Genomics, Proteomics, and Metabolomics in Nutraceuticals and Functional Foods

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Dedication

We dedicate this book to our beloved Teacher, Professor Amareshwar Chatterjee, Ph.D.

Debasis and Manashi Bagchi

I dedicate this book to my family, with love and gratitude.

Francis C. Lau

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Preface

The relationship between food and health was well established nearly two and a half millennia ago as indicated by Hippocrates, who famously proclaimed "Let food be thy medicine and medicine be thy food." Hippocrates, regarded as the Father of Medicine, also prophesied the importance of individualized nutrition that "if we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health." These immortal words became the tenet for nutrition science. Indeed, more than 2000 years later, Thomas Edison concurred with Hippocrates by stating that "the doctor of the future will no longer treat the human frame with drugs, but rather will cure and prevent disease with nutrition."

In this regard, functional foods and nutraceuticals have received considerable interest in the past decade largely due to increasing consumer awareness of the health benefits associated with food. A functional food is a foodstuff that provides health or medical benefits beyond the basic nutrients it contains. When a functional food facilitates the prevention or treatment of certain diseases or disorders, it is a nutraceutical. The founder of the Foundation for Innovation in Medicine, Dr. DeFelice, coined the term "nutraceutical," which combines the words nutrition and pharmaceutical emphasizing its therapeutic properties. Functional foods and nutraceuticals are often used interchangeably because a functional food to one consumer may serve as a nutraceutical to another.

Consumers in the past were mainly reactive to existing health problems, but today they are more proactive and increasingly interested in the health benefits of functional foods in the prevention of illness and chronic conditions. This combined with an aging population that focuses not only on longevity but also quality of life has created a market for functional foods and nutraceuticals. The global nutraceuticals market is defined as the cumulative sales of nutraceutical foods, beverages, and supplements fortified with bioactive ingredients. This market was worth $117.3 billion in 2007 and it is projected to increase to $176.7 billion in 2013.

It is estimated that the functional food and nutraceutical market is growing at a rate surpassing the traditional processed food market. As this market expands, so will the demand for the identification of new bioactive food ingredients and the discovery of beneficial components in existing foods. The completion of the Human Genome Project and the advances in genomics technologies have revolutionized the field of nutrition research. Nutritional genomics or

nutrigenomics provides the means for a high-throughput platform for simultaneously evaluating the expression of thousands of genes at the mRNA (transcriptomics), protein (proteomics), and metabolites (metabolomics) levels.

This book is divided into five main sections starting with a brief introductory section (chapters 1 and 2) followed by the second section (chapters 3–12), which covers recent advances in nutrigenomics. The third section (chapters 13–16) focuses on nutriproteomics and the fourth section (chapters 17-19) examines nutrimetabolomics. The final section summarizes the application of nutrigenomics to the development of phytomedicine (chapter 20) and to the prevention of cancer (chapter 21). The intent of this book is to bring together current advances and comprehensive reviews of nutritional genomics by a panel of experts from around the globe, with emphasis on the nutrigenomics approach to functional foods and nutraceuticals.

Debasis Bagchi
Francis C. Lau
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Section 1

Introduction

1
Recent Advances in Nutraceuticals and Functional Foods

Francis C. Lau, Debasis Bagchi, Shirley Zafra-Stone, and Manashi Bagchi

INTRODUCTION

Postgenomic advances have revolutionized nutrition research. Traditional nutrition science focused on the investigation of nutrient deficiencies and impairment of health. In the past few years, an emerging discipline of nutrition research, functional genomics, has provided new approaches and techniques to elucidate how nutrients modulate gene expression, protein synthesis, and metabolism [1]. It has become apparent that nutrients not only fuel our life but also participate in gene regulation [2]. Functional genomics as applied to nutrition research includes nutrigenomics and nutrigenetics. Whereas nutrigenomics investigates the impact of nutrients on gene regulation, nutrigenetics studies the effect of genetic variations on individual differences in response to specific food components [3].

Nutrigenomics and nutrigenetics offer the promise to unravel complex interactions among genes, gene products, genetic polymorphisms, and functional food components [4]. The ultimate goal is to devise strategies for personalized nutrition and dietary recommendations aiming at improving human health [5]. Indeed, nutrigenomic approaches based on ethnopharmacology and phytotherapy concepts have demonstrated the interaction of nutrients and botanicals with the genome to cause significant changes in gene expression [6]. This observation has resulted in the commercial launch of nutraceuticals and functional foods that can regulate health effects of individual genetic profiles [7].

Nutraceuticals or functional foods are bioactive food components that provide medical or health benefits [8]. In addition to essential nutrients such as carbohydrates, proteins, fatty acids, minerals, and vitamins, there are various nonessential bioactive food components capable of modulating cellular processes. These nutraceuticals or functional foods contribute to the prevention of diseases such as cancer, cardiovascular disease (CVD), obesity, and type II diabetes [9]. For instance, obesity and CVD are multifactorial diseases influenced by a number of environmental and genetic factors [10]. Nutrigenomics approach has begun to reveal that obesity and CVD may be susceptible to dietary interventions and these interventions may modulate the onset and progression of the disorders [9]. In the case of CVD, there is evidence for interactions between dietary fat and three common polymorphisms in the apolipoprotein (apo) E, apoAI, and peroxisome proliferator-activated receptor-gamma (PPARγ) genes [11]. Therefore, a clear understanding of how these genes affect the response of individuals to certain nutrients should facilitate the progression of personalized nutrition for people with high propensity for CVD.

Although the traditional Food Guide Pyramid by the U.S. Department of Agriculture (USDA) was based on the estimated average nutrient requirements for the U.S. population as a whole, USDA has recently updated the Food Guide Pyramid to MyPyramid, focusing on individual

nutrition needs [12]. The 2005 Dietary Guidelines for Americans published by USDA emphasizes on meeting adequate nutrients within calorie needs [13]. The guidelines further provide the Dietary Approaches to Stop Hypertension (DASH) Eating Plan designed to integrate dietary recommendations into a healthy way of eating [13]. It is foreseeable that nutritional recommendations will be subpopulation-based with variations according to ethnicity, age, gender, disease susceptibility, and genetic polymorphisms [14].

NUTRITIONAL GENOMICS

In the past, nutrition research was conducted mainly to retrace the importance of a nutrient through its deficiency, which manifested as health-related problems. The notion of the interrelationship between diet and health was firmly established. However, it was only until recently that nutrition research evolved to focus on the direct nutrient–genome interactions. This is largely due to the wealth of genomic information generated by the Human Genome Project (HGP), the largest international scientific research endeavor [15]. The HGP spent 13 years to sequence the entire 3 billion bases of genetic information in every human cell and was officially completed in 2003 [16]. The completion of the HGP significantly facilitated the identification of single nucleotide polymorphisms (SNPs) within populations leading to differential responses to specific nutrients [7]. Thus, nutritional genomics has emerged as a result of the genomic revolution. Nutritional genomics includes nutrigenetics and nutrigenomics. Nutrigenetics evaluates gene-nutrition regulation by showing how genetic variations (i.e. SNPs) among individuals affect their responses to certain nutritional components. On the other hand, nutrigenomics investigates nutrition-gene regulation by demonstrating how nutrients interact with the genome and modify the expression of certain genes.

NUTRIGENETICS

There exists a small fraction (about 0.1%) of variation in the human genome sequence, which manifests in the form of differences in phenotypes and in an individual's response to certain food components [17]. These genetic polymorphisms create diversity within the human population. Ninety percent of all human genetic polymorphisms are variations of a single base within a DNA sequence known as SNPs. SNPs may contribute to the inconsistencies observed in epidemiological studies concerning the effects of diet on chronic diseases. Mounting evidence indicates that bioactive food components may disrupt cellular pathways through alteration of gene expression, thereby increasing risks for developing various chronic diseases such as CVD and type II diabetes [18]. Thus, genetic information on the differences in response to dietary factors may aid in identification of candidate genes with functional variations that alter nutrient metabolism.

The classical example of folate and methyltetrahydrofolate reductase (MTHFR) gene interaction demonstrated that SNP at position 677 of MTHFR gene results in two variants of MTHFR protein. MTHFR protein plays an important role in providing the essential amino acid methionine. The wild-type MTHFR protein metabolizes folate normally, whereas the thermal-labile variant of MTHFR protein exhibits a significant reduction in its activity giving rise to higher homocysteine levels, leading to increased risk of CVD and accelerated cognitive decline [19]. Therefore, individuals with the thermal-labile form of MTHFR would greatly benefit by personalized diet supplemented with folate to reduce excess homocysteine levels [20, 21].

Although a plethora of SNP information exists, it has been slow in converting SNP information into individual-based nutritional practices. This is because it takes tremendous time and effort to collect and catalogue population SNP information, to integrate and assimilate such information in nutrient-disease scenarios, and to develop specific diagnostic tools [7].

NUTRIGENOMICS

Nutrigenomics appears to exploit the omics revolution at a rapid pace providing an ever-growing body of information on nutrition-gene regulation due to advances in omics technologies such as genomics, transcriptomics, proteomics, and metabolomics [22].

Transcriptomics

Transcriptomics seems to be the most successful omics technology in nutrigenomics studies because of its efficiency and high throughput characteristics [22]. Transcriptome consists of the entire complement of mRNA or transcripts generated from genes being actively transcribed or expressed. Therefore, transcriptomics is a powerful tool for profiling gene expression patterns. A wide variety of bioactive food components can influence the expression of genes leading to altered biological processes including cell proliferation and differentiation, cell metabolism, and cell death. The imbalance of these cellular processes may lead to diseases such as diabetes and cancer. In this regard, genome-wide interrogation of gene expression by nutrients is particularly relevant in nutrigenomics research. Microarray technology markedly facilitates the simultaneous quantification of thousands of mRNA, thereby providing detailed profiles of gene expression in scenarios such as before and after exposure to certain food components [22, 23]. Interfaced with bioinformatics platforms, it is possible to construct the pathways for the observed gene expression profiles.

Transcriptomics has been used to investigate the effect of bioactive dietary components on gene expression in a variety of experimental paradigms including cell cultures, animals, and humans. Dietary intervention human clinical trials in obese subjects have been conducted to examine the effects of energy-restricted diets on gene expression in adipose tissue using transcriptomics technology [24, 25]. Other transcriptomics human studies include the evaluation of impact of high-protein and high-carbohydrate breakfasts on transcriptome of human blood cells and the investigation of the influence of *Lactobacillus GG* on gene expression profiles of duodenal mucosa [26, 27]. Transcriptomics has also been applied to food safety evaluations [22]. The rapid accumulation of nutritranscriptomic microarray data has prompted the establishment of a Web-based database infrastructure. This integrated database, built on an open-source database platform, ensures the efficient organization, storage, and analysis of the immense amount of microarray data generated from each nutritranscriptomic experiment [28].

Proteomics

Proteomics is the large-scale analysis of a proteome expressed by a genome. A proteome is the entire complement of proteins synthesized in a biological system at a given time and under defined conditions, reflecting the expression of a set of specific genes in the situation pertaining to that time point [29]. The proteome is dynamic and more complex than the genome. A proteome continuously changes in the temporal continuum according to cell type and functional state of the cell [30]. Whereas the human genome encodes about 25,000 functional genes, the human proteome comprises an order of magnitude more proteins (about 250,000) due to alternative splicing and posttranslational modifications [31]. In order to assess the complex proteome, new proteomics tools have been developed. Indeed, protein analysis has rapidly progressed from gel-based techniques to technologies such as mass spectrometry, multiple reaction monitoring, and multiplexed immunoassays in recent years [32]. Currently, proteomics allows for the high-throughput investigation of numerous proteins simultaneously in cells, tissues, or biological fluids [30]. Proteomics also enables the discovery of novel proteins. As an integral part of nutrigenomics, nutritional proteomics examines the effects of food components on protein expression and provides the potential to identify biomarkers sensitive to dietary interventions [33].

Several cell culture studies using nutritional proteomics demonstrated the effects of food components such as butyrate, flavonoid, and genistein on protein profiles [34–39]. Animal studies also showed the potential of proteomics in nutritional research. Proteomics analysis of brain homogenates from rats fed a grape seed extract (GSE) supplemented diet identified 13 candidate proteins [40]. Many candidate proteins were regulated by GSE-supplementation in opposite direction from previous findings for the same proteins in Alzheimer's disease and mouse models of neurodegeneration, indicating that these candidate proteins may be modulated by GSE to confer neuroprotective benefits [40]. Proteomic analysis of liver tissues of atherosclerosis-susceptible and atherosclerosis-resistant mice identified 30 proteins significantly altered by atherogenic diet [41]. The findings revealed a clear distinction in differential expression of proteins involved in oxidative stress and lipid metabolism between the two strains of mice in response to atherogenic diet, suggesting that the candidate proteins may contribute to differences in susceptibility to atherogenesis [41]. Combining nutritional transcriptomics and proteomics, the enzymes and transporters responsible for fatty acid metabolism, sequestration, and their transcriptional control in zinc-deficient rats were identified and pathways for the observed increase in hepatic lipid accumulation were constructed [42].

In a randomized cross-over human study, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) was used to isolate serum biomarkers in subjects taking control or cruciferous-supplemented diet [43]. Serum protein B-chain of α_2-HS glycoprotein was identified as a diet-related biomarker involved in insulin resistance and immune function [43]. With advances in proteomic methodology, nutritional proteomics has the potential to rapidly generate new knowledge pertaining to the complex interplay of nutrition–protein regulation, to identify novel biomarkers for nutritional status, and to devise new strategies for dietary prevention and intervention of diseases [44].

Metabolomics

Metabolomics is one of the newest omics technologies in nutritional research. The metabolome consists of the entire set of metabolites synthesized in a biological system. Metabolites are the end products of metabolic reactions, reflecting the interaction of the genome with its environment [45]. Metabolomics is the study of global metabolite profiles in a biological system under specific environmental conditions.

Nutritional metabolomics has the potential to provide insight into biochemical changes after dietary intervention and to impact food safety issues pertaining to genetically modified food [46]. Metabolomic techniques such as nuclear magnetic resonance (NMR) and MS combined with powerful bioinformatics platforms greatly enhance metabolomic approach to nutrition research [4, 47, 48]. The first nutritional metabolomic approach to determining biochemical modifications following dietary intervention showed that soy isoflavones induced changes in plasma components in healthy premenopausal women under controlled environmental conditions [49]. The plasma biochemical profiles showed strong variability in each subject, indicating the complex interaction of factors such as genetics, age, health status, diet, and lifestyle. Despite the individual variability, there were clear diet intervention-related differences in the plasma lipoprotein, amino acid, and carbohydrate profile, suggesting a soy-induced alteration in energy metabolism [49].

Metabolic responses to chamomile tea ingestion in human subjects were evaluated by high-resolution ^{1}H NMR spectroscopy coupled with chemometric methods [50]. Although metabolite profiles exhibited a high degree of variation among subjects, there was a clear differentiation in urinary excretion demonstrating an increased hippurate and glycine with decreased creatinine level after chamomile ingestion [50]. A similar study evaluated the effects of black and green tea intake on human urinary metabolites. The study showed that green tea consumption resulted in a stronger increase in several citric acid cycle intermediates suggesting an effect of green tea flavanols on human oxidative energy metabolism [51].

Although metabolomics has contributed significantly to the omics revolution, a global description of human metabolism is impossible at this point due to limitations in current technologies and diversity among individuals in terms of age, gender, diet, lifestyle, health status, and other internal and external factors [44]. Currently, the extent to which food components in the human diet induce changes in nutritional metabolic profiles is poorly understood. However, with technological advances, the challenges of applying metabolomics in nutrition research can be overcome.

OMICS IN FUTURE NUTRITION RESEARCH

Nutrition research has accelerated greatly by the omics revolution. Nutrigenomics has already contributed a vast amount of information to nutrition science. The major tasks of postgenomic nutrition research are: to understand how diet or food components affect the genome and how genetic variations affect individual response to food components. The ultimate goal of nutritional genomics is to personalize diets based on individual needs for the maintenance of health and prevention of diseases. Emerging disciplines branching from genomics such as RNomics, miRNomics, liponomics, fluxomics, toxigenomics, and the like will further facilitate nutritional genomics.

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Novel Omics Technologies in Nutraceutical and Functional Food Research

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ABSTRACT

The detection of physiological effects induced in the human body by the uptake of nutrients requires robust technologies to measure many parameters. New omics technologies including transcriptomics, proteomics, and metabolomics offer exciting opportunities to address complex issues related to human health, disease, and nutrition. Systems biology opens new doors to understanding the complex interaction network between nutrients and molecules in biological systems. It is expected that omics-based human nutrition research can provide recommendations for personalized medicine and nutrition.

INTRODUCTION

Many nutrients and nonnutrient components of foods have multiple functions. For example, fatty acids not only function as constituents of cell membrane phospholipids but also participate in numerous biochemical processes in a cell-specific and tissue-specific fashion, involving hundreds of genes, many signal transduction pathways, and a large number of biomolecules, such as transcription factors, receptors, hormones, apolipoproteins, and enzymes. Hence, the measurements of a single gene, single protein, or single metabolite do not provide us with sufficient and thorough information to elucidate mechanisms that underlie the beneficial or adverse effects induced in the human body by the uptake of dietary nutrients or components. In recent years, novel omics technologies including transcriptomics, proteomics, metabolomics, and systems biology are getting more attention due to their power in addressing complex issues related to human health, disease, and nutrition.

To study the molecular basis of health effects of specific components of the diet, nutritionists increasingly make use of these state-of-the-art omics technologies (Zhang *et al.*, 2008). The term "genomics" refers to the study of nucleotide sequences in the genome of an organism. Nutrigenomics refers to the study of the impact of specific nutrients or diets on gene expression. It should not be confused with another closely related discipline, "nutrigenetics," which investigates how genetic variability influences the body's response to a nutrient or diet. Thus, nutrigenomics and nutrigenetics approach the interplay of diet and genes from opposing start points. Transcriptomics measures the relative amounts of messenger RNAs (mRNAs) in a given organism for determining patterns and levels of gene expression. Proteomics is the study of proteins expressed in a cell, tissue, or organism, including all protein isoforms and posttranslational modifications. Metabolomics is defined as the comprehensive analysis of all metabolites generated in a given biological system, focusing on measurements of metabolite

concentrations and secretions in cells and tissues. It is not to be confused with "metabonomics," which investigates the fingerprint of biochemical perturbations caused by disease, drugs, and toxins (Goodacre, 2007). Systems biology aims at simultaneous measurement of genomic, transcriptomic, proteomic, and metabolomic parameters in a given system under defined conditions. The vast amount of data generated with such omics technologies requires the application of advanced bioinformatics tools to obtain a holistic view of the effects of nutrients or nonnutrient components of foods and to identify a system of biomarkers that can predict beneficial or adverse effects of dietary nutrients or components for promoting health and preventing disease.

TRANSCRIPTOMICS IN NUTRITIONAL RESEARCH

The classical gene analysis approach, such as Northern blotting and real-time RT-PCR, can only analyze gene expression for a limited number of candidate genes at a time. DNA microarray technology allows for measuring the expression level of thousands of genes, or even entire genomes, simultaneously. A typical DNA microarray experiment includes a number of characteristic steps: (1) RNA extraction from a sample; (2) reverse transcription of the RNA to obtain complementary DNA (cDNA) and labeling of the cDNA with specific dyes (usually fluorophores like Cyanine 3 and 5), or reverse transcription of the cDNA to obtain cRNA and labeling of the cRNA; (3) hybridization of the labeled cDNA or cRNA onto the microarray under given conditions; (4) washing of the slides to remove nonhybridized labeled oligonucleotides; (5) use of an appropriate scanning device to detect signal; and (6) data analysis by bioinformatics tools.

There are more examples of DNA microarray technology being performed in cell culture systems or laboratory animals to identify the cellular responses to dietary constituents and their molecular targets. For example, green tea catechins (McLoughlin *et al.*, 2004; Vittal *et al.*, 2004), soy isoflavones (Herzog *et al.*, 2004), polyunsaturated fatty acids (Kitajka *et al.*, 2004; Lapillonne *et al.*, 2004; Narayanan *et al.*, 2003), vitamins D and E (Johnson and Manor, 2004; Lin *et al.*, 2002), quercetin (Murtaza *et al.*, 2006), arginine (Leong *et al.*, 2006), anthocyanins (Tsuda *et al.*, 2006), hypoallergenic wheat flour (Narasaka *et al.*, 2006). In particular, very recently, Lavigne *et al.* (2008) used a DNA oligo microarray approach to examine effects of genistein on global gene expression in MCF-7 breast cancer cells. They found that genistein altered the expression of genes belonging to a wide range of pathways, including estrogen- and p53-mediated pathways. At physiologic concentrations (1 or 5 mu M), genistein elicited an expression pattern of increased mitogenic activity, whereas at pharmacologic concentrations (25 mu M), genistein generated an expression pattern of increased apoptosis, decreased proliferation, and decreased total cell number. Park *et al.* (2008) performed a comprehensive analysis of hepatic gene expression in a rat model of an alcohol-induced fatty liver using the cDNA microarray. They found that chronic ethanol consumption regulated mainly the genes related to the processes of signal transduction, transcription, immune response, and protein/amino acid metabolism. For the first time, this study revealed that five genes (including β-glucuronidase, UDP-glycosyltransferase 1, UDP-glucose dehydrogenase, apoC-III, and gonadotropin-releasing hormone receptor) were regulated by chronic ethanol exposure in the rat liver.

Furthermore, the number of microarray-based transcriptomics analyses for assessing the biological effects of dietary interventions on human nutrition and health is steadily increasing. van Erk *et al.* (2006) investigated the effect of a high-carbohydrate (HC) or high-protein (HP) breakfast on the transcriptome of human blood cells with RNA samples taken from eight healthy men before and 2 hours after consumption of the diets. About 317 genes for the HC breakfast and 919 genes for the HP breakfast were found to be differentially expressed. Specifically, consumption of the HC breakfast resulted in differential expression of glycogen metabolism genes, and consumption of the HP breakfast resulted in differential expression of genes involved in protein biosynthesis. Using GeneChip microarrays, Schauber *et al.* (2006) examined

the effect of regular consumption of low-digestible and prebiotic isomalt and the digestible sucrose on gene expression in rectal mucosa in a randomized double-blind crossover trial with 19 healthy volunteers over 4 weeks of feeding. They revealed that dietary intervention with the low digestible isomalt compared with digestible sucrose did not affect gene expression in the lining rectal mucosa, although gene expression of the human rectal mucosa can reliably be measured in biopsy material. Mangravite *et al.* (2007) used expression array analysis to identify molecular pathways responsive to both caloric restriction and dietary composition within adipose tissue from 131 moderately overweight men. They found that more than 1000 transcripts were significantly downregulated in expression in response to acute weight loss. The results demonstrated that stearoyl-coenzyme A desaturase (SCD) expression in adipose tissue is independently regulated by weight loss and by carbohydrate and saturated fat intakes, and SCD and diacylglycerol transferase 2 (DGAT2) expression may be involved in dietary regulation of systemic triacylglycerol metabolism. Kallio *et al.* (2007) assessed the effect of two different carbohydrate modifications (a rye-pasta diet characterized by a low postprandial insulin response and an oat-wheat-potato diet characterized by a high postprandial insulin response) on subcutaneous adipose tissue (SAT) gene expression in 47 persons with the metabolic syndrome. They detected that the rye-pasta diet downregulated 71 genes (linked to insulin signaling and apoptosis) and oat-wheat-potato diet upregulated 62 genes (related to stress, cytokine-chemokine-mediated immunity, and the interleukin pathway). Using microarray analysis, Niculescu *et al.* (2007) investigated the effects of dietary soy isoflavones on gene expression changes in lymphocytes from 30 postmenopausal women. They indicated that isoflavones had a stronger effect on some putative estrogen-responsive genes in equol producers than in nonproducers. In general, the gene expression changes caused by isoflavone intervention are related to increased cell differentiation, increased cAMP signaling and G-protein-coupted protein metabolism, and increased steroid hormone receptor activity.

However, there are some problems or limitations for transcriptomics approaches in nutritional research. One major problem is nonreproducibility of gene expression profiles. Different conclusions could be drawn from the same experiment performed at different times or in different labs or platforms. Fortunately, for reducing errors or variations, standards for reporting microarray data have been established under MIAME (minimum information about a microarray experiment) (Brazma *et al.*, 2001). Barnes *et al.* (2005) evaluated the reproducibility of microarray results using two platforms, Affymetrix GeneChips and Illumina BeadArrays. The results demonstrated that agreement was strongly correlated with the level of expression of a gene, and concordance was improved when probes on the two platforms could be identified as likely to target the same set of transcripts of a given gene. Another major issue is the analysis of data sets and their interpretation. Analyses only providing gene lists with significant p-values are insufficient to fully understand the underlying biological mechanisms; a single gene significantly upregulated or downregulated does not necessarily have any physiological meaning (Kussmann *et al.*, 2008). The combination of statistical and functional analysis is appropriate to facilitate the identification of biologically relevant and robust gene signatures, even across different microarray platforms (Bosotti *et al.*, 2007). An additional and more specific limitation in human nutritional applications is that microarray studies require significant quantities of tissues material for isolation of the needed RNA, whereas access to human tissues is obviously limited, although it is not impossible to obtain biopsies from control subjects involved in a nutrition research. If using human blood cell instead of tissue material, large interindividual variation exists in gene expression profiles of healthy individuals (Cobb *et al.*, 2005), this makes it challenging to identify robust gene expression signatures in response to a nutrition intervention. On the other hand, sample handling and prolonged transportation significantly influences gene expression profiles (Debey *et al.*, 2004). Highly standardized protocol across different labs is needed. Whole-blood samples require depletion of globin mRNA to enable detection of low-abundance transcripts, which have not been employed in human nutritional studies until now.

PROTEOMICS IN NUTRITIONAL RESEARCH

In the last 2 decades, proteomics has developed into a technology for biomarker discovery, disease diagnosis, and clinical applications (Beretta, 2007; Lescuyer *et al.*, 2007; Zhang *et al.*, 2007a, 2007b). The workflow for proteomics analysis consists of sample preparation, protein separation, and protein identification.

For gel-based proteomics experiments, proteins are extracted from cell or tissue samples, separated by two-dimensional polyacrylamide gel electrophoresis (2D gel), and stained. To identify differences in protein content between protein samples, images of the spots on the gels can be compared. Subsequently, the protein spots of interest are excised and the proteins are digested. Lastly, resulting peptides can be identified by mass spectrometry (MS). However, 2D-gel technology has many inherent drawbacks (Corthesy-Theulaz *et al.*, 2005; Kussmann *et al.*, 2005): (1) bias toward the most abundant changes, giving poor resolution for low abundant proteins, which might generate erroneous conclusions due to the fact that subtle variation may lead to important changes in metabolic pathways; (2) inability to detect proteins with extreme properties (very small, very large, very hydrophobic, and very acidic or basic proteins); (3) difficulty in identifying proteins.

Instead of the gel approaches, chromatography-based techniques have been developed for protein/peptide separation, such as gas chromatography (GC) and liquid chromatography (LC). When these separation technologies are combined with MS or tandem MS (MS/MS), the superior power of MS in the proteomic analysis is greatly enhanced. The MS instruments most used for proteomics experiments are ESI-MS (electrospray ionization MS), MALDI-TOF-MS (matrix-assisted laser desorption ionization with a time-of-flight MS), and its variant SELDI-TOF-MS (surface-enhanced laser desorption ionization with a time-of-flight MS). In addition, FTICR-MS (Fourier transform ion cyclotron resonance MS) is an increasingly useful technique in proteomic research, providing the highest mass resolution, mass accuracy, and sensitivity of present MS technologies, although with relatively high costs (Bogdanov and Smith, 2005).

In previous years, there were exponentially increasing publications on the application of proteomic techniques to nutrition research (Griffiths and Grant, 2006), but many investigations were performed in animal models (Breikers *et al.*, 2006; de Roos *et al.*, 2005; Kim *et al.*, 2006). Limited proteomics analysis in humans was involved in identifying the molecular target of dietary components in human subjects. For example, proteomic analysis of butyrate-treated human colon cancer cells (Tan *et al.*, 2002), identification of molecular targets of quercetin in human colon cancer cells (Wenzel *et al.*, 2004), and identification of cellular target proteins of genistein action in human endothelial cells (Fuchs *et al.*, 2005). Recently, Smolenski *et al.* (2007) applied 2D gel and MALDI-TOF-MS identified 15 proteins involved in host defense. Batista *et al.* (2007) employed 2D gel and MS method to identify new potential soybean allergens from transgenic and nontransgenic soy samples. Similarly, a proteomic analysis method based on 2D-gel and MALDI-TOF-MS was used to characterize wheat flour allergens and revealed that nine subunits of glutenins are the most predominant IgE-binding antigens (Akagawa *et al.*, 2007). Fuchs *et al.* (2007) conducted the proteomic analysis of human peripheral blood mononuclear cells (PBMC) from seven healthy men after a dietary flaxseed intervention. The results showed that flaxseed consumption affected significantly steady-state levels of 16 proteins, including enhanced levels of peroxiredoxin, reduced levels of the long-chain fatty acid beta-oxidation multienzyme complex, and reduced levels of glycoprotein IIIa/II. PBMCs are an important sample for monitoring dietary interventions and are accessible with little invasive means. Vergara *et al.* (2008) have established a public 2-DE database for human peripheral blood mononuclear cell (PBMCs) proteins, which have the potential of PBMCs to investigate the proteomics changes possibly associated with food or drug interventions.

In any proteomic study aiming at biomarker discovery, a critical question is: "How much of a given protein is present at a given time in a given condition?" Recently, a number of quantitative proteomic techniques have been developed, such as 2D DIGE (difference gel electrophoresis),