Learning Objectives

- To have understanding of the concepts of molecular nutrition research (signals and signaling pathways, use of animal models);
- To have understanding of identification of early biomarkers;
- To be able to read and understand literature of the field (molecular nutrition and nutrigenomics);
- To have some (practical) knowledge how to apply molecular nutrition and nutrigenomics in the lab;
- To be able to extract relevant data/information from internet for molecular nutrition research;
- To be able to understand a “nutrigenomics” experiment;
- To have understanding on the evolution of genomic versus food patterns. Dietary signaling and sensing.

Contents

1. Dietary signals: from nutrients to genes (Diet x Genes)
   1.1 Bile–salt sensing
   1.2 Fatty–acid sensing during feeding and fasting
2. Nutrigenetics and personalized diets (Diet x Genotypes)
3. Specific nutrients and foods for specific individuals or groups
4. Regulatory, legal and ethical considerations
5. Evolution of genomics versus food patterns
6. Concluding remarks
7. Glossary
8. References

Key Messages

- Discussion on basic mechanisms of dietary signaling and sensing (Diet x Genome);
- Discussion on nutrigenetics (Diet x Genotype);
- Introducing some regulatory, legal and ethical issues of nutrigenomics;
- Discussion on evolution of genomic versus food patterns;
- Nutrigenetics;
- Personalized diet;
- “Thrifty” genotype;
- Regulatory, legal and ethical issues.
1. Dietary signals: from nutrients to genes (Diet x Genes)

In some ways, the nutrigenomics agenda can be seen as analogous to that of pharmacogenomics. 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 (µM to 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 signaling pathways that it activates.

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

AP1 – activating protein 1; CAR – constitutively active receptor; C/EBP – CAAT/enhancer binding protein; ChREBP – carbohydrate responsive element binding protein; ER – estrogen receptor; FXR – farnesine 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; SREBP – sterol-responsive-element binding protein; USF – upstream stimulatory factor; VDR – vitamin D receptor.
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 ω-3 polyunsaturated fatty acids have a positive effect on cardiac arrhythmia, whereas saturated C16-18 fatty acids (stearic acid and palmitic acid) do not. Furthermore, ω-6 unsaturated C18 fatty acids (oleic acid and linoleic acid) decrease plasma levels of low-density lipoprotein (LDL) cholesterol. 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. 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). 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)). 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. 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.

1.1 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) ABCB. 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. Through this receptor, bile acids increase the expression of numerous gene products that are involved in lipid metabolism, including ileal bile-acid binding protein, PPARα, short heterodimeric partner, phospholipid transfer protein, apolipoprotein E (APOE), APOCII and the bile-salt export pump (ABCB11). 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 canalici.

1.2 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. 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 potently repress the hepatic expression of several genes involved in fatty acid synthesis. 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. 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. One of the three PPAR isotypes — PPAR-α — 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-α, 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-α. The target genes of PPAR-α 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. 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.

2. Nutrigenetics and personalized diets (Diet x Genotypes)

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. Population differences in 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 methylenetetrahydrofolate reductase (MTHFR) might require more folate than those with the wild-type allele. 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 (GSTM1) null genotype than there was in those lacking this genotype. 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. The goal of the Single Nucleotide Polymorphisms Consortium is to map all the important polymorphic sites in the human genome. The challenge for molecular epidemiology is to identify specific polymorphisms that are linked to altered risk of disease or sensitivity to diet.
Gene expression patterns produce phenotype, which represents the physical characteristics or observable traits an organism, e.g., hair color, weight, the presence or absence of a disease. Phenotypic traits are not necessarily produced by genes alone. Phenotypic expression is influenced by nutrition. For example, diets alter cholesterol levels and types (LDL, HDL, and their ratios), homocysteine levels, and obesity (nutritional component), and these responses differ among individuals (genetic component). At the molecular level, variations in one DNA building block result in variations in gene structure. That variation, or SNP, can lead to variations in the protein structure after the gene or its variant is expressed.

Fig. 3 The balance between genetic and epigenetic factors. R. Jaenisch, A. Bird, Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals, Nat. Genet. Suppl. 33 (2003) 245-254.

Fig. 4 Genetic and Epigenetic Disturbances
Some structural variations in a protein may have an impact on its function, while some may not. Multiple genes may contain one or more SNPs, in distinctive patterns, associated with phenotypic patterns of nutritionally related health states, such as homocystenemia, high (or low) cholesterol, or variations in HDL and LDL cholesterol and their subunits. Patterns of multiple SNPs are called haplotypes.

The key element that distinguishes nutrigenetics from nutrition research is that the observable response to diet, or phenotype, is analyzed or compared in different individuals (or genotypes). Classical nutrition research essentially treats everyone as genetically identical, even while realizing that some individuals require more or less of specific nutrients. Molecular biology and biochemical approaches often assume the same: that an enzyme or flux through a metabolic or signal transduction pathway is the same in each individual. Similarly, the key element that distinguishes nutrigenetics from both molecular and genomics research is that nutrigenetics analyzes genetic expression in response to variations in diet. Molecular and genomic research often assumes that the environment does not influence genetic expression. Nutrigenetics combines these concepts. An individual enzyme, pathway, or collection of pathways will be unique to groups or each individual, depending on the variation (defined by SNPs, haplotypes, and other polymorphisms) inherited. The expression or activity of these variant forms of normal genes differs depending on the amount and type of food ingested and the interactions between the food and the specific genotype.

Understanding these interactions has significant implications: turning genes on or off or changing the abundance of certain proteins in response to different dietary chemicals may affect the balance between health and illness. Causes of chronic diseases, such as cardiovascular disease, cancer, or cognitive decline in aging, are not well understood because they are multifactorial in nature. While we have clues to some dietary and other environmental or lifestyle factors that appear to contribute to occurrence of those and other chronic diseases, effects are not consistent among individuals. Thus, clear cause/effect relationships are still emerging. Occurrences of chronic diseases are "encoded" by a combination of factors, all acting on the body over time to create the disease phenotype of variable severity. These factors may include a number of genes, common genetic variants (i.e., SNPs, haplotypes), environmental factors, risk-conferring behaviors, and socioeconomic status. The genetic factors contributing to complex disease are difficult to identify because they typically exert small effects over long periods of time. Moreover, other unrelated genes and environmental factors, such as diet and lifestyle, can modify the magnitude of their effect. This is nicely depicted by the health pendulum shown on Fig. 3.
The most commonly used approach for analysis of the contribution of genetic variations in response to diet or food components is the case-control approach, where the frequencies of genotypes are compared between groups of exposed individuals with different responses. More than 100 studies are published to date. However, their conclusions should be considered cautiously, having in mind the study of Hirschhorn et al. (5) on more than 600 association studies on multifactorial (chronic) diseases, showing that only six of the associations have been confirmed in more than three studies. The "failure" to identify single genes responsible for chronic diseases led to the common variant/common disease (CV/CD) hypothesis, which is largely responsible for the current genome-centric approach to the study of chronic diseases. This hypothesis, in its simplest form, is that combinations of naturally occurring gene variants (i.e., alleles of unlinked genes) rather than mutations produce any given chronic disease. This is exemplified by the number of chromosomal regions associating with obesity (Fig. 7), illustrating that susceptibility to this disease is multigenic. The link to nutrigenetics is that some of these naturally occurring gene variants will alter metabolism of nutrients, which in turn will alter the regulation genes involved in maintaining health or promoting disease.

Fig. 5 CHRONIC DISEASES MAY RESULT FROM DIFFERENT MOLECULAR PATHWAYS
- Almost 1700 genes fit the description of oncogene
- Different tumors may be initiated and/or promoted by one or more different oncogenes, each activating its own cascade of altered regulatory processes. Cancers of the same organ or cell type that appear to be morphologically and histologically similar may have unique molecular expression profiles. Glioblastoma provides one example. Data from high-density oligonucleotide arrays showed that EGFR positive tumors express 90 genes differently from EGFR negative tumors.
- Mutations in many different genes such as leptin, leptin receptor, agouti signaling peptide, attractin, insulin signaling protein, and carboxypeptidase-E cause obesity in mice and humans.

Fig. 6 NUTRITION x GENOME: ASSOCIATION APPROACH
- Case/control studies – variations in candidate genes are associated with certain response to diet
- More than 100 studies
- Limitations: sample sizes that lack appropriate statistical power, control groups that are not appropriately matched to cases, population stratification that occurs because of genetic admixtures among the study participants, and overinterpreting data (among others)
- Examples: Lipid, diet, smoking, sex, activity, alcohol vs. APOA1, APOA4, APOB, APOC3, APOE, APOH, LPL, CETP, LCAT, LDLR, HL, Cholesterol 7a-hydroxylase, Intestinal FABP, Neuropeptidase Y, M/N blood group, Alcohol dehydrogenase-3, Paraoxonase, Microsomal transfer protein

Fig. 7 More than 10 chromosomal loci associate with obesity.
Multiple effects of gene modifiers

On the other hand, a single environmental factor (e.g. food component) can influence the activity of several genes simultaneously (Fig. 8), i.e. diet or environment will also affect the expression and, in some cases the abundance, of the enzymes and proteins. Altering the concentrations of enzymes in the pathways will alter flux through pathways and ultimately the physiology of the organism. For example, in response to fasting and sugar-fed conditions mice liver reacts with global changes in gene expression as shown by high density microarrays:

- starvation response correlated with processes promoting anti-aging and longevity;
- most of these potentially beneficial changes were suppressed by sugar feeding;
- down-regulation of fatty acid synthesis and upregulation of fat breakdown to provide energy. Fat catabolism also entails activation of lipid signaling cascade, which provides protection from genotoxic side products. This group includes CYP450 species and lipid-activated nuclear receptors.

Global changes in gene expression in mouse liver in response to fasting and sugar-fed conditions using high density microarrays (Bauer et al., 2004):

- starvation response correlated with processes promoting anti-aging and longevity;
- most of these potentially beneficial changes were suppressed by sugar feeding.

Mutations in many different genes such as leptin, leptin receptor, agouti signaling peptide, attractin, insulin signaling protein, and carboxypeptidase-E cause obesity in mice and humans:

- Up-regulation of amino acid catabolism and urea cycle, since endogenous proteins are broken down during starvation to provide fuel and essential metabolites. The urea cycle regulates the nitrogen level and also helps prevent conditions where excess nitrogen and ammonia become toxic. In this regard, the up-regulation of the urea cycle can be seen as a protective reaction that functions as an antioxidant lipid signaling cascade. Since excess amino acids and proteins cannot be stored, the increase in the urea cycle can be brought about by two opposite physiological conditions, i.e. the absence and the excess, of dietary proteins, as for example in the high protein 'Atkins' diet.

- Significant transcriptional regulation of IGFs (insulin-like growth factors) and IGFBPs (IGF binding proteins). IGF1 is a major growth signaling molecule that is transcriptionally activated by insulin and GH under good nutrient conditions, thereby allowing cell growth and proliferation. Under starvation, these signals are absent so that IGF1 expression is strongly reduced, while its deactivating binding proteins IGFBP1 and IGFBP2 are up-regulated.
• Unexpected response to starvation in steroid metabolism. Because of the shortage of acetylCoA, expression of enzymes that catalyze de novo synthesis of cholesterol is strongly reduced. However, due to the breakdown of fat and cell membranes cholesterol is still available as a starting metabolite for steroid synthesis. Strikingly, most of the enzymes for synthesis of DHEA are up-regulated, whereas those catabolizing DHEA or converting it to other hormones are down-regulated.

The common study design errors in the case-control studies include small sample size, poorly matched control groups, population stratification, overinterpretation of data, and others. These methods and approaches are being improved to eliminate such errors and to reliably identify genes associated with complex phenotypes.

Several recent association studies on multifactorial diseases have included dietary variables in studies testing whether a single gene variant is associated with a complex phenotype:

• **Hypertension**
  A variant (designated AA) of the angiotensinogen (ANG) gene is linked with circulating ANG protein, which in turn, is associated with increased blood pressure. The Dietary Approaches to Stop Hypertension (DASH) diet positively affects individuals with the AA genotype, but the same diet was less effective in reducing blood pressure in individuals with a GG genotype. A large percentage (~60%) of African-Americans have the AA variant, with the remainder heterozygotic (AG) at this position (6).

• **Cardiovascular health**
  Apo-A1 plays a central role in lipid metabolism and coronary heart disease. G to A transition in the promoter of APOA1 gene is associated with increased HDL-cholesterol concentrations, but the results across studies are not consistent. Ordovas et al. (7) found that the A allele (or variant) was associated with decreased serum HDL levels. The genetic effect was reversed, however, in women who ate more polyunsaturated fatty acids (PUFA) relative to saturated fats (SF) and monounsaturated fats (MUFA). In men, this type-of-fat effect was significant when alcohol consumption and tobacco smoking were considered in the analyses. If confirmed by other studies, the APOA1 gene shows a classical gene–environment interaction. Such interactions may help explain why candidate gene studies show inconsistencies. Food intake therefore may alter susceptibility to diseases mediated by increased HDL-cholesterol levels.

• **Cancer**
  Methyleneetetrahydrofolate reductase (MTHFR) is a key gene in one-carbon metabolism and indirectly in all methylation reactions. Several laboratories have noted that the C667T polymorphism (ala to val), which reduces enzymatic activity, is inversely associated with occurrence of colorectal cancer. Dietary recalls were used to assess intake of folate, vitamin B-12, vitamin B-6, or methionine (and in one study, alcohol) in individuals with the CC or TT phenotypes. Low intakes of these vitamins were associated with increased risk for cancer among those with the MTHFR TT genotype. MTHFR variants are also implicated in cardiovascular disease.
Another application of nutrigenetics information is expected to be in treatment of enzyme deficiencies. Some genetic diseases in humans are caused by defective enzymes. A subset of these enzymes is altered by naturally occurring SNPs which increase the Michaelis constant, Km, of coenzyme for enzyme. Km is a biochemical measure of the affinity of coenzyme or substrate for enzyme - an increased Km results in decreased affinity. In certain cases, increasing the coenzyme concentration may ameliorate the decreased enzymatic activity.

The medical applications for such cases would be that if genetic tests were available for the variant gene and if that variant was shown to be the only cause of a disease process, a physician or nutritional expert could recommend increasing or decreasing intake of a specific vitamin or food. For example, increased dietary intake of nicotinic acid or nicotinamide might increase NADPH coenzyme concentrations enough to alter the equilibrium of GPDH ↔ GPDH–NADPH (Table 2). The same approach will not work for ALDH because the NAD substrate concentration could not be increased enough to overcome the increased Km caused by the substitution of lysine for glutamic acid at position 487. Elson-Schwab and Ames (8) have established a Web site (www.kmmutants.org) that summarizes nutritional information for a large number of coenzyme-containing enzymes.

### Table 2: Examples of enzyme sensitive to cofactors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cofactor</th>
<th>∆bp</th>
<th>∆AA</th>
<th>%f</th>
<th>Km&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td>FAD</td>
<td>C609T</td>
<td>P187S</td>
<td>-15</td>
<td>Increase</td>
</tr>
<tr>
<td>ALDH</td>
<td>NAD</td>
<td>-</td>
<td>E487K</td>
<td>-50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>150-fold increase</td>
</tr>
<tr>
<td>GPDH</td>
<td>NADP</td>
<td>C131G</td>
<td>A44G</td>
<td>11</td>
<td>5-fold increase&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>MTHFR - methylene tetrahydrofolate reductase; ALDH - aldehyde dehydrogenase; GPDH - glucose 6-phosphate dehydrogenase; ∆bp - change in base pair; ∆AA - change in AA; %f - percent of reference activity; Km - Michaelis constant

<sup>b</sup>Increased Km for cofactor - decreased affinity

<sup>c</sup>Heterozygote + homozygote

<sup>d</sup>May be aided by increased intake

### 3. Specific nutrients and foods for specific individuals or groups

Significant contributions from genomics, molecular biology, and nutritional disciplines have been made toward understanding the different components of complex, chronic disease phenotypes. However, a comprehensive, integrated picture still eludes us. This is specifically true for the effect of food on health and disease process.

Epidemiological studies repeatedly have demonstrated associations between diet and cardiovascular disease, cancer, and other chronic diseases. However, the specific cause/effect linkages between nutrient type and amount and health or disease phenotype are only beginning to emerge. The promise of nutrigenetics is that scientific research can deliver scientific evidence of health benefits of specific nutrients and foods for specific individuals or groups.

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. 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. 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 will be crucial if this endeavour is to succeed. So, although personalized diets would be an interesting application of nutrigenomics the implementation of such an approach lies far ahead of us and over the next 10 years the focus should be on understanding how nutrients interact with the genome at the molecular level.

The analogy of pharmacogenetics to nutrigenetics is readily evident. The goals of these areas are similar: customization of therapy, prevention and management of disease, and market...
segmentation based on personalized criteria. Through analysis of gene expression, SNPs, haplotypes, and biochemical and physiological results, scientists are verifying individual and group differential responses to diet.

When asked, 75–90% of consumers state that they make food choices with the intent of benefiting their or their family's health. **Health management and market segmentation through diet are possible, well established, and continue to grow.** Consumers seeking cholesterol management solutions have an array of foods available, including oatmeal, fat type and amount, carbohydrate type and amount, and stanols/sterols. Dairy products and soy for bone health, cancer, weight management, and cardiovascular health represent additional well established and emerging health segments. Dietary choices based on genetics are not new. Phenylketonuria and alcohol dehydrogenase deficiency are well-known conditions and can be avoided by avoiding consumption of phenylalanine and alcohol, respectively. Currently, food selection for health is based largely on generalized information, and, in some cases, more specific information derived from effects of diet on biomarkers such as bone density, cholesterol, serum triglycerides, or blood glucose, among others.

Epidemiological studies support the role of diet in health but have not revealed cause–effect linkages that are emerging through the combination of the previously discussed scientific disciplines. Each of those disciplines contributes to unique, yet interrelated understanding of chronic disease and the role of diet in phenotypic expression of wellness or disease. The role of fatty acid intake and metabolism in depression (DHA/EPA); obesity, colon cancer, heart disease (PPARs); and partitioning of energy into adipose or muscle tissue (CLA) are but a few examples of gene–diet interactions for which phenotype variability is being unraveled. Food clearly represents a nearly ideal channel through which to realize the benefits that nutrigenetics promises.

### 4. Regulatory, legal and ethical considerations

Knowledge resulting from the scientific combination that underpins nutrigenomics leads to a potential **change in the borderline between medicine and foods**. The distinction between those current definitions will be challenged with growing evidence of nutrient effects on disease processes at the cellular level and a role for nutrients in disease prevention and management. **Modern pharmaceuticals evolved from thousands of years of traditional lore concerning the uses of plants and herbs as medicines.**

Research efforts over the past 100 years have led to the widespread adoption of Paul Ehrlich’s Principles of (Chemo)therapy:

- Drugs need not be of natural origin and could be developed by planned chemical synthesis;
- Systematic exploration of structure/function relationship distinguishing therapeutic activity from toxicity is needed;
- Maximization of ratio of dose required to cure disease to that producing toxicity (broad therapeutic index) is needed;
- The importance of developing animal models of diseases for quantitative measurements of both therapeutic potency and toxicity is needed.

Highly sophisticated methods are now used to identify, characterize, and test potential drugs for effectiveness in humans to meet the criteria of these principles. However, the growing interest, acceptance, and use by the public of dietary supplements, not to mention herbal medicines, has outpaced the scientific, medical, and food industry’s ability and capacity to carefully analyze the chemicals, their combined and independent activities, and their effectiveness and safety.

**Nutrigenomics, by definition, will require clinical validation of effects, including safety, in the target market segment.** Clearly there is an opportunity and a growing need for consideration of a regulatory framework that will accommodate emerging science as well as deliver consumer benefits and afford consumer protection. While scientists suspected that food, like drugs, had cellular-level effects, the extent of that truth could not be supported with scientific evidence. Today, the proof is here and is growing. It is not suggested that food be regulated as drugs. The suggestion is that thoughtful consideration be given to heretofore unexpected effects of nutrients and foods on health. Such consideration, if managed with foresight, ideally would support research to identify genes, adjunct diagnostic tests, and foods that would afford opportunity for optimal consumer health and wellness.

At the consumer level, nutrigenomics will first be encountered as diagnostic testing for genetic patterns of SNPs, coupled with food products or supplements, and diagnostic monitoring of biomarkers that will track genetic response to diet. **Consumer counseling will be essential to**
translate the meaning and recommended actions suggested by one’s genetic profile. Successful incorporation of any food into an individual’s diet will depend completely on whether the food fits an existing dietary pattern and has excellent sensory properties. In other words, like many other health trends in the food industry, nutrigenomics will thrive and deliver the benefits inherent in the concept only if the products deliver consumer benefits and satisfy consumer preferences. Questions of ethics, privacy, compliance, insurance reimbursement, value creation and capture, and economic return, and, as noted above, the need for additional physiological and biochemical studies to identify and validate effects of dietary modification on phenotypic expression must be resolved. The science from various disciplines that constitute nutrigenomics continues to emerge and is being integrated into useful information on which the food industry can act. Clearly, this is not the food industry as is has been in the past. Alliances, partnerships, or consortia among varied commercial partners will be essential to deliver on the scientific and commercial potential of nutrigenomics.

5. Evolution of genomics versus food patterns

Our evolution as human beings took millions of years, whereas the human civilization exists from thousands of years. The evolution of human genome has been shaped in favor of survival under limited food/calories intake, which resulted in formation of "thrifty" genotype. The selective advantage of this genotype is in putting together metabolic network aiming at survival with minimal food/calories intake and storage of the chemical energy exceeding this minimal requirement. However, with the radical changes in our diet and physical activity patterns in industrial societies (the major hallmarks being excess intake of food and restricted energy expenditure) during the last “seconds” of our human history, the "thrifty" genotype is turning more and more into "susceptibility" genotype. Recently, the WHO (2002) predicted significant growth during the next two decades in the prevalence of diseases resulting from this bio-social conflict, such as type 2 diabetes, obesity, atherosclerosis, etc.

---

Fig. 11

![Evolution of genomics versus food patterns](image-url)
Exposing our "thrifty" genes to a new diet, characterized by much higher calory intake is very well demonstrated with the story of the Aboriginal Canadians Ojie-Cree (Fig. 12). Population of Ojie-Cree migrated from their natural habitat at the Sandy Lake to Toronto area, Ontario, about 1980's. This population showed a high rate of coronary heart disease some two decades later, which occurred to be about three times higher than the one of the All Ontario population, as well as to the rate among Ojie-Crees inhabiting their original settlements. When one of the genes belonging to the "thrifty" genotype was studied (HNF1A) it has been found that the Ojie-Crees living in both Sandy Lake area and Toronto area are frequent carriers of a mutation (S 319), which is exotic among the non-Aboriginal Canadians. Apparently, the high frequency of this mutation among the Aboriginal Canadians is a result from selection in favor of survival advantages. However, the exposure of carriers to drastically changed environment (sedentary life style, high saturated fatty acids diet, etc.) in their new settlement is converting the "thrifty" into "susceptibility" genotype.

Fig. 12 The concept of "thrifty" genotype. Increased incidence of CHD among carriers of a "thrifty" gene exposed to rapid changes in their environment.

Similarly, Native Americans moving from Mexico to Texas, US, have several times higher risk to develop obesity and type 2 diabetes, when compared with their relatives inhabiting Mexican mountains.

6. Concluding remarks

Nutrigenomics is an integrative science, which seeks to provide a genetic and molecular understanding for how common dietary chemicals affect the balance between health and disease by altering the expression and/or structure of an individual’s genetic makeup. The conceptual basis for this new branch of genomic research can best be summarized by the following

Five tenets of nutrigenomics:

- Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases;
- Common dietary chemicals can act on the human genome, either directly or indirectly, to alter gene expression or structure;
- The degree to which diet influences the balance between healthy and disease states may depend on an individual’s genetic makeup;
- Some diet-regulated genes (and their normal, common variants) are likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases;
• Dietary intervention based on knowledge of nutritional requirement, nutritional status, and genotype (i.e., "personalized nutrition") can be used to prevent, mitigate or cure chronic disease.

The sum total of molecular studies shows that diet and the chemicals in diet, influence physiological processes. This is achieved by altering the expression (or structure) of a subset of genes in the human genome. Dietary chemicals have been shown to alter gene expression in a number of ways. For example, they may:

• act as ligands for transcription factor receptors;
• be metabolized by primary or secondary metabolic pathways thereby altering concentrations of substrates or intermediates or
• serve as signaling molecules.

The ability of cells to adapt to environmental change by regulation of gene expression is essential for organism survival. Organisms vary their gene expression in the absence or presence of nutrients by increasing and decreasing production of cellular proteins necessary for life sustaining function. Ultimately, the science of nutrigenomics promises to offer the health practitioner greater knowledge, enabling them to predict potential genetic responses to nutritional intake and to target and modify associated behavior. A major step will be to establish biomarkers needed to quantify a positive biological response to nutrient intake. This will be a valuable step that differentiates nutrigenomics from the general public health messages of the early 21st century, which led to punitive dietary restrictions unrelated to individual health outcomes. Once verifiable protocols, based on genomic biomarkers are established, nutrigenomics will revolutionize health care leading to the reduction of individual health risk.

7. Glossary

Acute-phase response
The early and immediate set of homeostatic control reactions that are induced during inflammation.

Allele
One of the variant forms of a gene at a particular location on a chromosome. Different alleles produce variation in inherited characteristics such as hair color or blood type. In an individual, one form of the allele (the dominant one) may be expressed more than another form (the recessive one).

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

Comparative genomics
A new field of biological research in which the genome sequences of different species: human, mouse and a wide variety of other organisms from yeast to chimpanzees are compared. By comparing the finished reference sequence of the human genome with genomes of other organisms, researchers can identify regions of similarity and difference. This information can help scientists better understand the structure and function of human genes and thereby develop new strategies to combat human disease. Comparative genomics also provides a powerful tool for studying evolutionary changes among organisms, helping to identify genes that are conserved among species, as well as genes that give each organism its unique characteristics.

Gene expression
Process by which genes are activated to make proteins that in turn carry out a range of functions within the body.

Genotype
The genetic identity of an individual that does not show as outward characteristics.

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 β-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.

Ligands
Atom, molecule, group or ion that is bound to a central atom of a molecule, forming a complex.

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.

Microarray Technology
A new way of studying how large numbers of genes interact with each other and how a cell’s regulatory networks control vast batteries of genes simultaneously. The method uses a robot to precisely apply tiny droplets containing functional DNA to glass slides. Researchers then attach fluorescent labels to DNA from the cell they are studying. The labeled probes are allowed to bind to complementary DNA strands on the slides. The slides are put into a scanning microscope that can measure the brightness of each fluorescent dot; brightness reveals how much of a specific DNA fragment is present, an indicator of how active it is.

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.

Phenotype
The observable traits or characteristics of an organism, for example hair color, weight, or the presence or absence of a disease. Phenotypic traits are not necessarily genetic.

Polymorphism
A common variation in the sequence of DNA among individuals. Single nucleotide polymorphism (SNP) is common, but minute, variation that occurs in human DNA at a frequency of one every 1,000 bases. These variations can be used to track inheritance in families. SNP is pronounced “snip”.

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.

Transcription factors
Bind to specific DNA sequences in the promoter region of specific genes, thereby either enhancing or suppressing gene expression.

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 signaling pathway (superrepressor) by competing with the endogenous protein.

More at http://www.genomicglossaries.com/content/Basic_Genetic_Glossaries.asp

References