tools such as INDUCIBLE EXPRESSION SYSTEMS, TRANSDOMINANT NEGATIVE ADENOVIRAL CONSTRUCTS and RNA INTERFERENCE (RNAi), remain the main investigative strategies (FIG. 1).

The use of LASER-CAPTURE MICRODISSECTION for single-cell gene-expression profiling<sup>43,44</sup> should greatly improve the cell-specific information that is derived from nutrition experiments with intact organisms (*in vivo*). In addition, primary cells and cell lines are wonderful tools for studying the effects of nutrients on gene expression; however, sometimes cell lines display large differences in the expression of important transcription factors compared with primary cells or *in vivo*. For example, the widely used HepG2 cells are of no use for investigating nutrient signalling

pathways mediated by sterol-response-element binding protein (SREBP), because the SREBP1c/SREBP1a ratio is different from that in normal hepatocytes<sup>45</sup>.

### Gene-expression profiling

Microarrays now make it possible to assess the effect of a specific diet or nutrient on the expression of a large proportion of the whole genome. Recent examples of this approach include gene-expression profiling during caloric restriction and fasting, and examination of the effects of single nutrient deficiency (TABLE 2).

In general terms, gene-expression profiling can be used for three different purposes in nutrition research. First, it can provide clues about the mechanism that underlies the beneficial or adversary effects of a certain nutrient or diet. Highly specific changes in gene expression might explain the beneficial or adversary effects of many nutrients. For example, the beneficial effect of poly-unsaturated fatty acids on plasma LDL levels might be linked to specific changes in the expression of genes that are involved in cholesterol metabolism. However, there are barriers to such studies. To conduct them requires knowledge of which tissue or organ is responsible for the specific effects of a nutrient: information that is not always available. Also, the specific functions of most of the genes included in gene-expression profiling experiments are still unclear. If you do not know the function of a gene the expression of which is modified by a specific nutrient, it is difficult to elucidate the mechanism underlying the specific beneficial or adversary effect that is observed. Second, gene-expression profiling can help to identify important genes, proteins or metabolites that are altered in the pre-disease state and that might, therefore, act as ‘molecular biomarkers’ (BOX 1). This pre-disease state is characterized by small metabolic perturbations that might slowly progress towards disease. Biomarkers at this early and reversible stage can have a high prognostic value and are of great importance for nutritional studies. Third, at a more basic level, gene-expression profiling can help to identify and characterize the basic molecular pathways of gene regulation by nutrients.

An important barrier to identifying molecular biomarkers in humans is the inaccessibility of human tissue, especially from healthy individuals. Blood is one of the few tissues that can be easily collected. Therefore, microarray-based measurements of human LYMPHOCYTE gene expression are one of the most promising potential diagnostic tools<sup>46,47</sup>. Certainly, it seems from studies of the gene-expression profiles of

Table 2 | Gene-expression profiling studies related to nutrition

Focus	Topic	Organism	Organ	References
Ageing and caloric restriction	Reversal of ageing-related gene expression by caloric restriction	Mouse	Skeletal muscle and brain	85
		Mouse	Liver	86
		Mouse	Heart	87
		Mouse	Brain	88
Metabolic syndrome	Insulin resistance	Human	Skeletal muscle	89
Diabetes	DNA methylation	Human	Various	90
		Human	Pancreatic islets	91
Role of specific transcription factors	HNF1	Mouse	Liver	92
	HNF4 $\alpha$	Mouse	Liver	93
	LXR $\alpha$	Mouse	Adipose	94
	PPAR $\alpha$	Mouse	Liver	95
	MTF1	Mouse	Embryonic liver	96
Gene regulation by nutrients	Zinc	Rat	Intestine	97
	Fatty acids	Rat	Pancreas	98
	Protein	Rat	Liver	99
	Short-chain fatty acids	Human	Colon	100

HNF, hepatocyte nuclear factor; LXR, liver X receptor; MTF1, metal-responsive transcription factor; PPAR, peroxisome proliferator-activated receptor; TGF, transforming growth factor.

large B-cell lymphoma (DLBCL)<sup>47,48</sup> and chronic lymphocytic leukaemia (CLL)<sup>49</sup> that the peripheral blood mononuclear cells can display disease-characteristic gene-expression signatures.

Until now, these 'lymphochips' have been mainly used for biomedical diagnostics<sup>46–48,50–53</sup>. However, this approach might be broadly useful for non-invasive diagnostics. These readily accessible cells could then function as devices that monitor the health or nutritional status of an individual. If this vision becomes reality, it could provide a bridge between basic nutritional science and human diet-intervention studies. More specifically, it would allow us to assess the effectiveness of specific nutrients in preventing disease.

#### Nutrigenetics and personalized diets

Nutrigenomics is focused on the effect of nutrients on the genome, proteome and metabolome, whereas NUTRIGENETICS examines the effect of genetic variation on the interaction between diet and disease or on nutrient requirements. Genetics has a pivotal role in determining an individual's risk of developing a certain disease<sup>54</sup>. Population differences in single nucleotide polymorphisms (SNPs) can have an important effect on disease risk.

Inter-individual genetic variation is also likely to be a crucial determinant of differences in nutrient requirements. For example, one study indicates that individuals with a C→T substitution in the gene for methyl-ene tetrahydrofolate reductase might require more folate than those with the wild-type allele<sup>55</sup>. Conversely, several studies indicate that diet has an important influence on the risk of developing certain diseases in which

genetic predisposition has a role. One interesting example of the complicated interaction between genetics, diet and disease comes from a study of the occurrence of **hepatocellular carcinoma** in Sudan; there was a stronger relationship between the risk of developing the disease and the consumption of peanut butter contaminated with aflatoxins in Sudanese people with the glutathione S-transferase M1 null genotype than there was in those lacking this genotype<sup>56</sup>.

The availability of the sequence of the human genome, coupled with the ongoing cataloguing of human genetic variation, provides nutrigenetics with an enormous resource with which to work<sup>3,57</sup>. The goal of the Single Nucleotide Polymorphisms Consortium is to map all the important polymorphic sites in the human genome<sup>57</sup>. The challenge for molecular epidemiology is to identify specific polymorphisms that are linked to altered risk of disease or sensitivity to diet<sup>5,58</sup>.

A recent high-resolution recombination map of the human genome has greatly improved our knowledge of the genetic order of polymorphic markers, the precision of estimates of genetic distances, and the SNP map of the human genome<sup>59</sup>. SNPs should provide powerful molecular tools for investigating the role of nutrition in human health and disease. Incorporating studies of SNPs into metabolic and epidemiological studies might also help to define optimal diets. The combination of twin studies<sup>60</sup> with advanced genetic analysis might allow us to understand the basis of complex traits and the impact of sensitizing genotypes on the development of polygenic diet-related diseases such as diabetes. In future, this might lead to the adjustment of dietary recommendations on the basis of genotype.

Although the implementation of this type of personalized diet is still in its infancy, progress in the next few years is likely to be rapid. Indeed, several small biotechnology firms have been founded that focus on nutrigenomics/nutrigenetics and the commercialization of personalized diets. However, if the use of genotypes in the dietary prevention of disease is to be established, the field of molecular nutrition must first be successful in identifying the mechanisms driving the connection between diet and phenotype according to specific genetic variations. Understanding how nutrient-sensing transcription factors mediate the effects of dietary components on gene expression (see above) will be crucial if this endeavour is to succeed.

So, although personalized diets would be an interesting application of nutrigenomics, we believe that the implementation of such an approach lies far ahead of us. Although there are many that disagree, we think that over the next 10 years the focus should be on understanding how nutrients interact with the genome at the molecular level.

#### Nutritional systems biology

Functional genomics and proteomics approaches, in conjunction with metabolic control analysis<sup>61</sup> (FIG. 1) are increasingly used to study the metabolic status of cells in an effort to understand the metabolic effects of specific perturbations at the gene and protein level<sup>62,63</sup>. Systems biology aims to understand phenotypic variation and build comprehensive models of cellular organization and function. It also seeks to elucidate the interaction and functions of cellular, organ and even organism-wide systems<sup>64,65</sup>. The optimism for using systems biology in nutrition research<sup>15,16,66</sup> relates to the implementation of metabolomics (FIG. 1). Metabolomics allows the extensive, sensitive and rapid measurement of metabolic profiles in blood or organ samples<sup>16,39</sup>.

As discussed earlier, a systems-biology-driven approach is likely to be the most promising nutrigenomics strategy in humans, in which it is impossible to determine the exact mechanisms of diet-related homeostatic control. Systems biology has so far been used mainly for metabolic studies with prokaryotic organisms and yeast, or for disease- or drug therapy-related mammalian models<sup>61,65,67</sup>. It will be extremely challenging (and expensive) to extract useful information from nutrigenomics experiments using humans or mice. We do not know the function of most of the 35,000–40,000 genes, >100,000 proteins and several thousand metabolites in humans.

## PERSPECTIVES

We are also dealing with complex genotypes of polygenic diseases. The integration of all these data will require both intellectual and financial investments in analytical platforms, dataware housing, laboratory information-management systems, new database structures, algorithms and so on.

So, for this form of nutrigenomics to move out of the realm of 'science-fiction' it will require a huge financial investment. To generate the critical mass of researchers that is required to attract this investment, several large national and international nutrigenomics programmes have been established, such as the National Center on Minority

Health and Health Disparities (NCMHD) Center of Excellence for Nutritional Genomics (at the University of California at Davis in the United States), the German Berlin-Brandenburg Nutrigenome Network, two Dutch nutrigenomics centres (the Centre for Human Nutrigenomics, and the Innovative Cluster Genomics project) and several other European initiatives that will hopefully collaborate in a large 'Network of Excellence' within the KP6 programme of the European Union.

### Conclusion and future perspectives

We have discussed the two different strategies in nutrigenomics research: first, the genome

wide discovery of dietary target genes and the elucidation of the regulatory pathways involved in homeostatic control; and second, the use of systems biology to identify molecular biomarkers of early changes in whole-body homeostatic control. However, in these euphoric pioneering times for nutrigenomics we should also recognize the potential barriers to its success. Food is typically a complex and variable mixture of nutrients and other components. Most nutrients are weak dietary signals and must be considered in the context of chronic exposure. It has still to be shown that nutrigenomics offers the tools to measure such weak dietary signals, or is able to detect modest nutritional deficiency in humans. Nutrigenomics researchers also must deal with the challenge of understanding polygenic diet-related diseases. Finally, we must be prepared to address genome-scale questions rather than limited specific hypothesis, to gain the highest profit from this wealth of data.

As discussed earlier, the expense of such research is another important barrier to be overcome before nutrigenomics reaches its full potential. Investment in nutrigenomics will never equal the amounts that have already been invested in pharmacogenomics. So, the strategy we advocate here is focused on the basics as we see them: to identify the dietary signals; to elucidate the dietary sensor mechanisms; to characterize the target genes of these sensors; to understand the interaction between these signalling pathways and pro-inflammatory signalling to search for sensitizing genotypes; and to find 'signatures' (gene/protein expression and metabolite profiles) that allow the discrimination of healthy versus unhealthy individuals to enable early dietary intervention.

Of course, nutrigenomics is not simply the pharmacogenomics of food components, but data generated by pharmacogenomics research will be important for the future of the field. Many transporter proteins, metabolizing enzymes and, in particular, transcription factors, are of interest to both fields and it is likely that the better-funded field of pharmacogenomics will produce most of the relevant information. In our view, the long-term goal of nutrigenomics is to help to understand how we can use nutrition to prevent many of the same diseases for which pharmacogenomics is attempting to identify cures.

Nutrigenomics is a potential goldmine for the discovery of genes that are important as dietary targets. It should also have an important role in elucidating nutrient signalling pathways that might contribute to certain diet-related diseases, such as cancer or metabolic syndrome. However, we should also be aware that simply accumulating microarray

### Glossary

#### ACUTE-PHASE RESPONSE

The early and immediate set of homeostatic control reactions that are induced during inflammation.

#### BILE CANALICULUS

A half tubule that is formed by the apical membranes of two hepatocytes, and is limited laterally by their smooth surfaces.

#### CANALICULAR MEMBRANE

The apical membrane of liver epithelial cells (hepatocytes) that lines the bile canaliculus. Members of the ABC-transporter superfamily that are localized in this membrane are responsible for bile secretion.

#### HEPATOCYTES

Epithelial cells that are the main functional units of the liver, and comprise 80% of the organ's cytoplasmic mass.

#### INDUCIBLE EXPRESSION SYSTEMS

Expression systems that regulate mammalian gene expression with, for example, tetracycline or its derivatives (Tet-On/Tet-Off gene expression systems).

#### INFLAMMATION

The complex series of reactions that occur in the host as a response to injury, trauma or infection of a tissue, which prevent ongoing tissue damage, isolate and destroy the infective organism and activate the repair processes that are necessary to return the organism to normal function.

#### KETOGENESIS

The production of ketone bodies — such as acetoacetate and  $\beta$ -hydroxybutyrate — which are the intermediate products of fatty-acid catabolism and can be used to provide energy.

#### LASER CAPTURE MICRODISSECTION

A method in which cells are cut out from a tissue sample using a laser beam, allowing single cell expression analysis.

#### LYMPHOCYTE

A type of white blood cell that is responsible for the adaptive immune response; for example, B lymphocytes and T lymphocytes.

#### MACRONUTRIENTS

Organic compounds, including proteins, amino acids, carbohydrates and lipids, that are required in large amounts in the diet.

#### METABOLOMICS

The study of the metabolome, which is the entire metabolic content of a cell or organism, at a given time.

#### MICRONUTRIENTS

Dietary compounds, including vitamins and minerals that are required in small amounts in the diet.

#### NUTRIGENETICS

The relationship between genotype and the risk of developing diet-related diseases, such as cancer, diabetes type II and cardio-vascular diseases.

#### NUTRIGENOMICS

The study of the genome-wide influences of nutrition or dietary components on the transcriptome, proteome and metabolome, of cells, tissues or organisms, at a given time.

#### PHARMACOGENOMICS

A term often used to mean the influence of DNA-sequence variation — in drug targets, Phase I or Phase II drug-metabolizing enzymes, and transporters — on the effect of a drug, which ultimately allows physicians to design individualized therapy.

#### PROTEOMICS

The study of proteomes (the complete collection of proteins in a cell or tissue at a given time), which attempts to determine their role inside cells and the molecules with which they interact.

#### RNA INTERFERENCE

(RNAi). The process by which double-stranded RNA silences homologous genes.

#### SATURATION

The binding state of a C-C bond in a fatty acid molecule.

#### SYSTEMS BIOLOGY

The study of whole biological systems (cells, tissues and organisms) using holistic methods.

#### TRANSCRIPTOME

The complete collection of gene transcripts in a cell or a tissue at a given time.

#### TRANSDOMINANT NEGATIVE ADENOVIRAL CONSTRUCT

A recombinant adenovirus that infects cells, resulting in the high-level expression of a mutant protein that, for example, specifically blocks a given signalling pathway (superrepressor) by competing with the endogenous protein.



datasets alone cannot lead to important insights. These data must be gathered in conjunction with smart functional studies using the knowledge of nutrient signals and sensors, or systems-biology driven analysis of signatures, to define molecular biomarkers of dietary responses (FIG. 1). It is our belief that by applying nutrigenomics wisely, nutritional science has the potential to evolve from an applied to a more exact science.

Although we think that the contribution of nutrigenomics to public health will be minor during the next five years, we believe that in the long-term, the field has the potential to make an important contribution. At some point, we will be able to get beyond the basic agenda outlined here, and start to address more sophisticated nutritional questions. To reach this point, we believe we need more nutrigenomics studies by disease-oriented biomedical scientists. Furthermore, we need traditional nutritional scientists to understand the potential for molecular nutrition research in mouse models to provide insights into human nutrition. Finally, we have to convince the food industry that the time has come to use nutrigenomics to develop evidence-based nutrition.

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