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Individualized nutritional recommendations: do we have the measurements needed to assess risk and make dietary recommendations?

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Is the information currently available to adjust nutritional recommendations and develop individualized nutrition? No. There is not even the information needed for setting dietary recommendations with confidence now at the group level. Will it be available soon? The answer to this question depends on the drive and will of the nutritional community, the success in recruiting funding to the area, the education of nutritionists and the spawning of great ideas and approaches. The emerging tools of genomics, proteomics and metabolomics are enabling the in-depth study of relationships between diet, genetics and metabolism. The advent of technologies can be compared with the discovery of the microscope and the new dimensions of scientific visualization enabled by that discovery. Nutritionists stand at the crest of new waves of data that can be generated, and new methods for their digestion will be required. To date, the study of dietary requirements has been based largely on a black box approach. Subjects are supplemented or depleted and clinical outcomes are observed. Few recommendations are based on metabolic outcomes. Metabolomics and nutrigenomics promise tools with which recommendations can be refined to meet individual requirements and the potential of individualized nutrition can be explored. As yet, these tools are not being widely applied in nutritional research and are rarely being applied by nutritionists. The result is often interesting research that is frequently nutritionally flawed, resulting in inappropriate conclusions. Nutritional education is needed to put nutritionists at the forefront of the development of applications for these technologies, creating a generation of nutrigenomicists. A new generation of nutritionists should be working interdisciplinarily with geneticists, molecular biologists and bioinformaticians in the development of research strategies. The present paper reviews the current status of nutrigenomic research, the current controversies and limitations, and developments needed to advance nutrigenomics and explore fully the promise of individualized nutritional recommendations.

Dietary recommendations: Metabolomics: Nutrigenomics: Individualized nutrition

A view of the future

The dream of improving individual health through tailored nutritional recommendations has been well described in the *New York Times* in May 2003: 'A trip to the diet doc, circa 2013. You prick your finger, draw a little blood and send it, along with a $100 fee, to a consumer genomics lab in California. There, it's passed through a mass spectrometer, where its proteins are analyzed. It is cross-referenced with your DNA profile. A few days later, you get an email message with your recommended diet for the next four weeks. It doesn't look too bad: lots of salmon, spinach, selenium supplements, bread with olive

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oil. Unsure of just how lucky you ought to feel, you call up a few friends to see what their diets look like. There are plenty of quirks. A Greek co-worker is getting clams, crab, liver and tofu – a bounty of B vitamins to raise her co-enzyme levels. A friend in Chicago, a second-generation Zambian, has been prescribed popcorn, kale, peaches in their own juice and club soda. (This looks a lot like the hypertension-reducing 'Dash' diet, which doesn't work for everyone but apparently works for him.) He is allowed some chicken, prepared in a saltless marinade, hold the open flame – and he gets extra vitamin D because there's not enough sunshine for him at his latitude. (His brother's diet, interesting enough, is a fair bit different.) Your boss, who seems to have won some sort of genetic lottery, gets to eat plenty of peanut butter, red meat and boutique cheeses . . .

Nobody is eating exactly what you are. Your diet is uniquely tailored. It is determined by the specific demands of your genetic signature, and it perfectly balances your micronutrient and macronutrient needs. Sick days become a foggy memory. (Foggy memory itself is now treated with extracts of ginkgo biloba and a cocktail of omega-3 fatty acids.) . . . Your cholesterol does not react much to diet so you can eat bacon sandwiches and don't need to spend money on vitamin supplements that aren't doing anything for you . . . You willingly take only the vitamins you need in precisely the right doses, which will postpone the onset of disease to which you are naturally susceptible' (Grierson, 2003; reproduced with permission). This position is the promise of nutritional genomics.

How is individualized nutrition obtained and why the sudden expectations about this? The publication of the human genome heralded a new era of understanding of how genetic profiles influence disease risk. The human genome project has provided extensive information on genes of man, along with those of other species. It has been humbling to recognize how little unique genetic material man has compared with other living beings and the new knowledge that there are only about 30 000 genes; much fewer than anticipated (Pennisi, 2003). The rapid success of the sequencing of the genome also demonstrated the limitations of knowing genes without knowing function. Thus, genomics, the study of the entire genome, moved towards transcriptomics (see Mathers, 2004), the study of mRNA in order to understand which genes are being transcribed. This approach presented new challenges. DNA sampling, with the use of either blood or buccal cell swabs had become very easy. The collection of mRNA, however, is much more challenging, especially in human studies. Furthermore, transcription of genes still does not ensure the activity of their product. The examination of proteins comes a step closer to the activity of the gene. The field of proteomics was born and buoyed by the technologies (described later) that developed, which allowed measurement of all the proteins in an organism.

Nutrinomics includes genomic, proteomic, transcriptomic, metabolomic and metabonomic measurements (see Mathers, 2004). Its promise lies in the study and understanding of how genetic polymorphisms affect requirements, how nutrition influences genetic expression and subsequently impacts metabolic pathways, and how regulation is disturbed in diet-related disease (Mueller & Kerston, 2003).

The emerging tools of genomics, proteomics and metabolomics are enabling the study of the relationships between diet, genetics and metabolism in entirely different depth. These technologies can be compared with the discovery of the microscope and the new, somewhat overwhelming, dimensions of scientific visualization that opened. It has created a need for bioinformaticians to decipher new signals. Nutritionists stand at the crest of new waves of data that can be generated rapidly with high-throughput technologies and that require new methods for their digestion.

Given the current status, there is scepticism about moving forward and whether public health can be improved with such tailored recommendations even if the data are gathered and processed. The high costs of individualized assessment and the low level of motivation by individuals to adhere to a tailored diet are the reasons for the question of whether this approach will become the luxury of an elite few who can afford testing and have the education and the will to become intensely involved in their health maintenance in the long term. Alternatively, will nutritional science find its power to prevent disease enhanced tremendously by knowing genetic profiles and using the tools spun off by the genomic revolution?

Nutrigenetics *v.* nutrigenomics

Nutrigenetics addresses the importance of genotype on the risk of nutritionally-related disease. It starts from the assumption that certain genetic polymorphisms measurably alter nutritional deficiency. In nutrigenetics genetic polymorphisms are identified and studied to see if they modulate the relationships between nutritional exposure and risk. The inverse relationship is addressed by nutrigenomics, which focuses on the effect of nutrition or food-borne components on gene transcription, proteomics and metabolism. The premise in this case is that diet influences disease through mechanisms regulating genetic expression. Nutrigenomics is more difficult to utilize in nutritional research than nutrigenetics. It challenges human nutritional research to carefully design studies that control superfluous influences, to gather samples that are reflective of the target tissue, to do so on a critically-important timeline of responsiveness to the nutritional impact, to conduct highly-sensitive measurements with internal and external validity and reproducibly, to be able to handle and interpret tremendous amounts of data generated from single samples at single time points in single individuals and to combine those results to determine patterns that are interpretable. This approach is a systems biological approach (i.e. the study of the complete biological system of a tissue, cell or organism, using the complement of tools from genomics to metabolomics) that attempts to integrate the measures across the multiple 'omics' for genes, proteins and metabolites to allow understanding and robust conclusions.

Nutrigenetics addresses the questions of gene–mutant interactions relating to whether polymorphisms matter, whether there is compensation for alterations of single genes in a pathway or compensation in the expression of

other genes. A major concern here, as with biomarker-based studies, is how it can be ensured that the gene under study is the pivotal one and not just linked to the causal gene.

Nutrigenetics and recommended intakes

Nutrigenetics may lead to the identification of individuals who are less efficient in specific metabolic pathways and to the recommendation of greater intakes. The evidence of gene–nutrient interactions was first identified with monogenetic inborn errors of metabolism such as phenylketonuria, which is a model of the ability to turn such knowledge into prevention of disease. In phenylketonuria, to prevent mental retardation, infants are screened at birth and, where necessary, dietary exposure to phenylalanine is restricted (National Institutes of Health Consensus Development Panel, 2001). Subtler effects of dietary needs of individuals have been discussed at length elsewhere. They include the understanding of the relationship between various apoE genotypes 2/2, 2/3 and 4/2 to dietary responsiveness to the fat in the diet, the relationships between folic acid needs and polymorphisms of methylenetetrahydrofolate reductase and data suggesting that salt sensitivity is genospecific, and in the aetiology of carcinogenesis genotypes of glutathione *S*-transferase, and *N*-acetyltransferase may modulate diet-related cancer risk (for example, see Frosst *et al*. 1995; Campos *et al*. 2002).

Currently-recommended intakes are intended to cover the needs of 95% of healthy populations, and the neglected 5%, as well as others with health conditions. Subgroups with greater needs may be identifiable and addressed directly through nutrigenetics. An example of where the data are currently accumulating in a way that might justify polymorphism-based recommendations is in individuals with specific mutations of the methylenetetrahydrofolate reductase gene who appear to have a greater need for folic acid. Nutrigenetics could identify homozygotes and heterozygotes at risk of undernutrition and ensure that all individuals receive the greater intake (Cortese & Motti, 2001).

Nutrigenomics

Nutrigenomics is that area of science that employs the high-throughput technologies developed for genomic research to assess the entire set of responses to dietary exposures at the time the sample was taken. Nutrients are dietary signals that influence gene and protein expression and metabolic production, working as 'dietary signatures' (Mueller & Kersten, 2003). Nutrients such as Se can influence gene expression directly through transcription factors, such as nuclear hormone receptors (see Table 1). These receptors bind retinoic acid, fatty acids, vitamin D and other fat-soluble food components. *In vitro* research has led to understanding the effect of many nutrients on transcription factors and this knowledge base is expected to grow. Nutrigenomics also includes proteomic and metabolomic responses as measurable indicators of dietary effect. To date, nutritional research stems largely from transgenic and knock-out mouse models, and *in vitro* experiments using inducible expression systems, trans-dominant negative adenoviral constructs and RNA interference (Mueller & Kersten, 2003).

Table 1. Examples of modulation of gene expression by selenium in breast cancer cells (from Dong *et al.* 2002)

Cell cycle regulatory genes	Signalling molecules	Apoptosis regulatory genes
Cyclin B1	ERK1	Apo-3
cdc25C	mm-1	C-jun
PLK1	MAPK1	Cdk5
KKIALRE	JNK2	Cyclin D1
Activator 1	cdc42	bcl2A1
	AKT2	PIG 12

The challenge will be to develop useful ways of incorporating metabonomics into *in vivo* human research. Human metabolic profiles change from hour to hour and from day to day, and are influenced by factors other than diet, including lifestyle (physical activity, cigarette smoking, environmental exposures and medication use). Determining the relationship between mRNA and protein levels depends totally on when they are measured. As illustrated in Fig. 1, the ratio of the two variables changes radically at each of the three given time points, as the transcription ends and the protein synthesis continues to rise. Strategies for responding to this disparity include multiple measures at various time points, choosing the peaks for each variable or integrating measures over time and calculating the area under the curve.

Apparent major obstacles to the use of metabolomics in human research are access to the right tissue, measurement of metabolites at the right time, measurement variability within individuals and between runs and laboratories. Another issue is that the same metabolic profile may be good for some diseases and bad for others. There is a

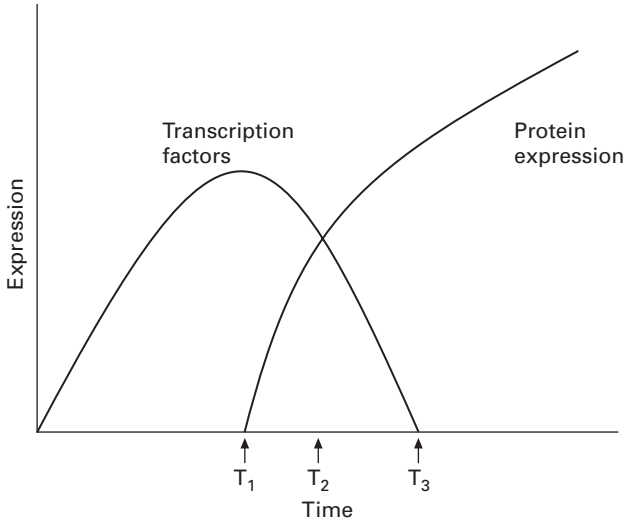

Fig. 1. Time cause and relationship between levels of transcription and protein expression.

danger of misinterpreting metabolic data; the butyrate paradox is one of the examples, in that butyrate shows cell stimulation in normal colonic epithelium but induces apoptosis in transformed cells (National Cancer Institute, 2003).

There are a number of issues critical to the experimental designs that apply these methods. Dietary effects are often weak, and chronic exposure may be most important; therefore, short-term feeding studies may not suffice and, for some effects, hypothetical long-term studies will be necessary. Also, as food intake is complex, studying the effect of single substances in isolation may be misleading. Many nutritionally-related diseases are associated with multiple genes, and as yet their multidimensional interactions cannot be measured.

At present, these tools are not being widely applied in nutritional research and are rarely applied by nutritionists. The result of this situation is interesting, often nutritionally -flawed research that may result in inappropriate conclusions. The May 2002 National Cancer Institute-sponsored meeting on nutritional genomics and proteomics in cancer prevention highlighted numerous experiments on knock-out mice and responses to specific nutrients in tissue culture, but showcased few human studies. The need for interdisciplinary teaching and research has become evident.

Proteomics

Proteomics is an exciting research front, and valuable breakthroughs are being made in the area of diagnostics and therapeutics. These breakthroughs include two-dimensional gel differential display, high-resolution MS, such as the QSTAR, which can yield 900 000 data points per sample (as compared with previous levels of 15 000 points) and protein microarrays, including reverse-phase protein arrays along with surface-enhanced laser–adsorption ionization chip processes in combination with MS. Intelligence-based bioinformatic tools that involve supervised and unsupervised learning systems to develop algorithms and to obtain a proteomic signature are essential to the application of these methods. Challenges in the general application of protein microarrays for proteomic research include the complication of measuring proteins in the same medium, with log orders of magnitude between the most-abundant and least-abundant proteins. Another challenge derives from the fact that proteins cannot be amplified and, therefore, must be tagged in some way.

The best examples of proteomic advances to date are new proteomic-based diagnostic approaches to the identification of difficult diseases, such as early-stage ovarian cancer. Based on the concept that perfusion of cancerous tissues will result in abnormal protein profiles in the blood, and that the identification of the specific proteins is not necessary as long as patterns are recognizable, proteomic spectra (generated by MS, using surface-enhanced laser desorption and ionization) of serum of women with and without ovarian cancer have provided information for learning algorithms that have successfully discriminated between diseased and non-diseased women with 100% sensitivity and 100% specificity (Petricoin *et al*. 2002).

Two major issues thwart the use of proteomics in a nutritional context. One is the importance of the site of the protein in the interpretation of the importance of protein differences. Direct measurement of adrenal, or liver, or target tissue proteins is desirable for many lines of research, but is clearly not feasible for human subjects. In addition, protein chemistry is extraordinarily difficult and high-throughput approaches for studying multiple proteins are limited. As blood and urine are easily accessible as possible media, and the technology is available for studying small-molecular-weight substances, metabonomics has become the champion of the '-omics'. Metabonomics is the study of the entire set of metabolites (small-molecular-weight substances) of an organism, as contrasted with metabolomics, the study of the set of metabolites in a tissue or organ.

Current requirements

The study of dietary requirements has been based largely on black box measures; outcomes of clinical relevance, rather than metabolic end points. Typically, subjects are experimentally supplemented or depleted and monitored to see what happens. Clinical outcomes at various levels of intake of a diet that is complete in all other nutrients except the one under study are monitored for the levels that trigger measurable or visible responses. When information of this nature is lacking, which is often for many outcomes, or is available but only in subpopulations that are not reflective of the population at large, recommendations are founded on information on intakes in seemingly healthy populations, as is the case with adequate intake levels as defined by the National Academy of Sciences. In the case of upper tolerable limits, most recommendations are based on animal experiments and adjusted with safety factors (Institute of Medicine Food and Nutrition Board, 2000). To individualize nutritional recommendations will increase the complexity and require similar information on subgroups with different genetic profiles and the information on the effects of combinations of polymorphisms with different diets on multiple outcomes.

This information is available for very few nutrients and only in relation to single genes. The information needed to individualize recommendations is largely unavailable for most nutrients. In fact, anyone who has been involved in the development of dietary recommendations, such as the dietary reference intakes of the National Academy of Sciences in the USA, can attest to the fact that the data for setting exact recommendations for intakes of groups are severely limited. This situation applies to estimates of the levels needed to keep half a population adequately nourished (estimated average requirement), as well as the levels that are safe (upper tolerable limits) for most of the population most of the time.

It would, however, be wrong to assume that there is currently no individualization of requirements; recommended intakes differ depending on physiological state (age, pregnancy, lactation) and genetics (gender). Most recently, they are also adjusted for lifestyle (cigarette smoking and vitamin C). However, going beyond this position suggests that other genetic factors will play a part

in recommendations and at least one, probably dozens, of genetic polymorphism states of multiple genes in each individual and their physiological responses will be involved in future generations of recommendations. It remains doubtful that recommendations will be based on metabolomics alone.

Few dietary recommendations are based on metabolic markers for a number of reasons, ranging from the lack of confidence in these factors as outcome markers, their unproven linkage to the health outcome of interest and their variability both within and between individuals. The extensive information available, for example, from the superb studies conducted at the National Institutes of Health on ascorbic acid defines clearly the intake levels associated with leucocyte saturation (Levine *et al*. 1996). However, the National Academy of Sciences committee involved in developing the estimated average requirement for ascorbic acid did not find saturation a strong enough outcome to be the basis for recommended intakes for vitamin C (Institute of Medicine Food and Nutrition Board, 2000). Thus, the use of metabolomic outcomes as a primary basis for determining individual recommendations is of questionable utility. The need to couple these measures with outcome data will remain.

What do nutritionists need to learn?

The next generation of nutritionists will need enough genetics to understand terminology, enough metabolism to understand the pathways, enough molecular biology to understand how nutrients work at the cellular level, enough statistics to appreciate informatic challenges and to understand the strategies, and enough laboratory experience to appreciate measurement error in the methods used. They will require enough epidemiology to design studies in appropriate populations with adequate power and robust measures, where the noise does not dominate over any signals coming from the data, and to ensure internal and external generalizability of studies. They will need to know the pathways of interest, all the genes that might be involved, the compensatory mechanisms in place and the frequencies of alleles.

A set of growing resources on genes and metabolic pathways, such as those listed in Table 2, provides support. These resources include websites such as the Human Genic Bi-Allelic Sequences database (see Table 2), which provides sequence variations and their physical relationship to the gene for over 22 000 single nucleotide polymorphisms, the Genbank accession number, location of polymorphism and population allele frequencies. The Kyoto Encyclopedia of Genes and Genomes for Pathways is another resource of the Genome Net (see Table 2) for understanding cellular function from genome information. This resource integrates information about genes and proteins with molecular pathways in the cell.

Nutritional education needs to evolve to put nutritionists at the forefront of the development of applications for these technologies, creating a generation of nutrigenomicists capable of working interdisciplinarily with geneticists, molecular biologists and bioinformaticians.

Table 2. A sampling of website resources to identify genes

Human Genic Bi-Allelic Sequences http://hgbase.interactiva.de/
Genome Net http://www.genome.ad.jp/kegg/kegg2.html
Environmental Genome Project http://egp.gs.washington.edu/
National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov/
Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html
National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov/Omim/
The Cancer Genome Anatomy Project http://cgap.nci.nih.gov/
The SNP Consortium Ltd http://snp.cshl.org/
UNC–CH Center for Bioinformatics http://bioinformatics.unc.edu/bioinformatics

Conclusions

The Human Genome Project and its offspring have provided enormous resources and tools that promise to enhance the depth of understanding about gene–nutrient interactions.

The risk of human disease has long been recognized to be the result of interaction between genetic susceptibility and environmental risk factors, in the longstanding nature *v*. nurture arguments. Now the ability to quantify effects and examine pathways in exquisite detail is becoming possible. In order to utilize this potential, education of nutritional professionals who can direct the research to areas relevant to public health is needed and possibly new subspecialities developed.

It is my hope that for this area of nutritional research there will be hypothesis-driven research on nutritional pathways in the study of the genetic input and effects of diet or environment on the metabolic cascade. This research should address the differences between individuals under controlled conditions and the effects within individuals as diet changes. The study of specific gene and specific metabolite changes in well-controlled feeding studies will help to isolate relevant dietary factors and vulnerable populations.

Nutrigenetic research presents a number of challenges. The pursuit of nutrigenetics requires relatively frequent genes to allow the detection of effect in the populations studied, a relatively strong effect, the fortune or design to study the effective dose and consideration of the polygenetic nature of most nutritionally-related diseases. For the epidemiologist the challenge is to identify the genes that may be of central importance, identify whether they are causal or just correlated through linkage disequilibria, measure them and design studies with enough power to test clearly whether the genotype impacts disease risk. To meet this challenge, the level of nutrient at which an effect is evident needs to be known. According to experience to date in gene–nutrient interactions, the relationship suggests less of a dose–response effect than that of a plateau effect. The greatest differences appear to be among deficiencies not among individuals at the higher intakes. Most of the interaction that takes place is in the low dietary exposures, as is the case with methylenetetrahydrofolate reductase and folic acid requirements.

The flaw with the image of the future drawn by Grierson (2003) in the introduction to the present paper is that it suggests that alterations of diet in adulthood may not affect the risk of chronic diseases. In fact, the more realistic follow-through of this scenario would be parents testing their infants at birth for their nutrigenetic profiles, and determining at that time the most ideal diet (according to current opinion) for the child in order to intervene before the fetal origins of disease can develop into the later decade realities and so that dietary damage cannot accumulate. It will require confidence that the proposed dietary recommendations and avoidances do not interfere with health in other ways, from fertility to asthma, from brain development to infectious disease risk and performance in other areas. The impact of individual foods and nutrients on all relevant time periods in development is not yet understood. However, concerted research in this area will present opportunities for nutritional scientists to refine recommendations.

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