REVIEW

Nutrigenomics in human intervention studies: Current status, lessons learned and future perspectives

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Nutrigenomics applications comprise transcript-, proteome- and metabolome-profiling techniques in which responses to diets or individual ingredients are assessed in biological samples. They may also include the characterization of heterogeneity in relevant genes that affect the biological processes. This review explores various areas of nutrition and food sciences in which transcriptome-, proteome- and metabolome-analyses have been applied in human intervention studies, including nutrigenetics aspects and discusses the advantages and limitations of the methodologies. Despite the power of the profiling techniques to generate huge data sets, a critical assessment of the study outcomes emphasizes the current constraints in data interpretation, including huge knowledge gaps, the need for improved study designs and more comprehensive phenotyping of volunteers before selection for study participation. In this respect, nutrigenomics faces the same problems as all other areas of the life sciences, employing the same tools. However, there is a growing trend toward systemic approaches in which different technologies are combined and applied to the same sample, allowing physiological changes to be assessed more robustly throughout all molecular layers of mRNA, protein and metabolite changes. Nutrigenomics is thereby maturing as a branch of the life sciences and is gaining significant recognition in the scientific community.

Keywords:
Intervention studies / Metabolomics / Nutrigenetics / Proteomics / Transcriptomics

1 Introduction

Functional genomics arose with the sequencing of the human genome [1] and the development of high-throughput technologies to investigate genes (genomics), transcripts (transcriptomics), proteins (proteomics) and metabolites (metabolomics). With the adoption of these technologies by the nutritional science field, the term "nutrigenomics" was coined to describe how nutritional factors affect gene and protein functions with a translational output to human health. The last ten years have seen a growing number of human intervention studies in which nutrigenomics technologies were applied for the identification of new biomarkers for the nutritional status, for a better understanding of how gene polymorphisms affect the individual response to nutrients and for defining how food components alter the gene expression as well as downstream processes [2]. It is the aim of the present paper to critically assess the deliverables of these studies for the progress in nutrition and food science and for a better understanding of the influence of human diversity in response to dietary interventions.

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Abbreviations: CVD, cardiovascular disease; **DHA,** docosahexaenoic acid; **EPA,** eicosapentaenoic acid; **MTHFR,** 5,10-methylenetetrahydrofolate reductase; **PBMCs,** peripheral blood mononuclear cells; **SNPs,** single nucleotide polymorphisms; **VOO,** virgin olive oil

2 Transcriptomics

The use of cDNA fragments for the detection of mRNA expression patterns dates back to 1987 when DNA arrays were used to identify changes in the gene expression as modulated by interferon [3]. In 1995, Schena and coworkers published the first application of transcriptomics using microarray glass plates [4]. However, the methods at that time lacked reproducibility, were prone to produce a lot of artifacts and genes were not yet fully annotated. Most of these technological hurdles have been overcome in the recent years; microarray designs have improved, hybridization can be better controlled and gene annotation is much more advanced [5]. Regardless of the transcriptomics platform used, post-technical steps like data normalization is of utmost importance to remove variation derived from different sources (*e.g.* locally weighted scatterplot smoothing or total intensity normalization). A better standardization of the exchange of microarray data between different microarray platforms and technologies has also been accomplished. The Microarray Gene Expression Data organization for example has established Minimum Information About a Microarray Experiment (MIAME) guidelines for microarray data annotation, reporting, sharing and interpretation [6]. This development project is a part of the ''Minimum Information for Biological and Biomedical Investigations'' project, which promotes coherent minimum reporting guidelines for several technologies in biological and biomedical investigations [5].

Although transcriptomics now is highly customized with various platforms available (*e.g.* Affymetrix, Illumina, ABI) and relatively fast and simple, there is still no commonly accepted consensus on how microarray data are best analyzed and which algorithms and statistical tools should be used for producing coherent and reproducible results. In addition, data interpretation also remains a challenge [7].

In nutrition, transcriptomic studies seem unlimited when applied to mammalian cells in culture, or when using primary human cells or tissue samples from animal models, and hundreds of differentially regulated transcripts have been identified following different dietary interventions in different models [8]. Within the last four years, the number of transcriptomics data sets derived from human intervention studies has also steadily increased (Table 1). A wide range of applications have been reported on effects of dietary fatty acids [9–13], probiotics, folic acid [14], olive oil [15–17], different diets [18–31], creatine [32], vitamin E and selenium [33], CoQ_{10} [34], soy isoflavone supplements [35] and broccoli extracts [36]. Although each study reported large numbers of transcripts as up or downregulated, their numbers depend on the cut-off values used and those are generally set low. In this respect, data from most human studies reveal that the intervention produced rather subtle changes in transcript levels. Based on the robustness of the biological systems this may be expected and nutritional means may produce in general less pronounced alterations in mRNA levels than, for example, drugs. Rather short intervention times and the use of healthy subjects in most studies may as well contribute to the rather small changes generally found. Since mRNA levels not necessarily translate into corresponding changes in protein levels or protein functions, the small expression changes reported in these studies must be interpreted with cautions.

The RNA material in the human intervention studies was either derived from whole human blood [27], peripheral blood mono-nuclear cells (PBMCs) [9, 10, 15–17] or subfractions thereof [35]. In addition, tissue samples obtained *via* biopsies were profiled [11, 12, 18–26, 28–34, 36–38]. In human studies, blood or biopsy samples are the two main sources of RNA material and, although these biosamples are readily obtained through minimally or less invasive techniques, they do always comprise a heterogeneous mixture of different cell types with each possessing an unique gene transcript profile [39]. In comparison to whole blood with the over representation of hemoglobin mRNA, for example, or the presence of aberrant mRNA of aged erythrocytes, the use of cellular RNA from a less variable cell population (*e.g.* from PBMCs) appears advantageous [39], although also in these cells, variation in gene expression profiles exists in healthy volunteers [40]. PBMCs for example were used to assess the responses to a 3 wk consumption of 44 g/day of a virgin olive oil (VOO) [17] with the resulting changes in the gene expression, indicating an activation of cell death pathways in cell subpopulations and immune response mechanisms that suggested that there might be an anti-atherogenic activity.

As a general problem in all analyses, the extraction of tissue samples harbors some hurdles. Mutch and coworkers compared the gene expression profiles of needle-aspirated adipose tissue samples with those of surgically obtained samples [41]. They concluded that needle-aspirated biopsies of adipose tissue may result in changes in expression of factors involved in inflammatory and extracellular pathways, which may not be activated when more laborious surgical biopsies, especially for the stroma vascular fraction of adipose tissue, were used. Similar findings were reported for prostate biopsy samples, demonstrating that the expression of genes involved in acute-phase response or cell proliferation may rapidly change due to the sample collection procedure [42].

Validation of observed changes in mRNA profiles by a second method, such as quantitative RT-PCR, is advisable. In most cases, changes in expression levels are confirmed; yet the magnitude of change can be quite different. Bouwens *et al.* reported that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake in humans resulted in a decreased expression of genes in PBMCs involved in inflammatory- and atherogenic-related pathways [9], with most changes observed using microarray analysis being confirmed by quantitative RT-PCR.

In summary, high-throughput gene expression array-based analysis improves constantly with new technologies,

Table 1. Compilation of dietary intervention studies using transcriptomics

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
Camargo *et al.* (2010) [15]	R, DB, Cr study with VOO	Metabolic syndrome patients (20) Age range: 40–70 years	Breakfasts with 40 mL of (i) VOO high (398 ppm) (ii) VOO low (70 ppm) in phenolic compounds	Acute (4 h)	RNA from PBMCs[a)] taken 0, 30, 60 and 240 min after breakfast. GeneChip arrays from all subjects. qRT-PCR for four genes was performed for validation	VVO-based breakfast is able to repress *in vivo* expression of several pro-inflammatory genes; → peripheral blood mononuclear cells switch to a less deleterious inflammatory profile
Bouwens *et al.* (2010) [10]	R, SB, Cr study with fatty acids (PUFA/MUFA/SFA)	Healthy young subjects (21) Age range: 19–27 years	Shakes consisting of low-fat yogurt, skim milk, sugar and (i) 55 g of 65% PUFA (ii) 55 g of 80% MUFA (iii) 55 g of 70% SFA	Acute (6 h)	RNA from PBMCs taken 0 and 60 min after shake intake. GeneChip arrays from all subjects	While PUFA intake decreased, SFA increased the expression of genes in liver X receptor signalling. PUFA also upregulated cellular stress response genes. MUFA intake had an intermediate effect on several genes
Bouwens *et al.* (2009) [9]	R, DB, PI study with fish oil (EPA/DHA)	Healthy elderly subjects (111) Age: >65 years	(i) 0.4 g EPA+DHA (*36*) (i) 1.8 g EPA+DHA (*37*) (ii) Placebo: HOSF (*38*)[a)]	26 wk	RNA from PBMCs; GeneChip arrays of 23 subjects (1.8 g) and 25 subjects (HOSF). qRT-PCR for 6 genes in all 111 subjects	EPA+DHA intake resulted in a decreased expression of genes involved in inflammatory- and atherogenic-related pathways
van Baarlen *et al.* (2009) [37]	R, DB, Cr study with living and heat-killed *Lactobacillus plantarum* bacteria	Healthy young subjects (8); Age: 24 ± 4 years	(i) Living bac. in log-phase (ii) Living bac. in stationary-phase; (iii) Placebo: heat-killed ''stationary'' bacteria	Acute (6 h)	RNA from gastroduodenoscopy; GeneChip arrays. qRT-PCR for six genes for each person and intervention	Consumption of *L. plantarum* prevents adaptive immune responses. Genes involved in i nflammatory immune responses were not expressed or not modulated
van Oostrom *et al.* (2009) [14]	PI pilot study with Folic acid	Patients with Diabetes Mellitus type 1 (20) and healthy subjects (20) Age: 34.2 ± 6.4 years	(i) 5 mg folic acid	4 wk	RNA from endothelial progenitor cells. GeneChip arrays of 11 DM1 patients and 11 healthy controls	Folic acid normalizes endothelial progenitor cell gene expression profiles of patients with type 1 diabetes
Ong *et al.* (2009) [19]	R, SB, PI study with dietary energy restriction	Overweight or obese women at increased risk of breast cancer (19) Age range: 35–45 years	(i) Energy restriction liquid diet (864 kcal/day) (10) (ii) Normal diet (*9*)	28 days	RNA from breast and abdominal tissues (biopsies) before and after intervention. GeneChip arrays from all subjects	Common differentially expressed genes and pathways in breast and abdominal tissue suggests that short-term energy restriction may influence breast cancer risk at the molecular level
Tsavachidou *et al.* (2009) [33]	R, PI, Cr study with selenium and Vit E	Men with prostate cancer (39) Age range: 44–70 years	(i) 400 IU Vit E (ii) 200 μg selenium (iii) Combination of both	3–6 wk	RNA from prostatectomy tissue samples (normal, stromal and tumor cells). GeneChip arrays from all patients. qRT-PCR from 21 genes for validation	Differential gene expression related to selenium and/or vitamin E treatments was identified that was cell type specific and tissue zone specific and that may have clinical implications
Konstantinidou *et al.* (2009) [16]	Pilot study with VOO	Healthy young subjects (60) Age range: 22–28 years	(i) 50 mL of VOO (75% MUFAs; 18.6% PUFAs; 6.4% SFA).	Acute (6 h)	RNA from PBMCs extracted prior and 6h after VOO consumption; GeneChip arrays from all subjects; qRT-PCR from nine genes for validation	VOO supplementation increased expression of genes related to metabolism, cellular processes, cancer and atherosclerosis

Table 1. Continued

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
Van Dijk *et al.* (2009) [11]	PI, study with SFA and MUFA	Abdominally overweight subjects (20) Age range: 45–60 years	(i) SFA diet (19% SFAs/11% MUFAs (*10*) (ii) MUFA (11% SFAs/20% MUFAs (10))	8 wk	RNA from adipose tissue biopsies. GeneChip arrays from all subjects. qRT-PCR from nine genes for validation	SFA increased expression of genes involved in inflammation, without changes in morphology or insulin sensitivity. MUFA led to an anti-inflammatory gene expression profile
Capel *et al.* (2008)[b)] [20]	R, PI study with hypoenergetic diets	Obese subjects from NUGENOB cohort (47) Age range: 20–50 years	Diets 600 kcal/day less than individuals energy requirement consisting of: (i) Low-fat, high-carbohydrate diet (LF) (ii) Moderate-fat, low-carbohydrate diet (MF)	10 wk	RNA from adipose tissue biopsies. GeneChip arrays and candidate gene approach was done for all patients. qRT-PCR was done for validation	Energy restriction had pronounced impact on variations in human adipose tissue gene expression than macronutrient composition. The macronutrient-sensitive regulation of a subset of genes may influence adipose tissue function and metabolic response
Khymenets *et al.* (2008) [17]	Pilot study with VOO	Healthy males (60) and females (4); Age range males: 22–28 years and females 20–44 years	(i) First intervention day: 50 mL (44 g) of VOO(ii) Following days: 25 mL (22 g) of VOO	3 wk	RNA from PBMCs; GeneChip arrays from all subjects. qRT-PCR for 23 genes which were changed most and which have a role in atherosclerosis	VOO supplementation activated the cell death pathway in certain cells and was modulating the immune response toward antiatherogenic protection
Ornish *et al.* (2008) [21]	Pilot study with low-fat diet and lifestyle modifications	Men with low-risk prostate cancer (30); Age: 62$\pm$15 years	Lifestyle modification: (i) Low-fat, whole-foods, plant-based diet, stress management, moderate aerobic exercise, 1-h group support session *per* week	3 months	RNA from prostate needle biopsies; GeneChip arrays from all subjects. qRT-PCR done for validation	Significant modulation of biological processes that have critical roles in tumorigenesis, including protein metabolism and modification, intracellular protein traffic and protein phosphorylation (all $P<0.05$)
Safdar *et al.* (2008) [32]	R, DB, Cr study with creatine monohydrate	Young and healthy non-obese men (12); Age: 26$\pm$3 years	(i) Creatine monohydrate drink (CELL-Tech (10 g CrM)) (ii) Placebo: 75 g dextrose	10 days	RNA from muscle biopsies; GeneChip arrays of all subjects. qRT-PCR for 4 kinases upregulated in the GeneChip	CrM upregulated genes encoding kinases involved in DNA replication and repair, RNA transcription control and cell survival, osmosening and signal transduction *etc.*
Kolehmainen *et al.* (2008) [22]	R, study with weight reduction program	Subjects with impaired fasting glycemia or impaired glucose tolerance and features of metabolic syndrome (46) Age range: 60–77 years	(i) Weight reduction (WR) (28) (ii) Control (normal lifestyle) (*18*)	12 wk	RNA from adipose tissue biopsies; GeneChip assays from WR (10) and control group (10); qRT-PCR validation done in seven genes	Genes regulating the extracellular matrix (tenomodulin-gene) and cell death showed a strong downregulation after long-term weight reduction
Niculescu *et al.* (2007) [35]	R, DB, PI study with soy isoflavones	Healthy, non-obese, postmenopausal women (30) Age: data not available	(i) Soy isoflavones (558 mg genistein; 296 mg daidzein; 44 mg glycitein) (20) (ii) Placebo (10)	84 days	RNA from peripheral lymphocytes extracted at day 1, 84 and 112. GeneChip arrays from all subjects	Isoflavone intake increased steroid hormone receptor activity had some estrogen agonist effects, increased cell differentiation *etc*
Gasper *et al.* (2007) [36]	R, Cr study with broccoli soup	Healthy men and women (16) Age range: 18–46 years	(i) High glucosinolate broccoli soup (2.3 mmol/L) (ii) Standard broccoli soup (0.7 mmol/L) (iii) Placebo (water)	Acute	RNA from gastric mucosa biopsis prior and after intervention. GeneChip arrays done for four subjects, three interventions, two time-points. qRT-PCR from three relevant genes	Only high glucosinolates broccoli consumption led to induction of genes involved in xenobiotic metabolism and other anticarcinogenic, metabolic processes

Table 1. Continued

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
Kabir *et al.* (2007) [12]	R, DB, PI study with PUFA	Women with type 2 diabetes without hypertriglyceridemia (27) Age range: 40–60 years	(i) 3 g fish oil (1.8 g *n*-3 PUFAs) (ii) Placebo (paraffin oil)	2 months	RNA from adipose tissue biopsies prior and after intervention. GeneChip assays done from all subjects. qRT-PCR validation for seven genes in placebo and PUFA group	PUFAs reduced adiposity and atherogenic markers without deterioration of insulin sensitivity in subjects with type 2 diabetes. Some adipose tissue inflammation-related genes were also reduced
Kallio *et al.* (2007) [23]	R, PI study with rye–pasta or oat–wheat–potato diet	Subjects with metabolic syndrome (53) Age: 55 $\pm$ 6 years	Replacement of normal breads and baked products with: (i) Rye–pasta diet (25) (ii) Oat-wheat-potato (28) diet	12 wk	RNA from adipose tissue biopsies prior and after intervention. GeneChip assays done for 10 subjects in both diets. qRT-PCR validation for seven genes	Rye-pasta down-regulated 71 genes, incl. genes linked to insulin signalling and apoptosis. Oat-wheat–potato diet upregulated 62 genes related to stress, cytokine-chemokine-mediated immunity and the interleukin pathway
Lin *et al.* (2007) [24]	Pilot study with low-fat/low-glycemic diet	Newly diagnosed male prostate cancer patients (8) Age range: 59–69 years	(i) Low-fat/low-glycemic diet (4) (ii) Standard American diet (4)	6 wk	RNA from prostate biopsies prior and after intervention. GeneChip arrays from all subjects. qRT-PCR validation done for selected genes	The low-fat/low-glycemic load diet was associated with multiple gene expression changes (*e.g.* they alter proliferation, metabolism and redox potential of prostate epithelial cells
Mangravite *et al.* (2007) [25]	R, PI study with different carbohydrate-(carb) containing diets	Moderately overweight men (131) Age: data not available	Basal diet, then: Randomized diet (ii) Basal diet (ii) Moderate-carb diet; (iii) Low-carb/low-SF diet (iv) Low carb/high-SF diet Then weight loss diet (-1103.0 ± 216.5 kcal/day)	1 wk 3 wk 5 wk	RNA from adipose tissue biopsies prior and after each intervention. Gene Chip arrays from 12 subjects. qRT-PCR validation done for five genes	Stearoyl-coenzyme A desaturase (SCD) expression in response to isocaloric dietary change was most strongly correlated with carbohydrate intake and, with the low-carbohydrate diet, SCD expression was inversely correlated with saturated fat intake
Meugnier *et al.* (2007) [26]	Pilot study with energy excess diet	Lean, young, healthy men (8) Age: 23 $\pm$ 1 years	(i) Diet supplying 30% energy excess ($\sim$550 kcal/day) than the baseline energy requirements	4 wk	RNA from skeletal muscle biopsies prior, after 2 and 4 wk of the intervention. GeneChips arrays were done from all subjects. qRT-PCR validation done for four genes	Energy excess diet stimulated synthesis of triacylglycerol, inhibited lipolysis, reduced fatty acid oxidation and development of adipocytes. By that, it promotes the storage of the excess energy
van Erk *et al.* (2006) [27]	R, DB, Cr study with isocaloric breakfasts	Young and healthy men (8) Age: 20.5 $\pm$ 2.5 years	(i) 400 g high carbohydrate, dairy-based, moderate protein (HC) (ii) 400 g low carbohydrate, dairy-based, high protein (HP)	Acute	RNA from whole blood taken before and 2 h after intake of breakfasts; GeneChip arrays from all subjects	HC: differential expression of glycogen metabolism genes; HP: differential expression of genes involved in protein biosynthesis
Gorjão *et al.* (2006) [13]	Pilot study with fish oil (FO)	Healthy men (10) Age range: 25–45 y	(i) 3 g/day of oil containing 2 6% EPA and 54% DHA	2 months	RNA from lymphocytes taken before and after intervention. GeneChip arrays from all subjects. qRT-PCR done for validation	EPA-rich FO exhibits immunossupressor effects, whereas DHA-rich FO stimulate several aspects of immune function

Table 1. Continued

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
Sparks *et al.* (2006) [28]	Pilot study with high-fat/low-carb diet	Healthy young men who are insulin-sensitive (10) Age: 23±3.1 years	(i) Isoenergetic high-fat/low-carb diet (HF/LCD)	3 wk	RNA from muscle biopsies taken before and after intervention. GeneChip arrays were done from all subjects. qRT-PCR validation for three genes	Diet changed expression of genes involved in the oxidation, storage, or glucose transporter expression. Results suggest a mechanism whereby HF/LCD regulates the genes necessary for glucose utilization and storage by transcriptional control
Di Caro *et al.* (2005) [38]	R, study with probiotic (*Lactobacillus GG)*	Male oesophagitis patients (6) Age range: 38±5 years	(i) *Lactobacillus GG* and Esomeprazole (80 mg/day) (*3*) (ii) Esomeprazole (80 mg/day) (*3*)	1 month	RNA from duodenal mucosa biopsies prior and after intervention. GeneChip arrays from all subjects. qRT-PCR done for validation (10 genes)	*Lactobacillus GG* mainly affected the expression of genes involved in immune response and inflammation, but also *e.g.* apoptosis, cell growth and cell differentiation, cell adhesion
Clément *et al.* (2004) [29]	PI study with very low calorie diet (VLCD)	Obese women (21), severely obese women (8) and healthy women (17) Age range: 39–41 years	(i) VLCD (800 kcal/day) (*21 obese/17 healthy*) (ii) VLCD (650 kcal/day) (*8 obese/17 healthy*)	28 days	RNA from adipose tissue biopsies prior and after intervention. GeneChip assays from all subjects. qRT-PCR validation for 10 genes	Gene expression in obese subjects after 28 day VLCD was close to the profile of lean subjects. Weight loss decreases proinflammatory factors and increases anti-inflammatory molecules

a) R: Randomized, SB: Single-blind, DB: Double-blind, PI: parallel, Cr: cross-over, HOSF: sunflower oil high in oleic acid content, SFA: saturated fatty acids.
b) Three other micro-array studies derived from the NUGENOB cohort are published: Dahlman *et al.* [18], Viguerie *et al.* [30] and Mutch *et al.* [31].

better suited analysis and data interpretation tools and by its high level of customization and automation. Yet, there are still both technical and biological (*i.e.* sample collection and treatment) problems to be addressed before changes in mRNA levels can be taken as predictive for protein expression and functions or as markers of human physiology.

3 Proteomics

Initially, proteomics attempted to deliver an inventory of the proteins present in a cell, an organelle or even in human plasma or other bodily fluids. It has also emerged as a potent technique in understanding the molecular basis of cellular and physiological processes, based on the composition and stoichiometry of protein complexes and their functional organization within pathways [43]. The advantage of proteomics is clearly that it not only allows the identification and quantification of specific proteins that carry the biological function, but also the elucidation of their cellular localization, the identification of protein interactions and of protein modifications by co- or post-translational processing.

Various proteomic technologies have been developed in the recent years including protein microarrays. From all these methods, MS in combination with LC-MS/MS has emerged as the dominant methodology for identification of proteins/peptides and for detecting modifications, due to its ability to acquire high-content quantitative information [44] replacing more and more the classical, but semi-quantitative, methods of separation of proteins *via* two-dimensional gel-electrophoresis (2-DE). In gel-based methods protein spots are isolated and subsequently to proteolytic digestion (usually by trypsin) protein identification is accomplished through peptide mass fingerprinting, mostly *via* MALDI-TOF analysis [45]. So-called shotgun proteomics involves protease-based digestion of the protein sample by multidimensional chromatographic separation of the generated peptide fragments first [46] followed by sequencing and identification *via* MS. It also allows quantitative analysis by employing different labeling techniques. Further technological advances make proteomics a more quantitative and reliable technique, by using different isotopes or tags that can be more efficiently detected by MS. The stable isotope labeling with amino acids (SILAC) method, isotope-coded affinity tags (ICAT) method and isotope tags for relative and quantitative quantization (ITRAQ) method are only a few of the great variety of new technologies that can be applied to proteins derived from human biological samples (for review, see [47]).

In nutrition research, the most common application of MS-proteomics is the comparison of protein expression patterns between two conditions or phenotypic states in biological samples. Although proteomics is accepted as a tool for biomarker identification [48], its use in human dietary intervention trials is still limited [45]. Human studies employing proteomics approaches (listed in Table 2) are aimed mainly at understanding the physiological responses to individual food components [49, 50], revealing new biomarkers for a specific pathological/physiological condition [51, 52] or both [53–55]. Additionally, one study was designed as a proof-of-concept study and validation of the technology for later use of proteomics as a diagnostic tool [56]. A human intervention study with vitamin E used proteomics for biomarker discovery in prostate cancer [57]. In most human studies, PBMCs or plasma samples were used for proteome analysis. From a technical point of view, 2-DE followed by MALDI-TOF and/or LC-MS/MS was employed in six of the reviewed studies, the other two used only MS for fingerprinting [56, 57] or used Western blotting [55]. In one study prior to 2-D fractionation, digestion and identification of proteins, PBMCs obtained after a dietary intervention were incubated with autologous plasma containing ^{35}S-methyonine/cysteine allowing metabolic labeling for the detection of newly synthesized proteins [52].

A major limitation of MS-based proteomic analysis used in human intervention studies is the difficulty to detect proteins present at low concentrations and low abundance. This is a key problem particularly in human plasma in which a few proteins (albumins and immunoglobulins) comprise over 90% of the proteome. Therefore, in most studies using plasma, samples were pre-treated to increase sensitivity by either using ammonium sulfate to deplete albumin [51] or by immunodepletion to remove all high-abundant proteins prior to analysis [50, 54]. Another limitation of proteomics is relatively high costs as well a time needed for sample treatment, handling and analyses, putting constraints on the analysis of large sample sets. Therefore, in the majority of studies only a subset of the samples collected from the study population was submitted to proteome profiling [50, 53], or pooled samples were used [54]. However, in sample pooling a high variability among the individuals may mask the identification of significant effects of treatment. Like other profiling technologies, proteomics is also prone to deliver a high rate of false-positive findings and therefore new methods for data processing have been proposed [58]. Ideally, a second independent method such as antibody-based approaches should be used to confirm the findings. Examples are the study by Aldred *et al.* [51] in which proteomics results were validated by ELISA-based measurements or Griffiths *et al.* [55] using Western blot and enzyme function assays to match the proteome profiling data. However, proteomic analysis appears in some cases to be more sensitive for the detection of diet-induced changes than the analysis of established plasma inflammation markers [53] as shown in a study in which postmenopausal woman underwent dietary isoflavone supplementation. A folic acid supplementation trial in humans applied proteome analysis to plasma samples, combined with measurements in homocysteine and folate levels, and identified changes in proteins of the complement system and coagulation process [50].

Similar to the other profiling methods applied in human nutritional intervention studies, proteomics approaches

Table 2. Compilation of dietary intervention studies using *Proteomics*

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology[a)]	Outcome
Duthie *et al.* (2010) [50]	R, DB, PI study with folic acid	Healthy males (5) and females (15) Age suppl.: 39.9 ± 3.5 years Age placebo: 40.2 ± 2.6 years	(i) 1.2 mg folic acid (10) (ii) Placebo (10)	12 wk	Fasting blood samples; 2DE and LC-MS/MS; determination of folate, 5-methyltetrahydrofolate, *S*-adenosylhomocysteine, homocysteine and uracil misincorporation	Thirty proteins showed subtle changes. This study does not provide strong evidence in either support or against fortification of the population with folic acid
Griffiths *et al.* (2009) [55]	R, DB, PL study with vitamin C	Healthy volunteers (220) Age: data not available	(i) 100 mg Vit C (55) (ii) 500 mg Vit C (55) (iii) 2000 mg Vit C (55) (iv) Placebo (55)	10 wk	Fasting blood samples; Determination of Vit C, potassium, caspase activity and WB of phophoinositol transfer protein	The effect of Vit C on phophoinositol transfer protein was confirmed *in vivo* after indication of this effect by proteomic analysis of cell culture samples
Hoelzl *et al.* (2008) [52]	Pilot study with Brussels sprouts	Healthy males (3) and females (2) Age: 33 ± 7 years	(i) 300 g Brussels sprouts	5 days	PBMC samples were metabolically labelled; 2DE and LC-MS/MS	Two proteins with anti-carcinogenic properties were upregulated
de Roos *et al.* (2008) [54]	R, DB, PI study with fish oil	Healthy males and females (81) Age range: 50–70 years	(i) 3.5 g HOSF (42)[a)] (ii) Placebo: 3.5 g fish oil (39)	12 wk	Non-fasting serum samples in eight pools *per* group; 2DE and MALDI-TOF and LC-MS/MS	Ten serum proteins involved in inflammation and lipid-modulating pathways were downregulated after fish consumption. These proteins suggested as biomarkers of development of coronary heart disease
Fuchs *et al.* (2007) [49]	Pilot study with flaxseeds	Healthy males (7) Age range: 20–40 years	(i) Flaxseeds: 0.4 g/kg body weight	1 wk	Fasting PBMC samples; 2DE and MALDI-TOF; determination of enterolactone	Nine regulated proteins associated with atherosclerosis and stress response. Further studies required for determining health impact
Fuchs *et al.* (2007) [53]	Cr study with isoflavone	Postmenopausal women (10) Age range: 45–70 years	(i) Cereal bars with 50 mg isoflavone extract (ii) Placebo: cereal bars without isoflavone	8 wk	PBMC samples; 2DE and MALDI-TOF	Expression of 29 proteins altered involved in inflammation processes, but no alterations in clinically relevant inflammation markers observed
Aldred *et al.* (2006) [51]	R, DB, PI study with Vit E	Healthy males (21) and females (11) Age range: 21–46 years	(i) 200 IU Vit E (11) (ii) 400 IU Vit E (11) (iii) Placebo (10)	28 days	Fasting blood samples; 2DE and MALDI-TOF; determination α-tocopherol	Apolipoprotein A1 is a biomarker of α-tocopherol effects

Table 2. Continued

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology[a)]	Outcome
Mitchell *et al.* (2005) [56]	R, B, Cr with different cruciferous diets	Healthy men and women (38) Age range: 20–40 years	Basal diet with: (i) 436 g cruciferous vegetables (ii) 190 g allium vegetables (iii) 270 g apiceous vegetables (iv) Diet devoid of fruit and vegetables	7 days	Fasting blood samples after each diet; MALDI-MS	This intervention study was successfully used to assess the validity of this methodology for biomarker discovery in other studies
Kim *et al.* (2005) [57]	Pilot study with Vit E and selenium	Prostate cancer patients (48) and healthy age-matched males (29) Age: data not available	Only patients received: (i) 400 IU Vit E (11) (ii) 200 μg selenium (9) (iii) Combination of both (10) (iv) Placebo (9)	3–6 wk	Serum samples; MS profiles	Analysis of serologic protein patterns that could be used to follow-up the success of a prostate treatment

a) R: Randomized, B: Blind; DB: Double-blind, PI: parallel, cr: cross-over, HOSF: sunflower oil high in oleic acid content; WB: Western blotting

mostly revealed only subtle changes in protein levels. In contrast to transcriptomics, proteomics faces the problem of a huge dynamic concentration range of the proteins in a given sample and sensitivity limits for identifying low-abundant proteins. A current limitation in most trials is also the rather small sample number usually analyzed as well as the difficulties to reach meaningful conclusions from the findings.

4 Metabolomics

The metabolome in a biological sample reflects a highly complex mixture of small molecules from all chemical classes. Alterations in the metabolome are consequently derived from differences in food intake and an individual's metabolic condition, which itself results from gene expression and protein levels of all metabolically relevant control systems. In this respect, the metabolome serves as a surrogate of metabolic status and adaptation to changing environmental cues. Two terms – ''metabolomics'' and ''metabonomics'' – have been introduced to describe metabolite profiling in biological samples, such as blood or urine, or applied to cells and tissues. The term metabonomics was defined as the ''quantitative measurement of time-related multiparametric metabolic responses of multi-cellular systems to pathophysiological stimuli or genetic modification'', whereas metabolomics covers a wider application range [59–62]. The terms are interchangeable thus, for simplicity, we use the term metabolomics in the current review [63].

The technological advances in NMR- and MS-based analysis allow the measurement of several hundreds of metabolites in small volumes of a single sample. Data sets produced are usually huge, multidimensional and need to be explored using multivariate analysis tools to identify novel molecules that may serve as reporters or markers. A first draft of the human metabolome was reported in 2007 [64, 65]. Nutritional studies have taken advantage of metabolomics approaches to explore the efficacy of nutrients and diets and for identification of novel biomarkers with predictive power for clinical outcomes [47, 66]. NMR and MS methods are highly complementary to cover as much of the metabolome as possible [67–69]. ^{1}H-NMR spectroscopy allows quick analysis with no need for sophisticated sample treatment. Yet, the sensitivity for detection of low abundance metabolites is limited and MS-based methods are here by far superior. For MS-based analysis samples are submitted to different separation techniques (*e.g.* GC, LC, UPLC, HPLC or CE) followed by MS detection. The latter methods provide advantages when measuring specific categories of chemical compounds, in particular when ''known'' compounds are analyzed, and have enabled the establishment of subcategories such as lipidomics [70, 71].

Although the different technology platforms *per se* deliver large and different data sets in high-throughput mode,

interpretation of the data is still challenging. Different multivariate biostatistical approaches are applied to detect rather subtle signal changes when metabolomics is employed in human studies [72, 73]. The metabolite data sets can be mined using a range of pattern recognition techniques that include principal component analysis, partial least squares discriminant analysis and hierarchical clustering analysis. However, these analyses are solely descriptive in nature and do not allow any interpretation of the origin of the changes.

Only very recently, nutritional intervention studies have incorporated metabolomics approaches to monitor an individual's health status or to link food intake with metabolomic signatures (Table 3). In the majority of the studies either the effects of different diets or individual food items were analyzed [74–80]. Examples are the effects of tea [81–84], chocolate and cocoa [85–87] or vitamins and selected nutraceuticals [88–91], on plasma or urine metabolite profiles. Mostly non-targeted analyses were performed while only a few studies used targeted approaches. The very first nutritional supplementation study using metabolomics was published in 2005; Daykin *et al.* applied NMR methodology and identified a previously unknown black tea metabolite [81]. MS-based metabolomics in human studies was first used in 2007 [76]. Walsh *et al.* combined NMR and MS methods showing acute changes in urinary profiles after consumption of a standardized diet containing phytochemicals. In addition to urine and/or blood plasma samples, red blood cell lysate samples were also analyzed [91].

Although the 18 intervention studies identified in our literature search indicated some correlations between metabolites, diets and health status (Table 3), the overall outcome is rather moderate with respect to the knowledge gained. Yet, metabolomics is still in its infancy and metabolomes recorded usually include a huge number of ''unknowns'' meaning signals with as yet unidentified chemical structures. The targeted metabolomics approaches are limited by a lower number of quantifiable metabolites that represent only a small subset of all possible metabolites. Metabolomics applications in general are not as comprehensive as transcriptomics approaches.

5 Nutrigenetics

Nutrigenetics tries to elucidate how genetic heterogeneity and nutritional components act jointly in determining an individual's responses to food and/or the risk for developing a condition or disease [92]. Currently, the majority of nutrigenetics research efforts are focused on single nucleotide polymorphisms (SNPs), which are variations with a frequency of more than 1% and they account for 90% of all human genetic variation [93]. SNP-genotyping is a rapidly developing field and the choice of the most suitable technique for nutrigenetics depends strongly on the research question and the study material. In large epidemiological studies, typical methods used are fluorescence signal-based detection (*e.g.* TaqMan®, Applied Biosystems), chemiluminescence (*e.g.* Pyrosequencing™, Biotage, Sweden) or a combination of two or more allele discrimination approaches (*e.g.* BeadArray™, Illumina, CA). In nutritional studies, enzymatic cleavage for allele discrimination by RFLP (restriction fragment length polymorphism) [94] has regularly been employed. Detailed descriptions of the over 100 existing genotyping methods can be found elsewhere [95, 96].

One of the most thoroughly studied examples of human nutritional interventions using nutrigenetics is folate supplementation and genetic variations in the respective pathways of one-carbon metabolism; in particular, the common C677T polymorphism in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene. MTHFR has a role in supplying 5-methylenetetrahydrofolate, which is necessary for the remethylation of homocysteine to methionine, and the *MTHFR* C677T genetic variant impacts on MTHFR enzyme activity. Carriers of the mutant allele have up to a 60% decrease in enzymatic activity leading to mild hyperhomocysteinemia [97, 98], and which, together with low dietary intake of folate and other B vitamins, may contribute to the risk of cardiovascular disease. A recent randomised controlled trial in 203 healthy Japanese males demonstrated that *MTHFR* C677T TT homozygote participants, receiving 1 mg folic acid orally *per* day, showed the largest decrease in plasma total homocysteine (tHcy) levels at both 1 and 3 months after the start of the intervention, with an estimated effect size of 2.4 [99, 100].

Another example of nutrigenetics research concerns the role of antioxidant (vitamin E) supplementation in the prevention of cardiovascular disease. Numerous placebo-controlled, randomised clinical trials have been unable to demonstrate the benefit of vitamin E supplementation on cardiovascular disease risk and, in 2005, a meta-analysis by Miller *et al.* even indicated that high-dose vitamin E supplementation may increase all-cause mortality. However, Blum *et al.* [101] recently published a meta-analysis of two independent placebo-controlled trials (ICARE and HOPE), which revealed that haptoglobin 2-2 individuals significantly benefited from vitamin E supplementation, with an over 40% reduction in the combined end-point of stroke and cardiovascular death.

New epidemiological findings based on genome-wide association studies are being published almost every day in identifying SNPs or haplotypes associated with diet effects and diet–disease relationships. A detailed compilation of human intervention studies using nutrigenetics was not the focus of the present review. However, in view of developments in personalized medicine and individual nutritional needs, genotyping and identification of allele risk carriers will be an intrinsic part. In particular, nutrigenetics will enable a better characterization and stratification of subjects prior to enrollment in a human intervention study and should thus minimize variation. It can be expected that next

Table 3. Compilation of dietary intervention studies using *Metabolomics*

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
Lee *et al.* (2010) [91]	Pilot study with *N*-acetyl-L-cysteine (NAC)	Healthy untrained male (*1*) Age: 22 years	(i) 5.7 g NAC (1)	5 days	RBC lysates; untargeted analysis; capillary electrophoresis-electrospray ionization-mass spectrometry (CE-ESI-MS)	NAC supplementation dampened oxidative stress marker metabolites (oxidized glutathione (GSSG), reduced glutathione (GSH), 3-methylhistidine, L-carnitine, *O*-acetyl-L-carnitine and creatine)
Chorell *et al.* (2009) [79]	Cr, R study with low- and high-carbohydrate sports beverages	Young men in regular exercise training (24) Age: 25.7±2.7 years	(i) Low carbohydrate (16% w/v) (ii) High carbohydrate (24% w/v) (iii) Low carbohydrate-protein (16% w/v) and protein (iv) Placebo	Acute	Serum samples; untargeted analysis; GC-MS	Metabolomics approach suggested pseudouridine as a novel marker for pro-anabolic effect following low-carbohydrate–protein and protein ingestion
Lakinen *et al.* (2009) [80]	PI, R open study with different fish diets	Subjects that had myocardial infarction or unstable ischemic attack (33) Age: under 70 years	(i) 100–150 g fatty fish/meal at least four times a week (11) (ii) 100–150 g lean fish/meal at least four times a week (12) (iii) Control lean meat (10)	8 wk	Plasma samples; targeted analysis; GC FID and UPLC-ESI-MS	Serum lipids (*e.g.* ceramides, lysophosphatidylcholines, diacylglycerols) found affected by the fatty fish diet are associated with insulin signalling and inflammation
Llorach *et al.* (2009) [86]	Cr, R study with cocoa powder in milk	Healthy women (5) and men (5) Age range: 18–50 years	(i) 40 g cocoa powder in 250 mL milk (ii) 40 g cocoa powder in 250 mL water (iii) 250 mL milk	Acute	Spot urine samples; untargeted analysis; HPLC-q-ToF	After cocoa intake, 27 putative or confirmed metabolites were identified as the main contributors to the urinary metabolome modification
Martin *et al.* (2009) [87]	R, PI open study with dark chocolate	Healthy males (11) and females (19) Age range: 18–35 years	(i) 40 g of dark chocolate	2 wk	Plasma samples and spot urine; untargeted analysis; ^{1}H-NMR and GC and LC-MS/MS	Dark chocolate reduced the urinary excretion of the stress hormone cortisol and catecholamines and partially normalized stress-related differences in energy metabolism and gut microbial activities
McCombie *et al.* (2009) [89]	R, DB, PI study with fish oil LC *n*-3 PUFAs (DHA/EPA)[a)]	Overweight or obese and hyperinsulinaemic women (93) Age range: 21–69 years	(i) 1.3 g EPA+ 2.9 g DHA+ weight loss program (35) (ii) Placebo: 1.4 g OA+2.8 LA+weight loss program (32) (iii) Placebo: 1.4 g OA+2.8 LA+(26)	24 wk	Plasma samples; untargeted analysis; ^{1}H-NMR, GC- and LC-MS	The fish oil supplementation in conjunction with weight loss reduced the total serum TAG content. Weight loss without fish oil supplementation decreased fatty acid 22:5, which was increased in the fish oil weight loss group
Micceli *et al.* (2009) [83]	Cr, DB, R study with green tea-based sports drink	Male athletes (44) Age range: 18–34 years	(i) 700±250 mL green tea-based carbohydrate-hydroelectrolyte drink (ii) 750±250 mL oligomineral water	Acute	Plasma and spot urine samples; untargeted analysis; ^{1}H-NMR	Within-individual variance using multilevel PLS-DA showed that the sports drink had an effect on glucose, citrate and lactate in plasma and acetone, 3-OH-butyrate and lactate in urine after strenuous exercise. The observed changes were smaller than the biological variations between individuals
van Velzen *et al.* (2009) [84]	Cr, R, DB study with dried black tea extract	Non-smoking healthy men (20) Age range: 18–40 years	(i) 2.5 g dried black tea extract capsule (ii) Red grape extract capsule (iii) Placebo	Acute	Spot urine; untargeted analysis; ^{1}H-NMR metabolic profiling	A selected set of urinary biomarkers fitted the one compartment model. They include hippuric acid, 4-hydroxyhippuric acid and 1,3-dihydroxyphenyl-2-*O*-sulfate

Table 3. Continued

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
						and derived from microbial fermentation of polyphenols in the gut
Coolen *et al.* (2008) [88]	Cr open study with vitamin E and vitamin C combination	Patients diagnosed with intermittent claudication and healthy controls (17) Patients (*10 males, 3 females*) Age range: 51–79 years Controls (3 males, 1 *female*) Age range: 65–79 years	(i) Patients took 80 mg aspirin (13) daily (ii) All study participants got vitamins E and C (17)	4 wk	Plasma and spot urine samples; untargeted analysis; ^{1}H-NMR	^{1}H-NMR revealed an effect in line with anaerobic ATP production via glycolysis in exercising (ischaemic) muscle of the claudicants. Intervention altered muscle bioenergetics in claudicants (lower concentrations of lactate and glucose and several other citric acid cycle metabolites, whereas acetoacetate was increased)
Pers *et al.* (2008) [77]	Sub-cohort analysis of a multicenter study using a high-fat test meal	Obese women (100) selected from the original NUGENOB cohort	High-fat test meal containing double-cream with 40 g fat/100 g. Nearly 95% of the energy in the meal was provided as fat and with 60% as saturated fat (100)	Acute	Plasma samples; untargeted analysis; ^{1}H-NMR and LC-MS	The specificity and sensitivity values were moderate. Therefore, fat oxidation capacity might possibly be only reflected in subtle differences in the metabolic profiles analyzed
Schwab *et al.* (2008) [78]	PI, R open study applying a weight reduction program	Overweight or obese females (11) and males (8) with IFG or IGT and metabolic syndrome (19) Age range: 40–70 years	(i) Weight reduction with decreased energy intake (9) (ii) Control (10)	12 wk	Plasma samples; untargeted analysis; GC FID and UPLC-MS	Diet-induced weight loss resulted in marked changes in the lipidomic profile in middle-aged and older men and women with IFG or IGT and insulin resistance and metabolic syndrome. Saturated and short chain TG and PC levels were reduced
Rezzi *et al.* (2007) [85]	Cr, R study with chocolate	Healthy chocolate desiring men (11) and chocolate indifferent men (11) Age range: 19–54 years	(i) 50 g commercially available chocolate (ii) Placebo (bread)	2 days	Plasma and 24 h urine samples; untargeted analysis; ^{1}H-NMR	The specific dietary preferences can influence basal metabolic state and gut microbiome activity., which is independent of the ingested food as chocolate *versus* placebo has no direct effect
Walsh *et al.* (2007) [76]	Cr study with a phytochemical diet	Healthy women (12) and men (9) Age range: 20–34 years	(i) Standardized phytochemical diet (ii) Low-phytochemical diet	2 days	Spot urine samples; untargeted analysis; ^{1}H-NMR and LC-MS	Acute changes in urinary metabolomic profiles occur after the consumption of dietary phytochemicals. Dietary restrictions in the 24 h before sample collection may reduce diversity in phytochemical intakes to improve data interpretation in metabolomics using urine
Stella *et al.* (2006) [75]	Cr, R open study with standardized meals: low-meat diet; high red meat diet; vegetarian diet	Healthy men (12) Age range: 25–74 years	(i) Low-meat diet (60 g protein) (ii) High red meat diet (420 g protein) (iii) Vegetarian diet (420 g protein)	15 days	24 h urine samples; untargeted analysis; ^{1}H-NMR	Creatine, carnitine, acetylcarnitine and trimethylamine-*N*-oxide (TMAO) have been elevated in the high-meat consumption period. L-carnitine was absent in a vegetarian diet, while *p*-hydroxyphenylacetate was higher in the vegetarian than meat diet samples

Table 3. Continued

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
van Dorsten *et al.* (2006) [82]	Cr, R study with black tea and green tea	Non-smoking healthy men (17) Age range: 20–70 years	(i) 6 g of dissolved black tea solids (ii) 6 g of dissolved green tea solids (iii) 360 mg caffeine control	2 days	Plasma and 24-h urine samples; untargeted analysis; ^{1}H-NMR	Green and black tea intake also had a different impact on endogenous metabolites in urine and plasma. Green tea intake caused a stronger increase in urinary excretion of several citric acid cycle intermediates
Daykin *et al.* (2005) [81]	Pilot study with decaffeinated black tea extract	Healthy women and one man (3) Age range: 22–44 years	(i) 3 g of decaffeinated black tea extract dissolved (dose equivalent to six cups)	Acute	Urine samples; untargeted analysis; ^{1}H-NMR metabolic profiling; New compound identification by HPLC coupled to MS and ^{1}H NMR	Untargeted analysis interpreted with the use of pattern recognition techniques identified hippuric acid as the major urinary black tea metabolite. One previously unknown black tea metabolite was identified as a sulfate conjugate of pyrogallol
Solanky *et al.* (2005) [74]	PI study with conjugated and unconjugated soy isoflavone diets	Healthy premenopausal, non-vegetarian women (9) Age range: 21–29 years	(i) Diet with 60 g textured vegetable protein containing 45 mg conjugated isoflavone glucosides (6) (ii) Basal diet with 50 g miso containing 25 mg unconjugated isoflavones (*3*)	One full menstrual cycle period	24 h urine samples; untargeted analysis; ^{1}H-NMR and 2D ^{1}H-^{1}H-NMR spectroscopy	Observed changes in endogenous methylamine pathway intermediates and corresponding modification in the urine concentration of choline, betaine, glycine and acetate, suggest soy consumption generated changes in lipid and cholesterol metabolism and transport

a) R: Randomized, DB: Double-blind, PI: parallel, Cr: cross-over, RBC: red blood cells, OA: oleic acid, LA: linoleic acid, sunflower oil high in oleic acid content, TAG: triacylglycerides, UPLC-ESI-MS: ultra-performance liquid chromatography coupled to electrospray ionization mass spectrometry and gas chromatography, PC: phosphatidylcholines, LPC: lysophosphatidylcholines, PL: phospholipids, TG: triacylglycerols; IFG: impaired fasting glucose, IGT: impaired glucose tolerance, PLS-DA: partial-least squares-discriminant analysis, GC-FID: GC flame ionization detection.

Table 4. Compilation of dietary intervention studies applying multiple *"-omics"* technologies

Ref.	Dietary intervention[a)]	Target population (*n*)	Dose *per* day (*Subjects*)	Duration	Methodology	Outcome
Bakker *et al.* (2010) [90]	R, DB, PI study with nutritional supplement	Healthy overweight men with mildly elevated plasma CRP (36)	(i) Resveratrol: 6.3 mg, green tea extract: 94.5 mg, α-tocopherol: 90.7 mg, vitamin C: 125 mg, PUFAs: 380 mg EPA/260 mg DHA/60 mg other ω-3, tomato extract: 3.75 mg lycopene (ii) Placebo	5 wk	RNA from PBMCs and adipose tissue. GeneChip arrays done for all subjects. LC-MS was used for plasma lipids and FFAs; GC-MS was used for other metabolites in plasma and urine samples. Proteomics analysis for 33 subjects	Nutritional supplement modulated inflammation of adipose tissue, improved endothelial function, affected oxidative stress and increased liver fatty acid oxidation
Linnane *et al.* (2002) [34]	R, PI study with CoQ_{10}	Age: <55 years Healthy, elderly subjects (14)	(i) CoQ_{10} (300 mg) dissolved in oil (7)	4 wk	RNA from muscle biopsies prior and after intervention. GeneChip arrays were done for 3 CoQ_{10} and 2 placebo subjects. Proteomics analysis (2DE)	CoQ_{10} has wide effects on overall tissue metabolism. It also plays a major role in the determination of membrane potential
		Age: 57–79 years	(ii) Placebo (oil) (7)			

a) R: Randomized, DB: Double-blind, PI: parallel.

generation sequencing with whole genome analysis [102] will rapidly move also into the nutrition field expanding rapidly the knowledge on how the individual's genome responds to a dietary intervention.

6 Concluding remarks

The term nutrigenomics was coined ten years ago to describe a branch of nutrition and food research that applies new profiling techniques for transcripts, proteins and metabolites to better understand the interplay of the genome with its nutritional environment. In this respect, nutrigenomics is still in its infancy and it will need time until it really delivers what was hoped. Although all technologies are attractive to use and promise to deliver huge data sets with new insights into mechanisms for dietary adaptation, we have learned that it is not trivial to collect the data and it is even more difficult to analyze and extract meaning from them. Although the number of nutrigenomics studies is rapidly growing, most applications are still of descriptive nature. Technologies are maturing but reproducibility and robustness are still in need of improvement. In contrast, nutrigenetics with SNP or haplotype analysis is more easily performed is highly accurate and robust from a technological perspective. Genetic variations in numerous genes can be associated with disease risks and susceptibility, although each SNP alone may have only a minor impact. However, each individual's susceptibility can be modulated by diet and this forms one of the conceptual lay-outs for nutritional requirements on an individual level. It can be predicted that future studies will employ all combinations of the different nutrigenomics techniques in the same study (as shown in Table 4) and including comprehensive genotyping of the volunteers. Yet, it can also be foreseen that huge knowledge gaps remain in the understanding of human nutrition and physiology.

In conclusion, the published human studies employing functional genomics methods related to human nutrition have so far mainly "proof-of-concept" character. They have demonstrated that various dietary components do affect gene expression, protein levels and the metabolite spectrum. In addition to the technical limitations described for each methodology, huge challenges remain with respect to evaluation, interpretation and matching of the results derived from the different platforms. The studies reviewed lack in particular, comparability, reproducibility and validation with established biomarkers. This argues for more concerted actions, larger study cohorts and collaborative research efforts with expert from different disciplines.

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