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Novel Omics Technologies in Food Nutrition

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1.1 Introduction

Many nutrients and non-nutrient 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, enzymes, and so on. Hence, the measurements of single genes, single proteins, or single metabolites are not enough to provide us sufficient thorough information to understand the 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, have received increased attention due to their power in addressing complex issues related to human health, disease, and nutrition.

Currently, in order to study the molecular basis of health effects of specific components of the diet, nutritionists are making increasing use of these state-of-the-art omics technologies (Zhang *et al.*, 2008). The term "genomics" refers to the study of all nucleotide sequences in the genome of an organism. Nutrigenomics refers to the study of the impact of specific nutrients or diets on gene expression. Note that 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 all messenger RNAs (mRNAs) in a given organism for determining the patterns and levels of gene expression. Proteomics is the study of all proteins expressed in a cell, tissue, or organism, including all protein isoforms and post-translational modifications. Metabolomics is defined as the comprehensive analysis of all metabolites generated in a given biological system, focusing on the 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 for 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 the nutrients or non-nutrient components of foods, and to identify a system of biomarkers that can predict the beneficial or adverse effects of dietary nutrients or components. The ultimate goals are to understand how nutrients/foods interact with the body and the related mechanisms of action and hence to enhance health and treat diet-related diseases (Norheim *et al.*, 2012).

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1.2 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 us to measure 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 the slides to remove non-hybridized labeled oligonucleotides;
5. using an appropriate scanning device to detect signal; and
6. data analysis by bioinformatics tools.

There are more and 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), and hypoallergenic wheat flour (Narasaka *et al.*, 2006).

For example, 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 μM), genistein elicited an expression pattern of increased mitogenic activity, while at pharmacologic concentrations (25 μM), 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. It was 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 beta-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 analysis 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 a high-protein (HP) breakfast on the transcriptome of human blood cells with RNA samples taken from eight healthy men before and 2 h 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 the 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 the 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 the 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 people with metabolic syndrome. They detected that there are rye-pasta diet downregulated 71 genes (linked to insulin signaling and apoptosis) and

oat-wheat-potato diet up-regulated 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.

Rcently, using transcriptomics, Marlow *et al.* (2013) investigated the effect of a Mediterranean-inspired diet on inflammation in Crohn's disease patients. They observed significant changes in gene expression, totally, 1902 genes were up-regulated and 1649 genes were downregulated, after a 6-week diet intervention. By Ingenuity Pathway Analysis (IPA), key canonical pathways affected by diet intervention were identified, including EIF2 signaling, B-cell development, T-helper cell differentiation, and thymine degradation. Rosqvist *et al.* (2014) performed transcriptomics to investigate liver fat accumulation and body composition after overfeeding saturated (SFA) (palm oil) or n-6 polyunsaturated (PUFA)(sunflower oil) for 7 weeks in 39 young and normal-weight individuals. The results revealed that SFA markedly increased liver fat compared with PUFA, and PUFA caused an almost three-fold increase in lean tissue than SFA. The differentially regulated genes were involved in regulating energy dissipation, insulin resistance, body composition, and fat cell differentiation.

However, there are some problems or limitations for transcriptomics approaches in nutritional research. One major problem is non-reproducibility of gene expression profiles. Different conclusions could be drawn from the same experiment but performed at different times or different labs or different 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 also improved when probes on the two platforms could be identified as being likely to target the same set of transcripts of a given gene. Another major issue is the analysis of the 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 that is 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, while access to human tissues is obviously limited, although it is not impossible to obtain biopsies from a control subjects involved in a nutrition research. If using human blood cells instead of tissue material, large inter-individual 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), the highly standardized protocol across different labs is needed. In particular whole-blood samples require the depletion of globin mRNA for enabling detection of low-abundance transcripts. Shin *et al.* (2014) showed that the experimental globin depletion removed approximately 80% of globin transcripts, and allowed for reliable detection of thousands of additional transcripts. However, a concern is that globin depletion leads to the significant reduction in RNA yields.

1.3 Proteomics in Nutritional Research

In the last two 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, b). The workflow for the proteomics analysis essentially consists of sample preparation, protein separation, and protein identification.

For the gel-based proteomics experiments, proteins are extracted from cell or tissue samples, separated by two-dimensional polyacrylamide gel electrophoresis (2D-Gel), and stained. In order 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. Last, the 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 towards 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); and (3) difficulty in identification of the proteins, time-consuming and costly.

Instead of the gel approaches, chromatography-based techniques have been developed for protein/peptide separation, such as gas chromatography (GC), liquid chromatography (LC). When these separation technologies is combined with MS or tandem MS (MS/MS), the superior power of MS in the proteomic analysis is greatly enhanced. The mostly used MS instruments 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, which provides the highest mass resolution, mass accuracy, and sensitivity of present MS technologies, although its relatively expensive (Bogdanov and Smith, 2005).

In recent years, there have been exponentially increasing numbers of 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), and identification of molecular targets of quercetin in human colon cancer cells (Wenzel *et al.*, 2004), the identification of cellular target proteins of genistein action in human endothelial cells (Fuchs *et al.*, 2005). Smolenski *et al.* (2007) applied 2D-gel and MALDI-TOF-MS identified 15 proteins that are involved in host defense. Batista *et al.* (2007) employed 2D-gel and the MS method to identify new potential soybean allergens from transgenic and non-transgenic 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 the 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 cells (PBMCs) proteins, which have the potentiality of PBMCs to investigate the proteomics changes possibly associated with food or drug interventions.

Recently, Bachmair *et al.* (2012) evaluated the effect of supplementation with an 80:20 *cis*-9,*trans*-11 conjugated linoleic acid blend on the human platelet proteome. Forty differentially regulated proteins were identified by LC-ESI-MS/MS, which participate in regulation of the cytoskeleton and platelet structure, as well as receptor action, signaling, and focal adhesion. Keeney *et al.* (2013) examined the effect of vitamin D (VitD) on brain during aging from middle to old age. Proteomics analysis revealed that several brain proteins were significantly elevated in the low-VitD group compared to the control and high-VitD groups, such as 6-phosphofructokinase, triose phosphate isomerase, pyruvate kinase, peroxiredoxin-3, and DJ-1/PARK7. This demonstrates that dietary VitD deficiency contributes to significant nitrosative stress in brain and may promote cognitive decline in middle aged and elderly adults. Qiu *et al.* (2013) applied quantitative proteomics to investigate the effects of lycopene on protein expression in human primary prostatic epithelial cells. The proteins that were significantly upregulated or downregulated following lycopene exposure were identified, which were involved in antioxidant responses, cytoprotection, apoptosis, growth inhibition, androgen receptor signaling, and the Akt/mTOR cascade. This suggests the preventive role of lycopene in prostate cancer.

In any proteomic study aiming for biomarker discovery a critical question is "how much of a given protein is present at a given time in a given condition?" Now a number of quantitative proteomic techniques have been developed, such as 2D DIGE (difference gel electrophoresis), ICAT (isotope-coded affinity tag), iTRAQ (isobaric tags for relative and absolute quantification), and proteolytic O-18-labeling strategies (Chen *et al.*, 2007a; Miyagi *et al.*, 2007). Wu *et al.* (2006) conducted the comparative study of three methods (DIGE, ICAT, and iTRAQ) and demonstrated that all three techniques yielded quantitative results with reasonable accuracy, although iTRAQ is most sensitive than DIGE and ICAT. Due to the fact that these methods displayed limited overlapping among the proteins identified, the complementary information obtained from different methods should potentially provide a better understanding of biological effects of dietary intervention. However, there are still some potential problems: the protein comigration problem for DIGE, cysteine-content bias for ICAT and susceptibility to errors in precursor ion isolation for iTRAQ. It is noted that all quantification approaches discussed so far deliver relative quantitative information. Moreover, absolute or stoichiometric quantification of proteome is becoming feasible, in particular, with the development of strategies with isotope-labeled standards composed of concatenated peptides. On the other hand, remarkable progress has also been made in label-free quantification methods based on the number of identified peptides (Gerber *et al.*, 2003; Kito and Ito, 2008; Old *et al.*, 2005). To date, few sample of quantitative proteomics analysis in nutritional research is available. For example, using DIGE and MALDI-MS/MS,

Alm *et al.* (2007) performed proteomic variation analysis within and between different strawberry varieties. They found that biological variation was more affected by different growth conditions than by different varieties, the amount of strawberry allergen varied between different strawberry varieties, and the allergen content in colorless (white) strawberry varieties was always lower than that of the red ones. However, only three proteins were the same among the proteins correlated with allergen and the color and this means that it is possible to breed a strawberry with low amount of allergen. Thus, the proteomic-based method has the potential to be used for variety improvement of fruit and vegetables.

Furthermore, protein microarray technology is a promising approach for proteomics, which can be used to detect changes in the expression and post-translational modifications of hundreds or even thousands of proteins in a parallel way. Its advantages include high sensitivity, good reproducibility, quantitative accuracy, and parallelization. The details of protein microarray method are described in recent review (Kricka *et al.*, 2006). Protein microarray platforms should open new possibilities to gain novel insight into the molecular mechanisms underlying nutrient-gene or nutrient-drug interactions (such as grapefruit-cyclosporine interaction). Puskas *et al.* (2006) applied the Panorama protein microarray to analyze the cholesterol diet-induced protein expression and found that a different phosphorylation pattern could be detected as well. Lin *et al.* (2007) showed that coupling the diversity of protein array with the biological output of basophilic cells was able to detect allergic sensitization. This is of great interest in nutrition research.

1.4 Metabolomics in Nutritional Research

Changes in mRNA concentration do not necessarily result in changes in cellular protein levels, and changes in protein levels may not always cause changes in protein activity. Metabolites represent the real endpoints of gene expression. Thus, alterations in the concentrations of metabolites may be better suited to describe the physiological regulatory processes in a biological system and may be a better measure of gene function than the transcriptome and proteome. Biological effects in nutrition cannot be reduced to the action of a single molecule but actually result from the modulation of many metabolic pathways at the same time, which is the product of a complex interplay between multiple genomes represented by the mammalian host and its gut microflora, and environmental factors (e.g., food habits, diet composition, and other lifestyle components) (Nicholson *et al.*, 2004; Rezzi *et al.*, 2007a). Metabolomics in nutrition has already delivered interesting insights to understanding the metabolic responses of humans or animals to dietary interventions.

The workflow for metabolomics involves a tandem use of analytical chemistry techniques to generate metabolic profiles and various bioinformatics tools to extract relevant metabolic information. Currently, the widely used tool for metabolomics experiments in nutrition research is proton nuclear magnetic resonance (NMR) technology. For example, the determination of metabolic effect of vitamin E supplementation in a mouse model of motor neuron degeneration (Griffin *et al.*, 2002); the evaluation of biochemical effects following dietary intervention with soy isoflavones in five healthy premenopausal women (Solanky *et al.*, 2003); the detection of human biological responses to different diets (e.g., chamomile tea, Wang *et al.*, 2005; or vegetarian, low meat, and high meat diets, Stella *et al.*, 2006); the characterization of the metabolic variability due to different populations (e.g., American, Chinese, and Japanese – Dumas *et al.*, 2006a; or Swedish and British populations – Lenz *et al.*, 2004). Bertram *et al.* (2007) employed a NMR-based metabolomic method to investigate biochemical effects of a short-term high intake of milk protein or meat protein on 8-year-old boys; this was the first report to demonstrate the capability of proton NMR-based metabolomics in identifying the overall biochemical effects of consumption of different animal proteins. They found that the milk diet increased the urinary excretion of hippurate, while the meat diet increased the urinary excretion of creatine, histidine, and urea. Moreover, based on NMR analysis of serum, the results demonstrated that the milk diet slightly changed the lipid profile of serum, but the meat diet had no effect on the metabolic profile of serum. Fardet *et al.* (2007) investigated the metabolic responses of rats fed whole-grain flour (WGF) and refined wheat flour (RF) using a NMR-based metabolomic approach. The results showed that some tricarboxylic acid cycle intermediates, aromatic amino acids, and hippurate were significantly increased in the urine of rats fed the WGF diet. Moazzami *et al.* (2011) evaluated the effects of a whole grain rye and rye bran diet on the metabolic profile of plasma in prostate cancer patients using (1)H NMR-based metabolomics. They found that five metabolites were increased after rye bran product (RP), including 3-hydroxybutyric acid, acetone, betaine, N,N-dimethylglycine, and dimethyl sulfone. This suggests a shift in energy metabolism from anabolic to catabolic status. Rasmussen *et al.* (2012) assessed the effect of high or low protein diet on the human urine metabolome by (1)H NMR and chemometrics. The results showed that citric acid was increased by the low (LP) protein diet, while urinary creatine was increased by the high (HP) protein diet.

Another exciting and powerful tool for metabolomics is MS-based technology. The main advantage of MS technique is its high sensitivity and rapid determination of mass or structure information. MS instruments in combination with

some separation technologies (such as gas or liquid chromatography, GC or LC, or capillary electrophoresis, CE) can quantitatively profile molecular entities like lipids, amino acids, bile acids, and other organic solutes at high sensitivity (Fiehn *et al.*, 2000; Watkins and German, 2002). A typical MS-based metabolomics system is the HPLC system using sub-2-μm packing columns combined with high operating pressures (UPLC technology). Compared with conventional HPLC-TOF-MS systems using 3–5-μm packing columns, UPLC-TOF-MS systems allow a remarkable decrease of the analysis time, higher peak capacity, and increased sensitivity. Recently, a number of applications of MS-based metabolomics to nutritional research have been reported. For example, a HPLC-TOF-MS-based study of changes of urinary endogenous metabolites associated with aging in rats (Williams *et al.*, 2005); a noninvasive extractive ESI-Q-TOF-MS for differentiation of maturity and quality of bananas, grapes, and strawberries (Chen *et al.*, 2007b); and combined GC-MS and LC-MS metabolic profiling for comprehensive understanding of system response to aristolochic acid intervention in rats (Ni *et al.*, 2007).

Recently, Tulipani *et al.* (2011) examined urinary changes in subjects with metabolic syndrome following 12-week nut consumption by an HPLC-Q-TOF-MS-driven nontargeted metabolomics approach. Twenty potential markers of nut intake were identified, including fatty acid conjugated metabolites, microbial-derived phenolic metabolites, and serotonin metabolites. Through employing urinary metabolic-profiling analysis based on UPLC coupled with quadrupole time-of-flight tandem mass spectrometry, Wang *et al.* (2013) identified reliable biomarkers of calcium deficiency from the rat model. In particular, significant correlations between calcium intake and two biomarkers, pseudouridine and citrate, were further confirmed in 70 women. Astarita *et al.* (2014) applied a multi-platform lipidomic approach to compare the plasma lipidome between WT and fat-1 mice, which can convert omega-6 to omega-3 PUFAs and protect against a wide variety of diseases including chronic inflammatory diseases and cancer. Fat-1 mice exhibited a significant increase in the levels of omega-3 lipids (unesterified eicosapentaenoic acid [EPA], EPA-containing cholesteryl ester, and omega-3 lysophosphospholipids), and a significant reduction in omega-6 lipids (unesterified docosapentaenoic acid [omega-6 DPA], DPA-containing cholesteryl ester, omega-6 phospholipids, and triacylglycerides). These lipidomic biosignatures may be used to monitor the health status and the efficacy of omega-3 intervention in humans.

However, a major problem for metabolomics is that the experimental metabolic profile is influenced not only by the genotype but also by age, gender, lifestyle, nutritional status, drugs, stress, physical activity, and so on. To minimize the variations in studies with humans, some attempts were made, such as using standardized diet, avoiding any vigorous activity, excluding smokers, and so on. Unfortunately, even under the consumption of standard diet, the metabolic variability remains. Using 1H NMR spectroscopy, Walsh *et al.* (2006) investigated the acute effects of standard diet on the metabonomic profiles of urine, plasma and saliva samples from 30 healthy volunteers. There are important biochemical variabilities to be observed for all biofluids at both intra- and inter-individual levels, significant variations in creatinine and acetate for urine and saliva, respectively, exist. After the consumption of standard diet, a reduction in inter-individual variation was observed in urine, but not in plasma or saliva. Indeed, different diets consumption in different populations leads to different metabolic profiles (Rezzi *et al.*, 2007a): higher urinary levels in creatine, creatinine, carnitine, acetylcarnitine, taurine, trimethylamine-N-oxide (TMAO), and glutamine are the metabolic signature of high-meat diet; higher urinary excretion of p-hydroxyphenylacetate, a microbial mammalian co-metabolite, and a decreased level in N,N,N-trimethyllysine are associated with the vegetarian diet; elevated β-aminoisobutyric acid and ethanol in Chinese urinary samples; increased urinary excretion in TMAO in the Japanese and Swedish populations due to the high dietary intake of fish; and usually high level of urinary taurine in the British population as a consequence of the Atkins diet. It is noted that a report reveals a "natural", stable over time, and invariant metabolic profile for each person, although the existence of human metabolic variations resulting from various dietary patterns (Assfalg *et al.*, 2008). This provides the possibility of eliminating the day-to-day "noise" of the individual metabolic fingerprint and opens new perspectives to metabolomic studies for personalized therapy and nutrition.

Another important issue in nutritional metabolomics is gut microbiota-host metabolic interactions, such as the interaction between the microbiome and the human, which makes the human become a "superorganism" (Goodacre, 2007). More than 400 microbial species exist in the large-bowel microflora of healthy humans, which produce significant metabolic signals so that the true metabolomic signals of nutrients in the diet could be "swamped" and the metabolome of biofluids in human nutrition is altered. Dumas *et al.* (2006b) investigated the metabolic relationship between gut microflora and host co-metabolic phenotypes using the plasma and urine metabolic NMR profile of the mouse. They found that the urinary excretion of methylamines from the precursor choline was directly related to microflora metabolism, demonstrating significant interaction between the mammalian host and microbiota metabolism. Rezzi *et al.* (2007b) performed the NMR analysis of plasma and urine metabolic profiles in 22 healthy male volunteers with

behavioral/psychological dietary preference (chocolate desire or chocolate indifference). The results revealed that chocolate preference was associated with a specific metabolic signature, which is imprinted in the metabolism even in the absence of chocolate as a stimulus. Marcobal *et al.* (2013) applied the UPLC technique to investigate the effects of the human gut microbiota on the fecal and urinary metabolome of a humanized (HUM) mouse. They found that the vast majority of metabolomic features are produced in the corresponding HUM mice, the metabolite signatures can be modified by host diet, and simplified bacterial communities can drive major changes in the host metabolomic profile. This demonstrates that metabolomics constitutes a powerful avenue for functional characterization of the intestinal microbiota and its interaction with the host.

1.5 Systems Biology in Nutritional Research

In order to better understand the complex interplay between genes, diet, lifestyle, and endogenous gut microflora, and to understand how diet can be modified to maintain optimal health throughout life, the integrative use of various omics technologies-systems biology technology offer exciting opportunities to develop the emerging area of personalized nutrition and healthcare (Naylor *et al.*, 2008; Zhang *et al.*, 2008). Currently, there has been limited work in this arena.

Using an integrated reverse functional genomic and metabolic approach, Griffin *et al.* (2004) identified perturbed metabolic pathways by orotic acid treatment. In the searching for correlations between the 60 most differentially expressed genes and the largest changed metabolite trimethylamine-N-oxide, they found that the most significant negative correlation is stearyl-CoA desaturase 1, which highlights the relationship between transcripts and metabolites in lipid pathways. Herzog *et al.* (2004) performed proteome and transcriptome analysis of human colon cancer cells treated with flavone. About 488 mRNA targets were found to be regulated by flavone at least two-fold. On the other hand, many proteins involved in gene regulation, detoxification, and intermediary metabolism, such as annexin II, apolipoprotein A1, and so on, were found to be altered by flavone exposure. Dieck *et al.* (2005) conducted transcriptome and proteome analysis to identify the underlying molecular changes in hepatic lipid metabolism in zinc-deficient rats. The experimental findings provide evidence that an unbalanced gene transcription control via the PPAR-α, thyroid hormone, and SREBP-dependent pathways could explain most of the effects of zinc deficiency on hepatic fat metabolism. Mutch *et al.* (2005) used an integrative transcriptome and lipid-metabolome approach to understand the molecular mechanisms regulated by the consumption of PUFA. They identified stearoyl-CoA desaturase as a target of an arachidonate-enriched diet and revealed a previously unrecognized and distinct role for arachidonate in the regulation of hepatic lipid metabolism. By combining DNA microarray, proteomics, and metabolomics platforms, Schnackenberg *et al.* (2006) investigated the acute effects of valproic acid in the liver and demonstrated a perturbation in the glycogenolysis pathway after administration of valproic acid.

Recently, by applying transcriptomics, proteomics, and metabolomics technologies to liver samples from C57BL/6J mice, Rubio-Aliaga *et al.* (2011) revealed alterations of key metabolites and enzyme transcript levels of hepatic one-carbon metabolism and related pathways, suggesting the important role of coupling high levels of choline and low levels of methionine in the development of insulin resistance and liver steatosis. Vendel Nielsen *et al.* (2013) investigated the hepatic response to the most abundant trans fatty acid in the human diet, elaidic acid, using a combined proteomic, transcriptomic, and lipidomic approach in HepG2 cells. They found that many proteins involved in cholesterol synthesis and the esterification and hepatic import/export of cholesterol were upregulated. Moreover, at the phospholipid level, there existed a marked remodeling of the cellular membrane. This suggests that trans fatty acids from the diet induce abundance changes in several hepatic proteins and hepatic membrane composition to alter plasma cholesterol levels.

1.6 Conclusions

The main goal of omics-based nutrition research is to understand the relationships between diet and disease and the relationships between diet and health, and finally to make recommendations for personalized nutrition or individualized diets (Figure 1.1, modified from Zhang *et al.*, 2008). In order to better understand the complex interplay that occurs between the individual in terms of genetics, physiology, health, diet, and environment, comparative genetic, transcriptomic, proteomic, and metabolomic analyses for individuals and populations are highly required. In particular, systems biology, more than the

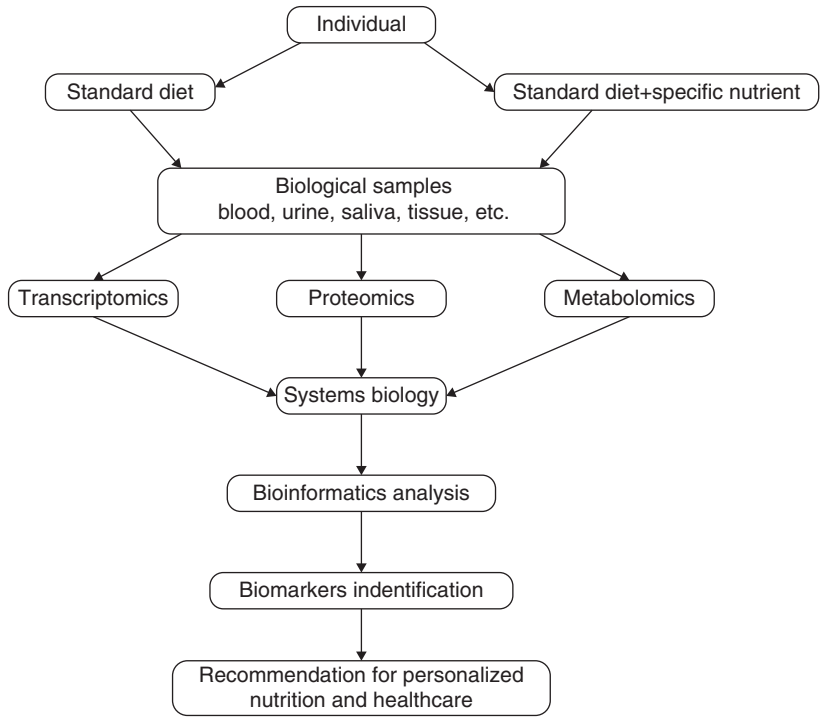

Figure 1.1 *Workflow for omics-based nutritional research.*

simple merger of various omics technologies (transcriptomics, proteomics, and metabolomics), aims for understanding the biological behavior of a cellular system in response to external stimuli, and opens up a new road to understanding the complex interaction network between nutrients and molecules in biological systems. An era of personalized medicine and nutrition is coming.

References

Akagawa, M., Handoyo, T., Ishii, T., Kumazawa, S., Morita, N., and Suyama, K. (2007) Proteomic analysis of wheat flour allergens. *J. Agr. Food Chem*. **55**(17), 6863–6870.

Alm, R., Ekefjard, A., Krogh, M., Hakkinen, J., and Emanuelsson, C. (2007) Proteomic variation is as large within as between strawberry varieties. *J Proteome Res*. **6**(8), 3011–3020.

Assfalg, M., Bertini, I., Colangiuli, D., Luchinat, C., Schafer, H., Schutz, B., *et al.* (2008) Evidence of different metabolic phenotypes in humans. *P Natl. Acad. Sci. USA* **105**(5), 1420–1424.

Astarita, G., McKenzie, J.H., Wang, B., Strassburg, K., Doneanu, A., Johnson, J., *et al.* (2014) Protective lipidomic biosignature associated with a balanced omega-6/omega-3 ratio in fat-1 transgenic mice. *PLoS One* **9**(4), e96221.

Bachmair, E.M., Bots, M.L., Mennen, L.I., Kelder, T., Evelo, C.T., Horgan, G.W., *et al.* (2012) Effect of supplementation with an 80:20 *cis*-9,*trans*-11 conjugated linoleic acid blend on the human platelet proteome. *Mol. Nutr. Food Res.* **56**(7), 1148–1159.

Barnes, M., Freudenberg, J., Thompson, S., Aronow, B., and Pavlidis, P. (2005) Experimental comparison and cross-validation of the Affymetrix and Illumina gene expression analysis platforms. *Nucleic Acids Res.* **33**, 5914–5923.

Batista, R., Martins, I., Jenoe, P., Ricardo, C.P., and Oliveira, M.M. (2007) A proteomic study to identify soya allergens – The human response to transgenic versus non-transgenic soya samples. *Int. Arch. Allergy Imm.* **144**(1), 29–38.

Bertram, H.C., Hoppe, C., Petersen, B.O., Duus, J.O., Molgaard, C., and Michaelsen, K.F. (2007) An NMR-based metabonomic investigation on effects of milk and meat protein diets given to 8-year-old boys. *British J. Nutr.* **97**(4), 758–763.

Beretta, L. (2007) Proteomics from the clinical perspective: many hopes and much debate. *Nat. Methods* **4**, 785–786.

Bogdanov, B. and Smith, R.D. (2005) Proteomics by FTICR mass spectrometry: Top down and bottom up. *Mass Spectrom. Rev.* **24**(2), 168–200.

Bosotti, R., Locatelli, G., Healy, S., Scacheri, E., Sartori, L., Mercurio, C., *et al.* (2007) Cross platform microarray analysis for robust identification of differentially expressed genes. *BMC Bioinformatics* **8**(Suppl. 1), 5.

Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., *et al.* (2001) Minimum information about a microarray experiment (MIAME) – toward standards for microarray data. *Nat. Genet.* **29**, 365–371.

Breikers, G., van Breda, S.G.J., Bouwman, F.G., van Herwijnen, M.H.M., Renes, J., Mariman, E.C.M., *et al.* (2006) Potential protein markers for nutritional health effects on colorectal cancer in the mouse as revealed by proteomics analysis. *Proteomics* **6**(9), 2844–2852.

Chen, X., Sun, L.W., Yu, Y.B., Xue, Y., and Yang, P.Y. (2007a) Amino acid-coded tagging approaches in quantitative proteomics. *Expert Rev. Proteomic.* **4**(1), 25–37.

Chen, H.W., Sun, Y.P., Wortmann, A., Gu, H.W., and Zenobi, R. (2007b) Differentiation of maturity and quality of fruit using noninvasive extractive electrospray ionization quadrupole time-of-flight mass spectrometry. *Anal. Chem.* **79**(4), 1447–1455.

Cobb, J.P., Mindrinos, M.N., Miller-Graziano, C., Calvano, S.E., Baker, H.V., Xiao, W., *et al.* (2005) Application of genome-wide expression analysis to human health and disease. *Proc Natl Acad Sci U S A.* Mar 29, **102**(13), 4801–4806.

Corthesy-Theulaz, I., den Dunnen, J.T., Ferre, P., Geurts, J.M.W., Muller, M., van Belzen, N., *et al.* (2005) Nutrigenomics: The impact of biomics technology on nutrition research. *Ann. Nutr. Metab.* **49**(6), 355–365.

Debey, S., Schoenbeck, U., Hellmich, M., Gathof, B.S., Pillai, R., Zander, T., and Schultze, J.L. (2004) Comparison of different isolation techniques prior to gene expression profiling of blood derived cells: impact on physiological responses, on overall expression and the role of different cell types. *Pharmacogenomics* **4**, 193–207.

de Roos, B., Duivenvoorden, I., Rucklidge, G., Reid, M., Ross, K., Lamers, R.J.A.N., *et al.* (2005) Response of apolipoprotein E*3-Leiden transgenic mice to dietary fatty acids: combining liver proteomics with physiological data. *FASEB J.* **19**(3), 813–838.

Dieck, H.T., Doring, F., Fuchs, D., Roth, H.P., and Daniel, H. (2005) Transcriptome and proteome analysis identifies the pathways that increase hepatic lipid accumulation in zinc-deficient rats. *J. Nutr.* **135**(2), 199–205.

Dumas, M.E., Maibaum, E.C., Teague, C., Ueshima, H., Zhou, B., Lindon, J.C., *et al.* (2006a) Assessment of analytical reproducibility of 1H NMR spectroscopy based metabolomics for large-scale epidemiological research: The INTERMAP Study. *Anal. Chem.* **78**(7), 2199–2208.

Dumas, M.E., Barton, R.H., Toye, A., Cloarec, O., Blancher, C., Rothwell, A., *et al.* (2006b) Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin resistant mice. *Proc. Natl. Acad. Sci. USA* **103**(33), 12511–12516.

Fardet, A., Canlet, C., Gottardi, G., Lyan, B., Llorach, R., Remesy, C. *et al.* Whole-grain and refined wheat flours show distinct metabolic profiles in rats as assessed by a H-1 NMR-based metabonomic approach. *J. Nutr.* **137**(4), 923–929.

Fiehn, O., Kopka, J., Dormann, P., Altmann, T., Trethewey, R.N., and Willmitzer, L. (2000) Metabolite profiling for plant functional genomics. *Nat. Biotechnol.* **18**, 1157–1161.

Fuchs, D., Erhard, P., Rimbach, G., Daniel, H., and Wenzel, U. (2005) Genistein blocks homocysteine-induced alterations in the proteome of human endothelial cells. *Proteomics* **5** (11), 2808–2018.

Fuchs, D., Piller, R., Linseisen, J., Daniel, H., and Wenzel, U. (2007) The human peripheral blood mononuclear cell proteome responds to a dietary flaxseed intervention and proteins identified suggest a protective effect in atherosclerosis. *Proteomics* **7**, 3278–3288.

Gerber, S.A., Rush, J., Stemman, O., Kirschner, M.W., and Gygi, S.P. (2003) Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6940–6945.

Goodacre, R. (2007) Metabolomics of a superorganism. *J. Nutr.* **137**(1 Supp.), 259S–266S

Griffin, J.L., Muller, D., Woograsingh, R., Jowatt, V., Hindmarsh, A., Nicholson, J.K., *et al.* (2002) Vitamin E deficiency and metabolic deficits in neuronal ceroid lipofuscinosis described by bioinformatics. *Physiol. Genomics* **11** (3), 195–203.

Griffin, J.L., Bonney, S.A., Mann, C., Hebbachi, A.M., Gibbons, G.F., Nicholson, J.K., *et al.* (2004) An integrated reverse functional genomic and metabolic approach to understanding orotic acid-induced fatty liver. *Physiol. Genomics* **17**(2), 140–149.

Griffiths, H.R., and Grant, M.M. (2006) The use of proteomic techniques to explore the holistic effects of nutrients *in vivo*. *Nutr Res Rev* **19**(2), 284–293.

Herzog, A,. Kindermann, B., Döring, F., Daniel, H., and Wenzel, U. (2004) Pleiotropic molecular effects of the pro-apoptotic dietary constituent flavone in human colon cancer cells identified by protein and mRNA expression profiling. *Proteomics* **4**, 2455–2564.

Johnson, A. and Manor, D. (2004) The transcriptional signature of vitamin E. *Ann. N.Y. Acad. Sci.* **1031**, 337–338.

Kitajka, K., Sinclair, A.J., Weisinger, R.S., Weisinger, H.S., Mathai, M., Jayasooriya, A.P., *et al.* (2004) Effects of dietary omega-3 polyunsaturated fatty acids on brain gene expression. *Proc. Natl. Acad. Sci. USA* **101**(30), 10931–10936.

Kallio, P., Kolehmainen, M., Laaksonen, D.E., Kekäläinen, J., Salopuro, T., Sivenius, K., *et al.* (2007) Dietary carbohydrate modification induces alterations in gene expression in abdominal subcutaneous adipose tissue in persons with the metabolic syndrome: the FUNGENUT Study. *Am. J. Clin. Nutr.* **85**, 1417–1427.

Keeney, J.T., Förster, S., Sultana, R., Brewer, L.D., Latimer, C.S., Cai, J., *et al.* (2013) Dietary vitamin D deficiency in rats from middle to old age leads to elevated tyrosine nitration and proteomics changes in levels of key proteins in brain: implications for low vitamin D-dependent age-related cognitive decline. *Free Radical Bio. Med.* **65**, 324–334.

Kim, H., Deshane, J., Barnes, S., and Meleth, S. (2006) Proteomics analysis of the actions of grape seed extract in rat brain: Technological and biological implications for the study of the actions of psychoactive compounds. *Life Sci.* **78**(18), 2060–2065.

Kito, K. and Ito, T. (2008) Mass spectrometry-based approaches toward absolute quantitative proteomics. *Curr. Genomics* **9**(4), 263–274.

Kricka, L.J., Master, S.R., Joos, T.O., Fortina, P. (2006) Current perspectives in protein array technology. *Ann. Clin. Biochem.* **43**, 457–467.

Kussmann, M., Affolter, M., and Fay, L.B. (2005) Proteomics in nutrition and health. *Comb. Chem. High T. Scr.* **8**, 679–696.

Kussmann, M., Rezzi, S., and Daniel, H. (2008) Profiling techniques in nutrition and health research. *Curr. Opin. Biotech.* **19**, 83–99.

Lapillonne, A., Clarke, S.D., and Heird, W.C. (2004) Polyunsaturated fatty acids and gene expression. *Curr. Opin. Clin. Nutr.* **7**(2), 151–156.

Lavigne, J.A., Takahashi, Y., Chandramouli, G.V.R., Liu, H.T., Perkins, S.N., Hursting, S.D., *et al.* (2008) Concentration-dependent effects of genistein on global gene expression in MCF-7 breast cancer cells: an oligo microarray study. *Breast Cancer Res. Tr.* **110**(1), 85–98.

Lenz, E.M., Bright, J., Wilson, I.D., Hughes, A., Morrisson, J., Lindberg, H., *et al.* (2004) A Metabonomics, dietary influences and cultural differences: a H-1 NMR-based study of urine samples obtained from healthy British and Swedish subjects. *J. Pharmaceut. Biomed.* **36**(4), 841–849.

Leong, H.X., Simkevich, C., Lesieur-Brooks, A., Lau, B.W., Fugere, C., Sabo, E. *et al.* (2006) Short-term arginine deprivation results in large-scale modulation of hepatic gene expression in both normal and tumor cells: microarray bioinformatic analysis. *Nutr. Metab.* **3**, 37.

Lescuyer, P., Hochstrasser, D.F., and Rabilloud, T. (2007) How shall we use the proteomics toolbox for biomarker discovery? *J. Proteome Res.* **6**, 3371–3376.

Lin, R., Nagai, Y., Sladek, R., Bastien, Y., Ho, J., Petrecca, K., *et al. (*2002*)* Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. *Mol. Endocrinol.* **16**, 1243–1256.

Lin, J., Renault, N., Haas, H., Schramm, G., Vieths, S., Vogel, L., *et al.* (2007) A novel tool for the detection of allergic sensitization combining protein microarrays with human basophils. *Clin. Exp. Allergy.* **37**(12),1854–1862.

Mangravite, L.M., Dawson, K., Davis, R.R., Gregg, J.P., and Krauss, R.M. (2007) Fatty acid desaturase regulation in adipose tissue by dietary composition is independent of weight loss and is correlated with the plasma triacylglycerol response. *Am. J. Clin. Nutr.* **86**(3), 759–767.

Marcobal, A., Kashyap, P.C., Nelson, T.A., Aronov, P.A., Donia, M.S., Spormann, A., *et al.* (2013) A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. *ISME J.* **7**(10), 1933–1943.

Marlow, G., Ellett, S., Ferguson, I.R., Zhu, S., Karunasinghe, N., Jesuthasan, A.C., *et al.* (2013) Transcriptomics to study the effect of a Mediterranean-inspired diet on inflammation in Crohn's disease patients. *Human Genomics* **7**, 24.

McLoughlin, P., Roengvoraphoj, M., Gissel, C., Hescheler, J., Certa, U., and Sachinidis, A. (2004) Transcriptional responses to epigallocatechin-3 gallate in HT 29 colon carcinoma spheroids. *Genes Cells* **9**(7), 661–669.

Miyagi, M., Rao, K.C.S., and Sekhar, K.C. (2007) Proteolytic O-18-labeling strategies for quantitative proteomics. *Mass Spectrom. Rev.* **26**(1), 121–136.

Moazzami, A.A., Zhang, J.X., Kamal-Eldin, A., Aman, P., Hallmans, G., Johansson, J.E., *et al.* (2011) Nuclear magnetic resonance-based metabolomics enable detection of the effects of a whole grain rye and rye bran diet on the metabolic profile of plasma in prostate cancer patients. *J. Nutr.* **141**(12), 2126–2132.

Murtaza, L., Marra, G., Schlapbach, R., Patrignani, A., Kunzli, M., Wagner, U., *et al.* (2006) A preliminary investigation demonstrating the effect of quercetin on the expression of genes related to cell-cycle arrest, apoptosis and xenobiotic metabolism in human COIIScolon-adenocarcinoma cells using DNA microarray. *Biotechnol. Appl. Bioc.* **45**, 29–36.

Mutch, D.M., Grigorov, M., Berger, A., Fay, L.B., Roberts, M.A., Watkins, S.M., *et al.* (2005) An integrative metabolism approach identifies stearoyl-CoA desaturase as a target for an arachidonate-enriched diet. *FASEB J.* **19**(1), 599.

Narasaka, S., Endo, Y., Fu, Z.W., Moriyama, M., Arai, S., Abe, K., *et al.* (2006) Safety evaluation of hypoallergenic wheat flour by using a DNA microarray. *Biosci. Biotech. Bioch.* **70**(6), 1464–1470.

Narayanan, B.A., Narayanan, N.K., Simi, B., and Reddy, B.S. (2003) Modulation of inducible nitric oxide synthase and related proinflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colon cancer cells. *Cancer Res.* **63**(5), 972–979.

Naylor, S., Culbertson, A.W., and Valentine, S.J. (2008) Towards a systems level analysis of health and nutrition. *Curr. Opin. Biotech.* **19**(2), 100–109.

Ni, Y., Su, M.M., Qiu, Y.P., Chen, M.J., Liu, Y.M., Zhao, A.H., *et al. (*2007*)* Metabolic profiling using combined GC-MS and LC-MS provides a systems understanding of aristolochic acid-induced nephrotoxicity in rat. *FEBS Lett.* **581**(4), 707–711.

Nicholson, J.K., Holmes, E., Lindon, J.C., and Wilson, I.D. (2004) The challenges of modeling mammalian biocomplexity. *Nat. Biotechnol.* **22**, 1268–1274.

Niculescu, M.D., Pop, E.A., Fischer, L.M., and Zeisel, S.H. (2007) Dietary isoflavones differentially induce gene expression changes in lymphocytes from postmenopausal women who form equol as compared with those who do not. *J. Nutr. Biochem.* **18**, 380–390.

Norheim, F., Gjelstad, I.M., Hjorth, M., Vinknes, K.J., Langleite, T.M., Holen, T., *et al. (*2012*)* Molecular nutrition research: the modern way of performing nutritional science. *Nutrients* **4**(12), 1898–1944.

Old, W.M., Meyer-Arendt, K., Aveline-Wolf, L., Pierce, K.G., Mendoza, A., Sevinsky, J.R., *et al. (*2005*)* Comparison of label-free methods for quantifying human proteins by shotgun proteomics. *Mol. Cell. Proteomics* **4**, 1487–1502.

Park, S.H., Choi, M.S., and Park, T. (2008) Changes in the hepatic gene expression profile in a rat model of chronic ethanol treatment. *Food Chem. Toxicol.* **46**(4),1378–1388.

Puskas, L.G., Menesi, D., Feher, L.Z., and Kitajka, K. (2006) High-throughput functional genomic methods to analyze the effects of dietary lipids. *Curr. Pharm. Biotechno.* **7**(6), 525–529.

Qiu, X., Yuan, Y., Vaishnav, A., Tessel, M.A., Nonn, L., and van Breemen, R.B. (2013) Effects of lycopene on protein expression in human primary prostatic epithelial cells. *Cancer Prev. Res.* **6**(5), 419–427.

Rasmussen, L.G., Winning, H., Savorani, F., Toft, H., Larsen, T.M., Dragsted, L.O., *et al.* (2012) Assessment of the effect of high or low protein diet on the human urine metabolome as measured by NMR. *Nutrients* **4**(2), 112–131.

Rezzi, S., Ramadan, Z., Fay, L.B., and Kochhar, S. (2007a) Nutritional metabonomics: applications and perspectives. *J. Proteome Res.* **6**, 513–525.

Rezzi, S., Ramadan, Z., Martin, F.P., Fay, L.B., van Bladeren, P., Lindon, J.C., *et al.* (2007b) Human metabolic phenotypes link directly to specific dietary preferences in healthy individuals. *J. Proteome Res.* **6**, 4469–4477.

Rosqvist, F., Iggman, D., Kullberg, J., Cedernaes, J., Johansson, H.E., Larsson, A., *et al.* (2014) Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* **63**(7), 2356–2368.

Rubio-Aliaga, I., Roos, B.D., Sailer, M., McLoughlin, G.A., Boekschoten, M.V., van Erk, M., *et al.* (2011)Alterations in hepatic one-carbon metabolism and related pathways following a high-fat dietary intervention. *Physiol Genomics* **43**(8), 408–416.

Schauber, J., Weiler, F., Gostner, A., Melcher, R., Kudlich, T., Lührs, H., *et al.* (2006) Human rectal mucosal gene expression after consumption of digestible and non-digestible carbohydrates. *Mol. Nutr. Food Res.* **50**, 1006–1012.

Schnackenberg, L.K., Jones, R.C., Thyparambil, S., Taylor, J.T., Han, T., Tong, W.D., *et al.* (2006) An integrated study of acute effects of valproic acid in the liver using metabonomics, proteomics, and transcriptomics platforms. *Omics* **10**(1), 1–14.

Shin, H., Shannon, C.P., Fishbane, N., Ruan, J., Zhou, M., Balshaw, R., *et al.* (2014) Variation in RNA-Seq transcriptome profiles of peripheral whole blood from healthy individuals with and without globin depletion. *PLoS One* **9**(3), e91041.

Smolenski, G., Haines, S., Kwan, F.Y.S., Bond, J., Farr, V., Davis, S.R., *et al.* (2007) Characterisation of host defence proteins in milk using a proteomic approach. *J. Proteome Res.* **6**(1), 207–215.

Solanky, K.S., Bailey, N.J.C., Beckwith-Hall, B.M., Davis, A,. Bingham, S., Holmes, E., *et al.* (2003) Application of biofluid H-1 nuclear magnetic resonance-based metabonomic techniques for the analysis of the biochemical effects of dietary isoflavones on human plasma profile. *Anal. Biochem.* **323**(2), 197–204.

Stella, C., Beckwith-Hall, B., Cloarec, O., Holmes, E., Lindon, J.C., Powell, J., *et al.* (2006) Susceptibility of human metabolic phenotypes to dietary modulation. *J. Proteome Res.* **5**, 2780–2788.

Tan, S., Seow, T.K., Linag, R.C.M.Y., Koh, S., Lee, C.P.C., Chung, M.C.M., *et al.* Proteome analysis of butyrate-treated human colon cancer cells (HT-29). *Int .J. Cancer.* **98**, 523–531.

Tsuda, T., Ueno, Y., Yoshikawa, T., Kojo, H., and Osawa, T. (2006) Microarray profiling of gene expression in human adipocytes in response to anthocyanins. *Biochem. Pharmacol.* **71**(8), 1184–1197.

Tulipani, S., Llorach, R., Jáuregui, O., López-Uriarte, P., Garcia-Aloy, M., Bullo, M., *et al.* (2011) Metabolomics unveils urinary changes in subjects with metabolic syndrome following 12-week nut consumption. *J. Proteome Res.* **10**(11), 5047–5058.

van Erk, M.J., Blom, W.A., van Ommen, B., and Hendriks, H.F. (2006) High-protein and high-carbohydrate breakfasts differentially change the transcriptome of human blood cells. *Am. J. Clin. Nutr.* **84**, 1233–1241.

Vendel Nielsen, L., Krogager, T.P., Young, C., Ferreri, C., Chatgilialoglu, C., Nørregaard Jensen, O., *et al.* (2013) Effects of elaidic acid on lipid metabolism in HepG2 cells, investigated by an integrated approach of lipidomics, transcriptomics and proteomics. *PLoS One* **8**(9), e74283.

Vergara, D., Chiriaco, F., Acierno, R., and Maffia, M. (2008) Proteomic map of peripheral blood mononuclear cells. *Proteomics* **8**(10), 2045–2051.

Vittal, R., Selvanayagam, Z.E., Sun, Y., Hong, J., Liu, F., Chin, K.V., *et al.* (2004) Gene expression changes induced by green tea polyphenol (-)-epigallocatechin-3-gallate in human bronchial epithelial 21BES cells analyzed by DNA microarray. *Mol. Cancer Ther.* **3**(9), 1091–1099.

Walsh, M.C., Brennan, L., Malthouse, J.P.G., Roche, H.M., and Gibney, M.J. (2006) Effect of acute dietary standardization on the urinary, plasma, and salivary metabolomic profiles of healthy humans. *Am. J. Clin. Nutr.* **84**(3), 531–539.

Wang, Y.L., Tang, H.R., Nicholson, J.K., Hylands, P.J., Sampson, J., and Holmes, E. (2005) A metabonomic strategy for the detection of the metabolic effects of chamomile (*Matricaria recutita* L.) ingestion. *J. AgrFood Chem.* **53**(2), 191–196.

Wang, M., Yang, X., Wang, F., Li, R., Ning, H., Na, L., *et al.* (2012) Calcium-deficiency assessment and biomarker identification by an integrated urinary metabonomics analysis. *BMC Med.* **11**, 86.

Watkins, S.M., and German, J.B. (2002) Toward the implementation of metabolomic assessments of human health and nutrition. *Curr. Opin. Biotechnol.* **13**, 512–516.

Wenzel, U., Herzog, A., Kuntz, S., and Daniel, H. (2004) Protein expression profiling identifies molecular targets of quercetin as a major dietary flavonoid in human colon cancer cells. *Proteomics* **4**, 2160–2174.

Williams, R.E., Lenz, E.M., Lowden, J.S., Rantalainen, M., and Wilson, I.D. (2005) The metabonomics of aging and development in the rat: an investigation into the effect of age on the profile of endogenous metabolites in the urine of male rats using 1H NMR and HPLC-TOF MS. *Mol. Biosyst.* **1**(2), 166–175.

Wu, W.W., Wang, G.H., Baek, S.J., and Shen, R.F. (2006) Comparative study of three proteomic quantitative methods, DIGE, cICAT, and iTRAQ, using 2D gel- or LC-MALDI TOF/TOF. *J. Proteome Res.* **5**(3), 651–658.

Zhang, X.W., Wei, D., Yap, Y.L., Li, L., Guo, S.Y., and Chen, F. (2007a) Mass spectrometry-based "omics" technologies in cancer diagnostics. *Mass Spectrom. Rev.* **26**, 403–431.

Zhang, X.W., Li, L., Wei, D., and Chen, F. (2007b) Moving cancer diagnostics from bench to bedside. *Trends Biotechnol.* **25**(4), 166–173.

Zhang, X.W., Yap, Y., Wei, D., Chen, G., and Chen, F. (2008) Novel omics technologies in nutritional research. *Biotechnol. Adv.* **26**, 169–176.