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COMPREHENSIVE REVIEW

Discovery of nutritional biomarkers: future directions based on omics technologies

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Abstract

Understanding the interactions between food and human biology is of utmost importance to facilitate the development of more efficient nutritional interventions that might improve our wellness status and future health outcomes by reducing risk factors for non-transmittable chronic diseases, such as cardiovascular diseases, cancer, obesity and metabolic syndrome. Dissection of the molecular mechanisms that mediate the physiological effects of diets and bioactive compounds is one of the main goals of current nutritional investigation and the food industry as might lead to the discovery of novel biomarkers. It is widely recognized that the availability of robust nutritional biomarkers represents a bottleneck that delays the innovation process of the food industry. In this regard, omics sciences have opened up new avenues of research and opportunities in nutrition. Advances in mass spectrometry, nuclear magnetic resonance, next generation sequencing and microarray technologies allow massive genome, gene expression, proteomic and metabolomic profiling, obtaining a global and in-depth analysis of physiological/pathological scenarios. For this reason, omics platforms are most suitable for the discovery and characterization of novel nutritional markers that will define the nutritional status of both individuals and populations in the near future, and to identify the nutritional bioactive compounds responsible for the health outcomes.

Keywords

Diet, genomics, metabolomics, nutrition, proteomics, transcriptomics

History

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Introduction

Foodomics is an emerging area that results from the application of –omics sciences to the study of nutrition and food disciplines (García-Cañas et al., 2012). Nutrition research has demonstrated a close interaction between diet, lifestyle and health. Currently, the ultimate goal for modern nutrition science is to characterize these associations at the molecular level, identifying which nutrients are responsible for the observed beneficial effects, and how they are processed, absorbed and metabolized in the organism. Understanding the mechanisms underlying these processes is of utmost importance since definition of the cellular pathways involved and the identification of the intermediary molecular effectors will provide novel biomarkers and intervention targets that are urgently needed for the development of more efficient nutritional strategies and applications (Ross, 2010). Emerging –omics and bioinformatics provide technical resources to investigate the correlation between diet/nutrients and health, based on the genome-wide analysis of elementary cellular components. In this context, new research areas have been developed, namely nutrigenetics, nutrigenomics, nutriproteomics and nutrimetabolomics to provide a comprehensive description of the interaction between diet and genes, proteins and metabolites, respectively (Ordovas Munoz, 2013). State of the art technologies including microarrays, next generation sequencing (NGS) and mass spectrometry allow the analysis of biological systems in unprecedented detail. Gene variants and expression, epigenetic regulation, protein levels, isoforms and postranslational modifications as well as low molecular weight molecules can be analyzed on a high throughput bases, leading to the generation of massive amount of data that is then integrated using bioinformatic approaches to deduce functional and regulatory information. These analytical tools have opened up new avenues for research and innovation and are indeed being extensively used for definition of the nutritional status of individuals and populations, predicting and analyzing responses or nutritional interventions, food safety, quality and traceability, development of transgenic food and detection of allergens (Herrero et al., 2012). In this review, we analyze and discuss recent studies that highlight the opportunities provided by the omics technologies for the development of food science and industry.

Biomarkers

During the last few years great effort has been directed towards the identification of biomarkers, with the clinical and pharmaceutical initiatives being pioneers in this area. As a general definition, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention (Biomarkers Definitions Working Group, 2001). Evident roles of biomarkers are diagnosis, prognosis, drug development, drug response, along with prevention and epidemiology. The natural development of a disease involves a progression from early stages that causes mild reversible alteration of cellular homeostasis, towards an increase in the pathological

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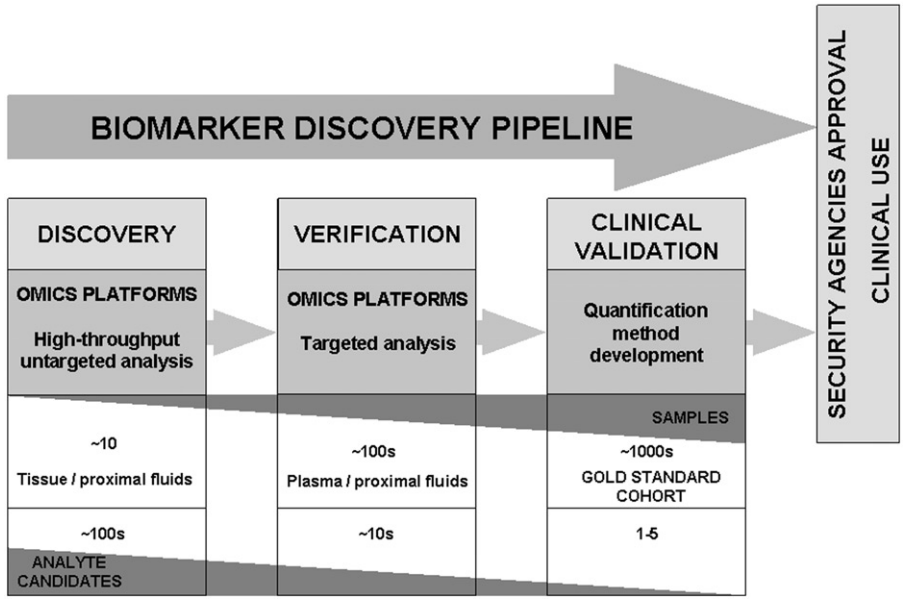

Figure 1. Workflow for the discovery of novel biomarker candidates supported by omics sciences. 'Analyte candidates' applies to the number of molecules expected to be evaluated as candidate biomarkers in each step of the pipeline. ''Samples'' applies to the sample requirements for each step.

abnormality until it becomes irreversible. One of the biggest challenges of modern medicine is the identification of biomarkers that allows early diagnosis of disease and facilitates the appropriate clinical intervention or that predicts responses to drug therapies (Gupta et al., 2014). In this sense, –omics disciplines are being increasingly applied in research and discovery of novel biomarkers in different areas. The high throughput approaches provide an overview of the pathogenic mechanisms analyzing the relative expression of hundreds of genes, proteins or metabolites and, therefore, profiles of the biochemical modifications occurring during the pathogenic process or application of therapy (Diamandis, 2014). The biomarker discovery pipeline is a multiphase process that includes biomarker candidate identification, verification and validation (Figure 1). Biomarker candidates are identified through comparative high throughput techniques, which are applied to a relatively low number of samples. This step requires complex bioinformatic tools to process and analyze the thousands of signals generated. Differentially expressed candidates must then pass through several verification and validation steps that involve the analysis of large number of samples using more selective and directed targeted methods in order to be established as real biomarkers (Rifai et al., 2006). Although it is generally agreed that biomarkers are pivotal in promoting clinical and epidemiologic development, and although many well-funded initiatives are currently underway, to date few novel biomarkers have been approved by regulatory organisms leading to their commercialization and common use in clinical practice. Limitations arise from several issues that still represent unsolved challenges throughout the process from candidate discovery to clinical application. Among the reasons that cause hallmark candidates to fail are the limited knowledge of the physiopathology of the disease, the transfer of the candidates from animal models to humans, the use of undefined signals rather than identified molecules, the need for very large and well characterized sample collections for validation, along with the long-term and high economical costs of these projects (Baker, 2005; Gupta et al., 2014; Lescuyer et al., 2007). Despite all these handicaps, successful examples exist of biomarkers that have been discovered, validated and approved by the Food and Drug Administration (FDA) for cancer diagnosis. The most recently approved in 2012 is phi, a prostate cancer predictor index, which includes three isoforms of the prostate specific antigen (PSA): total PSA, free PSA and pro-PSA that were identified with traditional protein purification methods, such as liquid chromatography. In 2011 and 2009, ROMA and OVA1 were approved. Both are algorithms to predict ovarian cancer malignancy. ROMA combines two proteins, carbohydrate antigen 125 (CA125) and human epididymis protein 4 (HE4) that were identified as over expressed mRNA in ovarian cancer (Hellstrom et al., 2003). And OVA1 includes five proteins (CA125, β2-microglobulin, transferrin, apolipoprotein A1, transthyretin), four of which were identified using the Surface-Enhanced Laser Desorption/Ionization–Time Of Flight (SELDI–TOF) platform (Høgdall et al., 2010). It is important to underline that all three biomarkers mentioned use a panel of multiple proteins to improve prediction performance.

Nutritional biomarkers

To understand interactions between diet, lifestyle and health, informative biomarkers for nutritional status must be defined. However, this is a relatively novel field and application of nutritional biomarkers requires careful attention to circumvent a number of challenging concerns. First, nutritional biomarker definition is still vague and there is no consensus on evaluation, application and purpose of biomarkers. Second, even the best-validated markers available are not sufficiently defined to support recommendation (Combs et al., 2013; Raiten et al., 2011). With the aim of resolving these constraints, several initiatives have been launched, such as the Marker Initiative on Nutrition Research (commissioned by the International Life Sciences Institute (ILSI)), the Institute of Medicine report (commissioned by the FDA), Biomarkers of Nutrition for Development (BOND) (commissioned by the Eunice Kennedy Shriver National Institute of Child Health and Development/National Institute of Health (NICHD/NIH)), the International Agency for Research on Cancer (IARC) Biomarker Group, Diet and Health Research Industry Club among others, to promote the identification and development of new nutritional biomarkers and to synchronize scientific communities in the definition of methods, conditions and settings of nutritional biomarker quantification to create applicable protocols. In addition to this strategic work, extensive research at several levels is also mandatory to provide a molecular understanding of the mechanisms driving the effects of nutrients on health and their interaction

with disease. In this regard, studies with cultured cells and animal models (rodent/non human primates) are of great use to test hypotheses and demonstrate causal effects so as to reveal molecules or combination of molecules that could be used as potential nutritional biomarkers. There are, of course, many practical aspects that must be addressed in order to transform a biochemical entity into a biomarker. These include cutoffs to determine biomarker deficiency or toxicity, standardization of methods for detection, sensitivity, specificity, adequacy of the different biological fluids or tissues, sample recovery and storage. Other issues to be considered are the restrictions inherent to biomarker biology, for example, the genetic variability that prevents applicability in different individuals or populations, and the interaction with other key parameters including age, gender or inflammation (Jenab et al., 2009).

Traditionally, nutritional studies defined the nutritional status of a human being by estimating the dietary intake of a compound through food frequency questionnaires (FFQs) and diet diaries (O'Sullivan et al., 2010). However, due to the limitations inherent to the method, such as external factors and genetic variability, dietary intake assessment methods are prone to measurement errors. Misleading conclusions give rise to variable and contradictory results (Kaaks et al., 1997). These techniques are self reported and dietary profiles are subject to personal considerations, resulting in low accuracy information. Furthermore, data quantification requires food composition databases that contain limited information on many nutrients (Gomez-Cabrero et al., 2014). Moreover, absorption can be influenced by external factors, such as the combination of nutrients, type and degree of processing and lifestyle (Potischman, 2003). It is also recognized that genetic variability affects processes, such as digestion and absorption of nutrients along with their metabolism, turn over and excretion (Allen & Wood, 1994; Borel et al., 1998; Jacob, 1994). These limitations can be at least partially overcome by the application of the –omics sciences, which provide objective measurement of nutritional status biomarkers that can be measured directly in the organism of target individuals (Ordovas Munoz, 2013; Potischman, 2003).

Depending on the information given, nutritional markers can be classified in four groups, although many nutritional biomarkers could be included in several different groups (Aggett et al., 2005; Combs et al., 2013; Raiten et al., 2011):

(1) Exposure/intake biomarkers: these reflect the level of consumption of a nutrient.
(2) Status biomarkers: these show the nutrient levels in the organism.
(3) Function biomarkers: these reveal the role of specific nutrients in the organism.
(4) Effect biomarkers: these reveal how a person responds to a treatment or intervention (biological response, health status or benefits and risk).

Applications of reliable and well-defined markers are many and of huge relevance as, for instance, validation of dietary assessment methods. The most valuable molecular biomarkers for this purpose are recovery biomarkers, such as doubly labelled water (Livingstone & Black, 2003), where the hallmark molecule is an excretion product of the nutrient metabolism. This method assumes that the metabolic balance is based on a correlation between the excretion level of the product and nutrient intake. Nutritional status of individuals can be also assessed through the measurements of nutritional markers, which would allow standardization of normal/pathological levels. Furthermore, regarding target populations, nutritional markers can be useful to infer disease risk associations with diet and the need for interventions. Concentration markers, for example, serum vitamins, lipids or urinary electrolytes are the choice for this purpose (Potischman, 2003). Finally and most importantly, molecular biomarkers allow the effect of specific nutrients upon nutritional interventions to be monitored (Puiggros et al., 2011).

Another emerging application of nutritional biomarkers is the functional description of bioactive compounds. In the last 50 years, a relationship has been established between diet and non-communicable chronic diseases, such as cancer, cardiovascular diseases, obesity and insulin resistance (World Health Organization, 2002). In 2003 the WHO/FAO gathered information that related particular dietary habits to non-desirable health outcomes, describing positive and negative associations for several chronic diseases (World Health Organization, 2003). The positive associations were: dental caries with sugar, obesity with high energy foods and cardiovascular disease with saturated fatty acids, trans-fatty acids and sodium. On the other hand, negative associations were: osteoporosis with vitamin D and calcium, obesity with fiber, fruits and vegetables and cardiovascular disease with linoleic acid, $n-3$ long chain polyunsaturated fatty acids, potassium, fruits and vegetables. This interest in healthy diets has led the food industry to engineer foods reducing the negative elements (low calories, fat free) and incorporating or supplementing them with beneficial nutrients (calcium, vitamins, fiber). These are the so-called functional foods, whose composition has been altered to obtain health benefits. Such nutritional health claims are subsequently evaluated by the competent regulatory agency. To cover the need for regulation in this new field of food claims, the UE developed the Regulation (EC) no. 1924/2006 according to which claims need to be based on and be substantiated by generally accepted scientific data. With respect to the scientific matters, an EU-sponsored and ILSI coordinated project: ''Process for the Assessment of Scientific Support for Claims on Foods'' (PASSCLAIM) created a document defining a set of criteria for the scientific validation of health claims in foods. Among these criteria it is mentioned that when the true endpoint of a claimed benefit cannot be measured directly, studies should use markers. These markers should be characterized on the basis of the molecular mechanisms that establish the association between the marker and the final outcome as well as the variability within the target population.

It is generally agreed that the discovery, development and use of nutritional biomarkers are an urgent need and the lack of these indicators prevents the achievement of diet-based health benefits in the populations and slows health progress. Several stakeholders, therefore, have an interest and should be involved in the biomarker development and validation process, including academia, the food industry, the health sector, regulation agencies, and of course, the consumer. Any biomarker must be carefully validated in three steps: (1) analytical validation: performance assessment, accuracy, precision, repeatability and other issue, such as sample type, sample handling, storage and so on; (2) biological validation, demonstrating that the surrogate measure is highly predictive of the targeted health or disease endpoint and (3) utilization step, determining whether the analytical and biological validation provide sufficient support for the proposed use of the biomarker. Omics technologies are valuable tools to measure biochemical changes associated with advantageous or disadvantageous health outcomes related to diet, to identify nutritional status biomarkers in humans and animals, to monitor nutritional intervention studies, to investigate the molecular mechanisms of bioactive compounds and to incorporate human variability (age differences, inter-ethnic differences or polymorphisms). Omics platforms can also investigate patterns of gene transcripts, proteins and metabolites using *in vitro* models to provide helpful means to validate integrated testing strategies using mechanistic *in vitro* assays to reduce animal studies and move towards predictive modeling (EFSA, 2010).

Omics approaches and biomarker discovery in nutrition sciences

Omics sciences intend to analyze biological systems on a genome-wide scale to provide non-supervised and comprehensive insights into the cellular processes mediating adaptive responses, pathogenic processes and response to therapy or nutritional interventions. According to the type of biomolecule under study, four disciplines can be distinguished: genomics, transcriptomics, proteomics and metabolomics, or more specifically in the nutrition science context, nutrigenomics, nutritranscriptomics, nutriproteomics and nutrimetabolomics. Genomics and transcriptomics are based on microarray technology or next-generation sequencing (NGS), while proteomics and metabolomics are based on combinations of separation procedures (electrophoresis or liquid chromatography) and mass spectrometry. Integration of the resulting data using bioinformatics is the key step for functional and regulatory interpretations of human biology.

As discussed in a recent EFSA scientific report (EFSA, 2014), the main advantage of these omics approaches is their high throughput nature, leading to the possibility of measurement of molecular profiles related to diet. Identification of biochemical signals obtained from nutritranscriptomic, nutriproteomic or nutrimetabolomic comparative experiments is expected to provide novel nutritional biomarkers to foster ambitious nutritional applications for the benefit of all society through the design of improved diets that fit the necessities of each individual according to genetic background and environmental factors.

Nutrigenomics

Since the first human genome was sequenced, the need for faster sequencing methods has greatly increased, leading to the development of second-generation sequencing methods or next-generation sequencing (NGS). NGS platforms achieve massive parallel sequencing in which millions of fragments of DNA from a single sample are simultaneously sequenced (Metzker, 2010). This high-throughput sequencing procedure allows analysis of the whole human genome in less than one day. NGS can also be applied to exome sequencing, providing a summary report of the genome, its expression program and its variants or targeted sequencing for the analysis of particular amplicons that have already proven their value as biomarkers (Grada & Weinbrecht, 2013). Genetic variation affects nutrition at several levels. It is known, for instance, that taste and food choices are genetically determined (Caruso et al., 2012; Garcia-Bailo et al., 2009). Interestingly, the interaction between genetics and nutrition can occur in both directions: genes would determine the effect of the diet on health (nutrigenetics), and on the other hand, nutrients can modulate gene expression through epigenetics or activation of targeted transcription programs (nutrigenomics) (Fenech, 2005). The intricate diet-genetics interaction suggests that some biomarkers could be only valuable for particular segments of a population depending on their specific genetic characteristics. This represents the general use of nutritional biomarkers to properly assess nutritional status or to monitor the effect of nutritional intervention studies in non-targeted populations. Human genetic variation is mainly due to single nucleotide polymorphisms (SNPs) (Wieczorek & Tsongalis, 2001), but also to copy number polymorphisms (CNPs) (Sebat et al., 2004) and specific allele combinations (haplotypes). Nutrigenetics studies have shown that SNPs can influence nutrient metabolism. Recent studies show that nutritional outcome is related to multiple SNPs variants. For instance, Ali et al. (2013) analyzed 91 SNPs from 55 candidate genes in relation to type 2 diabetes (T2D), and suggested five genes as being commonly associated with T2D susceptibility. Moreover, Loria-Kohen et al. (2014) reported the screening of 14 SNPs in nine genes involved in lipid metabolism looking for associations of dairy food consumption and susceptibility for developing cardiovascular disease (CVD), and concluded that one SNP variant of PPARA (rs135549 SNP) was significantly associated with a reduction in the total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) and low-density lipoprotein/HDL-C (LDL/HDL) ratios after 12 month of skimmed milk intake. Therefore, these results suggest that genetic analysis of PPARA rs135549 might be a valuable tool to identify those individuals who are more likely to benefit from reductions of saturated fatty acid in their diet. Delgado-Lista et al. (2014) examined the effect of 904 SNP in the context of metabolic syndrome, and found that several SNPs correlate with different variables of glucose metabolism: fasting glucose, rs26125 (*PPARGC1B*); fasting insulin, rs4759277 (*LRP1*); C-peptide, rs4759277 (*LRP1*); homeostasis assessment of insulin resistance, rs4759277 (*LRP1*); quantitative insulin sensitivity check index, rs184003 (*AGER*); sensitivity index, rs7301876 (*ABCC9*), acute insulin response to glucose, rs290481 (*TCF7L2*) and disposition index, rs12691 (*CEBPA*).

The extra complexity of individual gene regulation resulting from epigenetic events, including DNA methylation (Scarano et al., 2005), histone acetylation (Mai et al., 2005) as well as RNA interference (Almeida & Allshire, 2005), is also worth noting. Nutriepigenetics studies have demonstrated that exposure or restriction of selective nutrients during early stages of life can affect epigenetic patterns and long-term health outcomes (Bendik et al., 2014). For example, it has been shown that restriction of folic acid, methionine or vitamin B-12 before and during pregnancy, and during early childhood, decreases the methylation of a DNA sequence named the differentially methylated region (DMR) of the insulin growth factor 2 (IGF2) gene, increasing the expression of this growth factor (Jang & Serra, 2014).

Epigenetics can also provide markers to meet the need for specific nutrients and to monitor their activity. For instance, the polyphenol epigallocatechin-3-gallate, present in green tea, has been related to the decrease of cancer risk and of rate of ageing. At physiological level this molecule has also been shown to inhibit 5-cytosine DNA methyltransferase, inducing hypomethylation of p16 (*INK4a*), retinoic acid receptor β (*RARβ*), O(6)-methylguaninemethyltransferase (*MGMT*) and human mutL homologue 1 (*hMLH1*) genes in a cancer cell line (Fang et al., 2003). These observations are relevant as they are pioneers in highlighting the role of diet modulating DNA methylation (Fenech, 2005). In addition, Milagro et al. (2011) compare DNA methylation patterns of high and low responders to a hypocaloric diet, identifying novel potential epigenetic biomarkers of weight loss in specific genes. The *ATP10A* and *CD44* genes showed baseline methylation differences depending on the weight-loss outcome. At the treatment endpoint, DNA methylation levels of *WT1* promoter were statistically more methylated in the high than in the low responders.

Nutritranscriptomics

As mentioned above, transcriptomics is based on techniques that allow relative quantification of the expression levels of thousands of genes in a single experiment. SAGE (Serial analysis of gene expression), SuperSAGE and more recently DNA microarray technology have provided technical support for gene expression profiling (Nygaard & Hovig, 2009). The arrival of next-generation sequencing allowing the robust and easy sequencing of an entire human genome in a few days has also led to the possibility of analyzing complete expression programs by

RNA-Seq (RNA Sequencing) experiments, as an alternative to microarrays (Buermans & den Dunnen, 2014). This platform consists of a pre-defined arrangement of a large number of probe sequences, which serve as hybridization templates for RNA or DNA fragments generated from the sample under study (Nygaard & Hovig, 2009). Upon hybridization, statistically significant relative abundances for transcripts in the analyzed conditions are obtained from which a fold-change is deduced. Global expression analysis is often followed by a more detailed interrogation of those specific groups of genes identified as being affected in the first global study. At present, this can be achieved by using customized microarrays (Dondeti et al., 2004) or multi-PCR (multi-polymerase chain reaction) in microfluidic devices, TLDAs (TaqMan Low Density Arrays) (Sorby et al., 2010). In the particular case of nutritranscriptomics, sample availability is more restricted than in the rest of the omics since biological fluids lack cells for RNA extraction, and as such is useful only tissue biopsies or cell lines. For this reason, the use of animal and cellular models has provided valuable insights on gene expression responses to different nutrients or energy restriction (Maciel-Dominguez et al., 2013; Shahzad et al., 2014), circumventing the well-known drawbacks inherent in studies requiring human samples. Shahzad et al. (2014) generated transcriptome data from overfed (OF) or restricted fed (RE) cows to evaluate the impact of energy intake on cow livers during the peripartal period. They found a pivotal role for several lipid-related transcription factors (e.g. PPARs, SREBPs and NFE2L2) in priming the liver of RE cows to better adapt to the early postpartum metabolic and inflammatory challenges (Shahzad et al., 2014). Maciel-Dominguez et al. (2013) compared gene expression profiles of wild-type and peroxisome proliferator-activated receptor deficient mice (*PPARα−/−* mice) fed with specific fatty acids to assess their role in heart tissue and the implications of PPARα. This study showed several genes statistically regulated by all or many of the fatty acids studied, mostly representing well-described targets of PPARs (e.g. *Acot1*, *Angptl4*, *Ucp3*) but also including *Zbtb16/PLZF*, related to immune response and inflammation (Maciel-Dominguez et al., 2013). These results demonstrate the marked impact of dietary fatty acids on gene regulation in the heart *via* PPARα. Studies in animal or cell models give insights of new potential nutritional biomarkers and their mechanism of action, such as the PPAR-regulated pathways.

Hair follicles, epithelial cells from mucosa or desquamated intestinal cells have been proposed as potential human samples but the labile nature of the RNA molecules makes obtaining sufficient material to perform the experiments difficult. For this reason, peripheral blood mononuclear cells (PBMCs) are one of the best options to design human studies (O'Grada et al., 2014; Sanchez et al., 2014; Ulven et al., 2014). O'Grada et al. (2014) compared the gene expression pattern between PBMCs and white adipose tissue on the same individuals showing global similarities, irrespective of the type of metabolic challenge. Closer examination of individual genes revealed this similarity to be strongest in pathways mediating immune response/inflammation. Notably, the expression of metabolism-related nuclear receptors, including PPARs, liver X receptor (LXR), etc. was discordant between tissues (O'Grada et al., 2014). Sanchez et al. (2014) explored peripheral blood gene expression as a source of biomarkers of joint health improvement related to glycosaminoglycan (GAG) intake in humans. Approximately 157 coding genes were differentially expressed, a down-regulation of glucuronidase-beta (*GUSB*), matrix metallopeptidase 23B (*MMP23B*), xylosyltransferase II (*XYLT2*) and heparan sulfate 6-*O*-sulfotransferase 1 (*HS6ST1*) in the supplemented group among them. It is therefore suspected that expression levels of specific genes in blood cells, in particular those related to GAG metabolism and extracellular matrix dynamics, are potential biomarkers of beneficial effects on articular health (Sanchez et al., 2014).

Ulven et al. (2014) reviewed 14 studies of the effect of $n-3$ fatty acids on gene expression in PBMCs, including five genome transcriptome analyses, and highlighted that processes and pathways related to atherosclerotic plaque formation, such as inflammation, oxidative stress response, cell cycle, cell adhesion and apoptosis, were modulated after fish oil supplementation (Ulven et al., 2014). Taken together, these observations lead to the conclusion that PBMC gene expression profiling might clarify further the molecular effects of fish oil consumption on human health and therefore pave the way for the discovery of novel biomarkers.

Although transcriptomics has demonstrated to be a valuable resource for understanding human nutrition and nutritional interventions at the molecular level, it must be emphasized that besides the general concerns of human research regarding annotated sample accessibility and biological diversity, gene expression experiments require standardization, data quality control and data analysis to generate valid and comparable information. Page et al. (2003) and Potter (2003) reviewed the crucial aspects of microarray experimentation in nutritional genomics that must be considered before and during research, including experimental design, sample size, statistical analysis, data verification, data handling and experimental interpretation.

Nutriproteomics

Proteomic approaches can bridge the gap between the content of a particular ingredient in a food matter and its biological end point (Kussmann et al., 2010). Proteomic techniques allow two different type of analyses (Baker et al., 2012):

(1) untargeted high throughput (shotgun) analyses to obtain the sample protein fingerprint, focused on unsupervised mechanistic assessment and biomarker discovery (Picotti et al., 2007), and
(2) targeted analyses (selected reaction monitoring), which detect and quantitate only selected analytes with high sensitivity on triple quadrupole-based platforms (Picotti & Aebersold, 2012), focused on biomarker verification.

Traditionally proteomics has been the platform chosen for drug development and biomarker discovery (Kellner, 2000). The strategy is based on the notion that any pathological process or treatment produces a change at the protein level.

Comparative proteomic profiling combines different methods to analyze complex proteomes and peptidomes identifying differentially expressed proteins with mass spectrometric techniques. Sample collection, storage and protein solubilization, protein/peptide separation, protein identification and bioinformatic analysis are common steps of a typical proteomic analysis (Bodzon-Kulakowska et al., 2007). Proteome coverage is greatly enhanced by the use of fractionation strategies and the subsequent study of subproteomes (Gatto et al., 2010). After isolation and solubilization of the proteome, constituent proteins must be efficiently separated to allow the identification of polypeptides and comparative analysis (Fang & Zhang, 2008; Qian et al., 2006; Vuckovic et al., 2013). There are numerous methodologies to achieve this goal and these can be divided into two major categories. The first and most widely used category is the two-dimensional polyacrylamide gel electrophoresis (2-DE). Proteins are resolved according to two different biochemical parameters, the isoelectric point (pI) in the first dimension and the molecular mass (Mr) in the second dimension (Gorg et al., 2009). Separated proteins are visualized using staining procedures, such as Coomassie blue, silver staining or fluorescent dyes (Miller et al., 2006; Steinberg, 2009). More recently, gel-to-gel variation

was reduced by the so-called Difference Gel Electrophoresis (DIGE) technology where proteins are labelled using different fluorescence dyes (Minden, 2012). However, some restrictions are associated with 2-DE and this prevent a complete description of the proteome. The primary weakness of this method is the relatively low throughput since low abundant, hydrophobic, very acidic or basic and small (Mr lower than 10 kDa) proteins are hardly observed on 2-DE gels (Fey & Larsen, 2001). After separation, spots of interest are excised and analyzed by mass spectrometric methods to allow protein identification. The second major proteomic approach is shotgun proteomics. This is a gel-free alternative consisting of proteolytic digestion of the protein mixture to produce a large collection of peptides that are then subjected to multidimensional liquid chromatography (LC) and tandem mass spectrometry (MS/MS) analysis (Washburn et al., 2001; Wolters et al., 2001). This approach might circumvent some of the typical drawbacks of the 2-DE analysis listed above and provide a means to perform comparative studies by combination of shotgun peptide sequencing and stable isotope labelling. Peptide mixtures from different samples can be chemically (ICAT, isotope-coded affinity tag) (Gygi et al., 1999); ITRAQ, isobaric multiplexing tagging system (Ross et al., 2004), enzymatically (digestion in $H_2{}^{18}O$) (Schnolzer et al., 1996) or metabolically (SILAC, stable isotopic labeling with aminoacids in cell culture) (Ong et al., 2002) labeled using light and heavy isotopes, and then combined equally and analyzed by MS/MS. Differential protein expression profiling, protein interactions or identification of post-translational modifications can be alternatively accomplished by using protein chips (Casal & Barderas, 2010; Lueking et al., 1999; MacBeath & Schreiber, 2000). Although protein arrays have a tremendous potential, the development of protein microarrays for high-throughput proteomics investigation is slow because of the complex nature of proteins. Whatever the analytical procedure used, data obtained from high throughput proteomic analysis must be integrated and processed using appropriate bioinformatic tools to generate valuable biological information from the resulting complex molecular descriptions in formats allowing the validation of data and the exchange among different laboratories.

From a biological concern, nutrients can be considered as signals (Ryan & Seeley, 2013), interacting with transcription factors and modifying the expression of specific genes with the subsequent alteration of the protein profile and metabolite balance or can interact with proteins to regulate their function. In this context, nutriproteomics delivers both nutritional markers and targets for intervention since modulation of proteins may lead to the control of physiological outcomes. Although proteomics is still an emerging technology and has been rarely applied to elucidate the physiological activity of food components, several studies mainly focusing on 2DE have been published describing changes in proteomes upon intervention studies. Fuchs et al. (2007) described changes in the PBMC proteome as a consequence of flaxseed supplementation. Flaxseed contains flax lignans, a group of natural products converted to enterolactone by the intestinal microbiota, which has been shown to mediate atherosclerosis-protective effects. The authors reported the alteration of 16 proteins and interestingly glycoprotein IIIa/II, peroxiredoxin and long-chain fatty acid beta-oxidation multienzyme complex were regulated in a similar manner when blood mononuclear cells were exposed *ex vivo* to enterolactone (Fuchs et al., 2007); Fogle et al. (2010) described, using the ICAT technique, a panel of proteins altered by long-term consumption of alcohol in rat cardiac muscle, and speculated with the relation of this panel with the changes in myocardial wall thickness that was measured under the same conditions. Additionally, multiple search works have been published describing potential biomarkers. For instance, Cervi et al. (2010) describe platelet factor-4 as a biomarker indicative of protection against prostate cancer progression, upon administration of a vitamin E, selenium and lycopene micronutrient cocktail. Moreover, the effect of nutrients not only impact gene and protein expression, but they can also modify the post-translational modification pattern of proteins. Kheterpal et al. (2014) investigated the effects of an extract from *Artemisia dracunculus L.*, termed PMI 5011, on the human skeletal muscle phosphoproteome. PMI 5011 was shown to improve insulin sensitivity by activating cellular insulin signaling in *in vitro* and *in vivo* studies. This phosphoproteomics analysis demonstrated conclusively that PMI 5011 modified the phosphorylation levels of specific proteins and identified novel pathways by which PMI 5011 exerts its insulin-sensitizing effects in skeletal muscle (Kheterpal et al., 2014).

Nutritional research is also using proteomics resources to study food bioactive compounds and their biological effect. Interest in these compounds has increased in recent years due to their capacity to improve long-term health outcome. The characterization of these molecules: quantification in different foods, gut processing, absorption, bioavailability, interaction with target proteins and identification of altered signaling pathways can help development of personalized nutrition and identification of intervention requirements. These compounds include molecules with a different chemical nature: proteins, peptides, fatty acids, flavonoids, phytoestrogens, carotenoids and others (Puiggros et al., 2011). Proteomics has a specific role in the identification of bioactive proteins and peptides and in their functional characterization. Pioneering studies discovered bioactive peptide and proteins from bovine milk and dairy derivates, and more recently additional sources of this type of molecules have been discovered, including legumes, cereals, vegetables and seafood (Kussmann et al., 2010). Independently of the biochemical nature of the bioactive compounds, proteomics offers ample possibilities to decipher the targeted signaling pathways and cellular processes to explain the functional mechanisms and intermediate molecules involved. Also worthy of mention is the study by Frozza et al. who described the antioxidant and anticancer activities of red propolis in the Hep-2 cell line using a 2D approach, leading to the identification mainly of down-regulated proteins (GRP78, PRDX2, LDHB, VIM, TUBA1A, RPLP0 and RAD23B) involved in energy production and conversion, carbohydrate transport and metabolism, post-translational modification, protein turnover and chaperons, cytoskeleton, ribosomal structure and repair pathway (Frozza et al., 2014).

In spite of its indubitable potential, proteomics faces at present several challenges ahead, some of which are inherent to the nature of the human proteome and others which relate specifically to the nutrition context. It must be noted that the highly dynamic behavior together with an abundance and dynamic range spanning 6–12 orders of magnitude, still makes the study of the proteome in its full complexity a challenging task, far beyond the capabilities of current technology (Geiger et al., 2012). Currently, MS instruments have a detection range up to four orders of magnitude with a limit of sensitivity in the range of femtomol or attomol depending on the type of mass spectrometer (Cunsolo et al., 2014). For this reason pre-fractionation of the sample and exhaustive peptide separation is essential to cover the highest range of the proteome. Regarding nutrition, food nutrients and bioactive compounds, unlike drugs, are incorporated into the human body in low concentrations during long periods of time, performing their effect through relatively small changes in the molecular metabolism maintained in a chronic way. For this reason, it is very important to design carefully the experiments to increase the capacity to detect minute changes with statistical significance. Moreover, the application of proteomic techniques

in nutrition science is relatively recent compared with drug development or clinical biomarker discovery. Development of new techniques, with greater sensitivity and higher throughput, will allow the nutriproteomics discipline to contribute with new nutritional biomarkers and bioactive compounds that will foment the progress of personalized nutrition (Kussmann et al., 2010).

Nutrimetabolomics

Metabolomics profiles the type and concentrations of all metabolites of a biological sample, referred to as the metabolome. The metabolome is the consequence of all the genomic, transcriptomic and proteomic activities. Currently, there are two methods widely used for metabolomics experiments in nutrition: proton nuclear magnetic resonance (NMR) (Zhang et al., 2013) and mass spectrometry (MS) technologies (Swann & Claus, 2014). Both techniques have their own advantages and disadvantages. NMR spectroscopy is a particularly powerful tool in metabolic profiling as it enables the detection of a wide range of metabolites in different complex biological samples, with minimal sample preparation. It has been carried out to obtain comprehensive metabolite profiling and pathways of large biological sample set. The main disadvantages of this technique are the low sensitivity and the spectral overlap in complex samples (Claus & Swann, 2013). MS technologies have expanded significantly during the recent years likely due to their great capacity for high throughput screening of low molecular mass molecules as well as for its high sensitivity, rapid and accurate determination of mass and structural information. The most frequently used setup for an MS approach consists of the combination of MS instruments with electrophoretic separation procedures based on liquid or gas chromatography (Ma & Chowdhury, 2013). Both techniques should not be considered as exclusive but rather complementary with the ability to offer broad coverage of the metabolome (Claus & Swann, 2013). On the other hand, advanced data mining and bioinformatics analysis methods for large NMR or MS datasets are also an essential need (Ma & Chowdhury, 2013). Even though the development of new platforms and technologies has largely improved the coverage of the metabolic component of the cell, the huge variety of small molecules integrating the metabolome and their different chemical nature requires the application of enrichment strategies to select specific families according to compatible solubilization and/or chromatographic properties (Wishart, 2011).

Metabolomics has two main focuses in nutrition: research in nutrition and health, and characterization of food composition. Metabolites represent the real endpoints of metabolism and of underlying physiological regulatory processes. Individual metabolites, such as cholesterol, glucose, homocysteine and others are considered markers for health or disease status (Frishman, 1998). Metabolomics when applied to nutrition aims to obtain metabolite profiles to define the molecular mechanism of bioactive compounds, discover status biomarkers and to monitor dietary interventions studies (Brennan, 2013; Kinross et al., 2014). For instance, Batch et al. (2013) investigated the levels of 55 metabolites to identify novel biomarkers that might allow metabolic wellness to be distinguished from metabolic unwellness. They identified branched chain amino acids and related metabolites as promising biomarkers to refine the designation of cardiometabolic risk (Batch et al., 2013). Edmands et al. (2011) work aims to find measurable biomarkers of cruciferous vegetables (CVs) consumption which have been inversely correlated to cancer (breast, lung and bladder), diabetes and cardiovascular and neurological disease. Four NMR spectroscopic peaks were related to CVs intake, one of which was identified as *S*-methyl-l-cysteine sulfoxide (SMCSO) (Edmands et al., 2011).

Novel evidence has suggested that gut microbiota and diet contribute to chronic not transmissible diseases such as obesity, metabolic syndrome and cardiovascular disease. Ridaura et al. (2013) analyzed germ free mice receiving fecal microbiota from female twin pairs with different phenotypes (obese versus lean). Germ free mice developed obese or lean phenotypes when receiving the obese and lean microbiota, respectively. Moreover, when obese and lean mice were cohoused, obese phenotype was reverted, but only when mice were fed with low fat and high fruit and vegetable diet. These findings reveal the effects of diet-by-microbiota interactions (Ridaura et al., 2013). Wang et al. (2011) described three molecules of the dietary lipid phosphatidylcholine metabolism – choline, trimethylamine *N*-oxide (TMAO) and betaine – that predict risk for CVD. Using germ-free mice, they confirmed a critical role for dietary choline and gut flora in TMAO production and in dietary-choline-enhanced atherosclerosis promotion (Wang et al., 2011). In other examples of nutritional intervention studies, metabolic effects caused by the intake of cocoa, chocolate, tea or meat were examined (Cross et al., 2011; Llorach et al., 2013; Van Dorsten et al., 2006). All these studies show the implementation of metabolomic approaches to define bioactive food components and to discover new nutritional status biomarkers.

It is widely recognized that diet affects health outcomes through secondary metabolites but food may also contain biologically active molecules that can act as hormones interacting directly with transcription factors or other proteins (Ryan & Seeley, 2013). Identification of these compounds can lead to food improvement and promotion of health. High throughput metabolomics allows the analysis of hundreds of metabolites in foods. For instance, red propolis is a natural product produced by honeybees with antioxidant and anticancer activities, and its composition is complex. Frozza et al. (2013) use mass spectrometry methods to identify the different molecular ingredients of red propolis, identifying several isoflavones, flavones and high polyphenolic content.

Conclusion

Omics platforms provide both the untargeted high throughput techniques for unsupervised mechanistic studies and biomarker discovery, and the targeted techniques that allow high sensitive quantification of selected groups of molecules. For this reason, omics technologies are valuable tools as they allow the measurement of biochemical changes associated with an advantageous or disadvantageous health outcome related to diet, the identification of nutritional status biomarkers, the monitoring of nutritional intervention studies, the investigation of bioactive compounds molecular mechanisms and the incorporation of human variability (age differences, inter-ethnic differences or polymorphisms). Looking to the future, the greatest challenge for omics research is the integration of all information resulting from the four disciplines, since combination of gene and protein expression patterns with metabolic fingerprints will contribute to the knowledge of the molecular mechanisms responsible for the relationship. This surely promotes the discovery of novel nutritional biomarkers and more efficient interventions.

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Declaration of interest

All authors declare no conflict of interest concerning this supplement.

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References

Aggett PJ, Antoine JM, Asp NG, Bellisle F, Contor L, Cummings JH, Howlett J, et al. 2005. PASSCLAIM: consensus on criteria. Eur J Nutr 44:i5–i30.

Ali S, Chopra R, Manvati S, Singh YP, Kaul N, Behura A, Mahajan A, et al. 2013. Replication of type 2 diabetes candidate genes variations in three geographically unrelated Indian population groups. PLoS One 8: e58881.

Almeida R, Allshire RC. 2005. RNA silencing and genome regulation. Trends Cell Biol 15:251–258.

Allen LH, Wood RJ. 1994. Calcium and phosphorus. In: Shils ME, Olson JA, Shike M, editors. Modern nutrition in health and disease. Philadelphia: Lea & Febiger. p 144–163.

Baker ES, Liu T, Petyuk VA, Burnum-Johnson KE, Ibrahim YM, Anderson GA, Smith RD. 2012. Mass spectrometry for translational proteomics: progress and clinical implications. Genome Med 4:63–74.

Baker M. 2005. In biomarkers we trust? Nat Biotechnol 23:297–304.

Batch BC, Shah SH, Newgard CB, Turer CB, Haynes C, Bain JR, Muehlbauer M, et al. 2013. Branched chain amino acids are novel biomarkers for discrimination of metabolic wellness. Metabolism 62: 961–969.

Bendik I, Friedel A, Roos FF, Weber P, Eggersdorfer M. 2014. Vitamin D: a critical and essential micronutrient for human health. Front Physiol 5:248–262.

Biomarkers Definitions Working Group. 2001. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69:89–95.

Bodzon-Kulakowska A, Bierczynska-Krzysik A, Dylag T, Drabik A, Suder P, Noga M, Jarzebinska J, Silberring J. 2007. Methods for samples preparation in proteomic research. J Chromatogr B Anal Technol Biomed Life Sci 849:1–31.

Borel P, Grolier P, Mekki N, Boirie Y, Rochette Y, Le Roy B, Alexandre-Gouabau MC, et al. 1998. Low and high responders to pharmacological doses of beta-carotene: proportion in the population, mechanisms involved and consequences on beta-carotene metabolism. J Lipid Res 39:2250–2260.

Brennan L. 2013. Metabolomics in nutrition research: current status and perspectives. Biochem Soc Trans 41:670–673.

Buermans HP, den Dunnen JT. 2014. Next generation sequencing technology: advances and applications. Biochim Biophys Acta 1842: 1932–1941.

Caruso MG, Gazzerro P, Notarnicola M, Cisternino AM, Guerra V, Misciagna G, Laezza C, Bifulco M. 2012. Cannabinoid type 1 receptor gene polymorphism and macronutrient intake. J Nutrigenet Nutrigenomics 5:305–313.

Casal JI, Barderas R. 2010. Identification of cancer autoantigens in serum: toward diagnostic/prognostictesting? Mol Diagn Ther 14: 149–154.

Cervi D, Pak B, Venier NA, Sugar LM, Nam RK, Fleshner NE, Klotz LH, Venkateswaran V. 2010. Micronutrients attenuate progression of prostate cancer by elevating the endogenous inhibitor of angiogenesis, platelet factor-4. BMC Cancer 10:258.

Claus SP, Swann JR. 2013. Nutrimetabonomics: applications for nutritional sciences, with specific reference to gut microbial interactions. Annu Rev Food Sci Technol 4:381–399.

Combs Jr GF, Trumbo PR, McKinley MC, Milner J, Studenski S, Kimura T, Watkins SM, Raiten DJ. 2013. Biomarkers in nutrition: new frontiers in research and application. Ann NY Acad Sci 1278:1–10.

Cross AJ, Major JM, Sinha R. 2011. Urinary biomarkers of meat consumption. Cancer Epidemiol Biomarkers Prev 20:1107–1111.

Cunsolo V, Muccilli V, Saletti R, Foti S. 2014. Mass spectrometry in food proteomics: a tutorial. J Mass Spectrom 49:768–784.

Delgado-Lista J, Perez-Martinez P, Solivera J, Garcia-Rios A, Perez-Caballero AI, Lovegrove JA, Drevon CA, et al. 2014. Top single nucleotide polymorphisms affecting carbohydrate metabolism in metabolic syndrome: from the LIPGENE study. J Clin Endocrinol Metab 99:E384–E389.

Diamandis EP. 2014. Present and future of cancerbiomarkers. Clin Chem Lab Med 52:791–794.

Dondeti VR, Sipe CW, Saha MS. 2004. In silico gene selection strategy for custom microarray design. Bio Techniques 37:768–70, 772, 774–776.

Edmands WM, Beckonert OP, Stella C, Campbell A, Lake BG, Lindon JC, Holmes E, Gooderham NJ. 2011. Identification of human urinary biomarkers of cruciferous vegetable consumption by metabonomic profiling. J Proteome Res 10:4513–4521.

EFSA Scientific Committee. 2010. Guidance on human health risk-benefit assessment of foods. EFSA J 8:1673–1713.

EFSA Scientific Report. 2014. Modern methodologies and tools for human hazard assessment of chemicals. EFSA J 12:36–38.

Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, Welsh W, Yang CS. 2003. Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res 63:7563–7570.

Fang X, Zhang WW. 2008. Affinity separation and enrichment methods in proteomic analysis. J Proteomics 71:284–303.

Fenech M. 2005. The genome health clinic and genome health nutrigenomics concepts: diagnosis and nutritional treatment of genome and epigenome damage on an individual basis. Mutagenesis 20:255–269.

Fey SJ, Larsen PM. 2001. 2D or not 2D. Two-dimensional gel electrophoresis. Curr Opin Chem Biol 5:26–33.

Fogle RL, Lynch CJ, Palopoli M, Deiter G, Stanley BA, Vary TC. 2010. Impact of chronic alcohol ingestion on cardiac muscle protein expression. Alcohol ClinExp Res 34:1226–1234.

Frishman WH. 1998. Biologic markers as predictors of cardiovascular disease. Am J Med 104:18S–27S.

Frozza CO, RibeiroTda S, Gambato G, Menti C, Moura S, Pinto PM, Staats CC, et al. 2014. Proteomic analysis identifies differentially expressed proteins after red propolis treatment in Hep-2 cells. Food Chem Toxicol 63:195–204.

Frozza CO, Garcia CS, Gambato G, de Souza MD, Salvador M, Moura S, Padilha FF, et al. 2013. Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. Food Chem Toxicol 52: 137–142.

Fuchs D, Piller R, Linseisen J, Daniel H, 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.

Garcia-Bailo B, Toguri C, Eny KM, El-Sohemy A. 2009. Genetic variation in taste and its influence on food selection. OMICS 13:69–80.

García-Cañas V, Simó C, Herrero M, Ibáñez E, Cifuentes A. 2012. Present and future challenges in food analysis: foodomics. Anal Chem 84:10150–10159.

Gatto L, Vizcaino JA, Hermjakob H, Huber W, Lilley KS. 2010. Organelle proteomics experimental designs and analysis. Proteomics 10:3957–3969.

Geiger T, Wehner A, Schaab C, Cox J, Mann M. 2012. Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. Mol Cell Proteomics 11:M111.014050.

Gomez-Cabrero D, Abugessaisa I, Maier D, Teschendorff A, Merkenschlager M, Gisel A, Ballestar E, et al. 2014. Data integration in the era of omics: current and future challenges. BMC Syst Biol 8:I1.

Gorg A, Drews O, Luck C, Weiland F, Weiss W. 2009. 2-DE with IPGs. Electrophoresis 30:122–132.

Grada A, Weinbrecht K. 2013. Next-generation sequencing: methodology and application. J Invest Dermatol 133:e11.

Gupta S, Venkatesh A, Ray S, Srivastava S. 2014. Challenges and prospects for biomarker research: a current perspective from the developing world. Biochim Biophys Acta 1844:899–908.

Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. 1999. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol 17:994–999.

Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, Drescher C, et al. 2003. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 63:3695–3700.

Herrero M, Simó C, García-Cañas V, Ibáñez E, Cifuentes A. 2012. Foodomics: MS-based strategies in modern food science and nutrition. Mass Spectrom Rev 31:49–69.

Høgdall E, Fung ET, Christensen IJ, Yip C, Nedergaard L, Engelholm SA, Risum S, et al. 2010. Proteomic biomarkers for overall and progression-free survival in ovarian cancer patients. Proteomics Clin Appl 4:940–952.

Jacob RE. 1994. Vitamin C. In: Shils ME, Olson JA, Shike M, editors. Modern nutrition in health and disease. Philadelphia: Lea & Febiger. p 432–448.

Jang H, Serra C. 2014. Nutrition, epigenetics, and diseases. Clin Nutr Res 3:1–8.

Jenab M, Salvini S, van Gils CH, Brustad M, Shakya-Shrestha S, Buijsse B, Verhagen H, et al. 2009. Dietary intakes of retinol, beta-carotene, vitamin D and vitamin E in the European Prospective Investigation into Cancer and Nutrition cohort. Eur J Clin Nutr 63: S150–S178.

Kaaks R, Riboli E, Sinha R. 1997. Biochemical markers of dietary intake. IARC Sci Publ 142:103–126.

Kellner R. 2000. Proteomics. Concepts and perspectives. Fresenius J Anal Chem 366:517–524.

Kheterpal I, Scherp P, Kelley L, Wang Z, Johnson W, Ribnicky D, Cefalu WT. 2014. Bioactives from *Artemisia dracunculus* L. enhance insulin sensitivity via modulation of skeletal muscle protein phosphorylation. Nutrition 30:S43–S51.

Kinross J, Li JV, Muirhead LJ, Nicholson J. 2014. Nutritional modulation of the metabonome: applications of metabolic phenotyping in translational nutritional research. Curr Opin Gastroenterol 30: 196–207.

Kussmann M, Panchaud A, Affolter M. 2010. Proteomics in nutrition: status quo and outlook for biomarkers and bioactives. J Proteome Res 9:4876–4887.

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

Livingstone MB, Black AE. 2003. Markers of the validity of reported energy intake. J Nutr 133:895S–920S.

Loria-Kohen V, Espinosa-Salinas I, Ramirez de Molina A, Casas-Agustench P, Herranz J, Molina S, Fonolla J, et al. 2014. A genetic variant of PPARA modulates cardiovascular risk biomarkers after milk consumption. Nutrition 30:1144–1150.

Lueking A, Horn M, Eickhoff H, Bussow K, Lehrach H, Walter G. 1999. Protein microarrays for gene expression and antibody screening. Anal Biochem 270:103–111.

Llorach R, Urpi-Sarda M, Tulipani S, Garcia-Aloy M, Monagas M, Andres-Lacueva C. 2013. Metabolomic fingerprint in patients at high risk of cardiovascular disease by cocoa intervention. Mol Nutr Food Res 57:962–973.

Ma S, Chowdhury SK. 2013. Data acquisition and data mining techniques for metabolite identification using LC coupled to high-resolution MS. Bioanalysis 5:1285–1297.

MacBeath G, Schreiber SL. 2000. Printing proteins as microarrays for high-throughput function determination. Science 289:1760–1763.

Maciel-Dominguez A, Swan D, Ford D, Hesketh J. 2013. Selenium alters miRNA profile in an intestinal cell line: evidence that miR-185 regulates expression of GPX2 and SEPSH2. Mol Nutr Food Res 57: 2195–2205.

Mai A, Massa S, Rotili D, Cerbara I, Valente S, Pezzi R, Simeoni S, Ragno R. 2005. Histone deacetylation in epigenetics: an attractive target for anticancer therapy. Med Res Rev 25:261–309.

Metzker ML. 2010. Sequencing technologies – the next generation. Nat Rev Genet 11:31–46.

Milagro FI, Campion J, Cordero P, Goyenechea E, Gomez-Uriz AM, Abete I, Zulet MA, Martínez JA. 2011. A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in relation to diet-induced weight loss. FASEB J 25:1378–1389.

Miller I, Crawford J, Gianazza E. 2006. Protein stains for proteomic applications: which, when, why? Proteomics 6:5385–5408.

Minden JS. 2012. DIGE: past and future. Methods Mol Biol 854:3–8.

Nygaard V, Hovig E. 2009. Methods for quantitation of gene expression. Front Biosci 14:552–569.

O'Grada CM, Morine MJ, Morris C, Ryan M, Dillon ET, Walsh M, Gibney ER, et al. 2014. PBMCs reflect the immune component of the WAT transcriptome – implications as biomarkers of metabolic health in the postprandial state. Mol Nutr Food Res 58:808–820.

Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M, et al. 2002. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 1:376–386.

Ordovas Munoz JM. 2013. Predictors of obesity: the ''power'' of the omics. Nutr Hosp 28:63–71.

O'Sullivan A, Gibney MJ, Brennan L. 2011. Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. Am J Clin Nutr 93:314–321.

Page GP, Edwards JW, Barnes S, Weindruch R, Allison DB. 2003. A design and statistical perspective on microarray gene expression studies in nutrition: the need for playful creativity and scientific hard-mindedness. Nutrition 19:997–1000.

Picotti P, Aebersold R, Domon B. 2007. The implications of proteolytic background for shotgun proteomics. Mol Cell Proteomics 6: 1589–1598.

Picotti P, Aebersold R. 2012. Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods 9:555–566.

Potischman N. 2003. Biologic and methodologic issues for nutritional biomarkers. J Nutr 133:875S–880S.

Potter JD. 2003. Epidemiology, cancer genetics and microarrays: making correct inferences, using appropriate designs. Trends Genet 19: 690–695.

Puiggros F, Sola R, Blade C, Salvado MJ, Arola L. 2011. Nutritional biomarkers and foodomic methodologies for qualitative and quantitative analysis of bioactive ingredients in dietary intervention studies. J Chromatogr A 1218:7399–7414.

Qian WJ, Jacobs JM, Liu T, Camp 2nd DG, Smith RD. 2006. Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. Mol Cell Proteomics 5: 1727–1744.

Raiten DJ, Namaste S, Brabin B, Combs Jr G, L'Abbe MR, Wasantwisut E, Darnton-Hill I. 2011. Executive summary – biomarkers of nutrition for development: building a consensus. Am J Clin Nutr 94:633S–650S.

Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, et al. 2013. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341:1241214.

Rifai N, Gillette MA, Carr SA. 2006. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. Nat Biotechnol 24:971–983.

Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, et al. 2004. Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. Mol Cell Proteomics 3:1154–1169.

Ross SA. 2010. Evidence for the relationship between diet and cancer. Exp Oncol 32:137–142.

Ryan KK, Seeley RJ. 2013. Physiology. Food as a hormone. Science 339: 918–919.

Sanchez J, Bonet ML, Keijer J, van Schothorst EM, Mollller I, Chetrit C, Martinez-Puig D, Palou A. 2014. Blood cells transcriptomics as source of potential biomarkers of articular health improvement: effects of oral intake of a rooster combs extract rich in hyaluronic acid. Genes Nutr 9:417–429.

Scarano MI, Strazzullo M, Matarazzo MR, D'Esposito M. 2005. DNA methylation 40 years later: its role in human health and disease. J Cell Physiol 204:21–35.

Schnolzer M, Jedrzejewski P, Lehmann WD. 1996. Protease-catalyzed incorporation of 18O into peptide fragments and its application for protein sequencing by electrospray and matrix-assisted laser desorption/ionization mass spectrometry. Electrophoresis 17: 945–953.

Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Månér S, et al. 2004. Large-scale copy number polymorphism in the human genome. Science 305:525–528.

Shahzad K, Bionaz M, Trevisi E, Bertoni G, Rodriguez-Zas SL, Loor JJ. 2014. Integrative analyses of hepatic differentially expressed genes and blood biomarkers during the peripartal period between dairy cows overfed or restricted-fed energy prepartum. PLoS One 9:e99757.

Sorby LA, Andersen SN, Bukholm IR, Jacobsen MB. 2010. Evaluation of suitable reference genes for normalization of real-time reverse transcription PCR analysis in colon cancer. J Exp Clin Cancer Res 29:144–153.

Steinberg TH. 2009. Protein gel staining methods: an introduction and overview. Methods Enzymol 463:541–563.

Swann JR, Claus SP. 2014. Nutrimetabonomics: nutritional applications of metabolic profiling. Sci Prog 97:41–47.

Ulven SM, Myhrstad MC, Holven KB. 2014. Marine $n-3$ fatty acids and gene expression in peripheral blood mononuclear cells. Curr Cardiovasc Risk Rep 8:412–422.

Van Dorsten FA, Daykin CA, Mulder TP, Van Duynhoven JP. 2006. Metabonomics approach to determine metabolic differences between green tea and black tea consumption. J Agric Food Chem 54: 6929–6938.

Vuckovic D, Dagley LF, Purcell AW, Emili A. 2013. Membrane proteomics by high performance liquid chromatography–tandem mass spectrometry: analytical approaches and challenges. Proteomics 13:404–423.

Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, et al. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472:57–63.

Washburn MP, Wolters D, Yates 3rd JR. 2001. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat Biotechnol 19:242–247.

Wieczorek SJ, Tsongalis GJ. 2001. Pharmacogenomics: will it change the field of medicine? Clin Chim Acta 308:1–8.

Wishart DS. 2011. Advances in metabolite identification. Bioanalysis 3: 1769–1782.

Wolters DA, Washburn MP, Yates 3rd JR. 2001. An automated multidimensional protein identification technology for shotgun proteomics. Anal Chem 73:5683–5690.

World Health Organization. 2002. The world health report 2002: reducing risks, promoting healthy life. Available at: http://www.who.int/whr/2002/en/. Accessed on 13 April 2015.

World Health Organization. 2003. Technical report series: diet, nutrition and the prevention of chronic diseases, vol. 916, pp 1–149. Available at: http://whqlibdoc.who.int/trs/who_trs_916.pdf. Accessed on 13 April 2015.

Zhang AH, Sun H, Qiu S, Wang XJ. 2013. NMR-based metabolomics coupled with pattern recognition methods in biomarker discovery and disease diagnosis. Magn Reson Chem 51:549–556.