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## Inter-individual differences in response to dietary intervention: Integrating omics platforms toward personalised dietary recommendations

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### Abstract

Technologic advances now make it possible to collect large amounts of genetic, epigenetic, metabolomic, and gut microbiome data. These data have the potential to transform approaches toward nutrition counseling by allowing us to recognize and embrace the metabolic, physiologic and genetic differences among individuals. The ultimate goal is to be able to integrate these multi-dimensional data so as to characterize the health status and disease risk of an individual and to provide personalised recommendations to maximize health. To this end, accurate and predictive systems-based measures of health are needed that incorporate molecular signatures of genes, transcripts, proteins, metabolites, and microbes. Although we are making progress within each of these omics arenas, we have yet to integrate effectively multiple sources of biologic data so as to provide comprehensive phenotypic profiles. Observational studies have provided some insights into associative interactions between genetic or phenotypic variation and diet and their impact on health; however, few human experimental studies have addressed these relationships. Dietary interventions that test prescribed diets in well-characterized study populations and that monitor system-wide responses (ideally using several omics platforms) are needed to make correlation-causation connections and to characterize phenotypes under controlled conditions. Given the growth in our knowledge, there is the potential to develop personalised dietary recommendations. However, developing these recommendations assumes that an improved understanding of the phenotypic complexities of individuals and their responses to the complexities of their diets will lead to a sustainable, effective approach to promote health and prevent disease — therein lies our challenge.

### Keywords

human feeding study; metabolomics; gut microbiome; data integration

### Introduction

Technologic advances now make it possible to collect large amounts of genetic, epigenetic, proteomic, metabolomic, and gut microbiome data. Many of the applications of this multi-dimensional data have been in the areas of disease detection, prognosis, and treatment. However, such approaches may also lend themselves toward characterizing healthy phenotypes and more effectively informing dietary recommendations for maintaining or improving the health of individuals on a personal level. Omics data have the potential to

transform our approach toward nutrition counseling by allowing us to recognize and embrace the metabolic, physiologic and genetic differences among individuals. The ultimate goal would be to integrate these multi-dimensional data so as to characterize the health status and disease risk of an individual and to provide personalised dietary recommendations to maximize health. To this end, accurate and predictive systems-based measures of health are needed that incorporate molecular signatures of genes, transcripts, proteins, metabolites, and microbes.

Nutrition, as a science, has a long tradition of determining the nutrient requirements of heterogeneous populations eating a wide variety of diets and of providing dietary recommendations for health. This has typically involved simplifying the inherent complexity into manageable recommendations in the form of dietary guidance for the purpose of preventing disease in a population. Despite the application of biostatistical approaches with the goal to be as inclusive of the population as possible, there are limitations due to assumptions that metabolic organizational structure is uniform among individuals and that direct cause-effect relationships exist. In reality, the large number of functional redundancies and adaptive mechanisms that provide for homeostasis<sup>(1)</sup> make evaluating the complexities and nuances challenging.

The concept of a “nutritional phenotype”—i.e., an integrated set of genetic, proteomic, metabolomic, functional, and behavioral factors that, when measured, could provide the basis for assessment of human nutritional status—was introduced several years ago by Ziesel *et al*<sup>(2)</sup>. It was proposed as a way to integrate the effects of diet on disease/wellness and provide a quantitative indication of the paths by which genes and environment exert their effects on health<sup>(1)</sup>. The concept provides a good base from which to begin to establish approaches to personalised dietary recommendations; however, several questions need to be addressed. These include, but are not necessarily limited to: What data will we need on an individual in order to personalise dietary recommendations? How can we use controlled feeding studies and other dietary interventions to generate a nutritional phenotypic framework? How can we most effectively integrate omics data so as to be able to apply them toward personalised nutrition?

## **What data will we need on an individual in order to personalise dietary recommendations?**

Numerous factors contribute to variation in nutritional requirements and responses to diet, including sex, stage of life cycle, disease, physical activity level, genetic background, gut microbial community and environmental exposures. Several of these are already considered in the construction of personalised nutritional recommendations; for example, sex, age, adiposity, and activity level are routinely used in determining nutrient requirements in healthy individuals and understanding the contributions of disease state to nutritional requirements is a hallmark of therapeutic nutrition. To date, the more complex factors such as genomics, host microbial community structure, and environmental exposures are often not included in the equation.

Genetic polymorphisms are well-recognized sources of variation in human response to some aspects of diet, including taste preference, food tolerance, nutrient absorption, transport and metabolism, and effects at target tissues<sup>(3)</sup>. Typically, in past studies, one particular genetic variant has been considered in relation to intake of one particular nutrient. For example, two polymorphisms in the *MTHFR* gene (C677T and A1298C) are associated with reduced methylenetetrahydrofolate reductase activity and higher homocysteine concentrations<sup>(3)</sup>. Carriers of these polymorphisms are at higher risk of cardiovascular disease, thus sufficient intake of folate is particularly important. Other examples include iron overload and

hemochromatosis, copper malabsorption and Menkes disease, and glucose-6-phosphate dehydrogenase (G6PD) and consumption of fava beans, high in pro-oxidant glycosides (favism)(reviewed in<sup>(3)</sup>). Further, genomics may contribute to phenotypic differences in health behavior and modify response to interventions designed to change health behaviors<sup>(4)</sup>.

Several genome-wide association studies (GWAS) have evaluated the association between multiple single nucleotide polymorphisms (SNPs) and metabolomics profiles. In a sample of 284 men, Gieger *et al.*<sup>(5)</sup> integrated GWAS data with serum metabolomics-based quantitation of 363 metabolites. They reported associations of frequent SNPs with differences in the metabolic homeostasis, explaining up to 12% of the observed variance. Using ratios of certain metabolite concentrations as a proxy for enzymatic activity, up to 28% of the variance can be explained (p-values  $10^{-16}$  to  $10^{-21}$ ). Four variants in genes coding for enzymes (FADS1, LIPC, SCAD, MCAD) were identified where a corresponding metabolic phenotype (metabotype) clearly matched the biochemical pathways in which these enzymes are active.

More recently, Suhre *et al.*<sup>(6)</sup> conducted an analysis of genotype-dependent metabolic phenotypes using a GWAS with non-targeted metabolomics in a sample of 1768 individuals. They identified 37 genetic loci associated with blood metabolite concentrations, of which 25 showed effect sizes that accounted for 10–60% difference in metabolite levels per allele copy. These results provided functional insights into disease-related associations that have been reported in previous studies, including those for cardiovascular and renal disorders, type 2 diabetes, cancer, gout, venous thromboembolism and Crohn's disease.

The human gut microbial community also shapes host exposure to dietary constituents by modulating absorption, storage, and energy harvest from the diet. It is a large, complex ecosystem, with the number of different species of bacteria estimated to range from 300 and 1000 and the majority of the species diversity distributed between the phyla Firmicutes and Bacteroidetes<sup>(7,8)</sup>. There is high inter-individual variation in the composition of communities, mostly at the species level<sup>(9)</sup>, whereas the distribution of bacterial functional genes is less varied. This functional redundancy is a hallmark of a stable symbiosis in which many different species carry out the same functional role.

Recent studies suggest that individuals can be clustered into distinct groups based on their gut microbiome composition and functional metabolism<sup>(10)</sup>. The underlying metabolism of the dominant bacteria that define these groups is the degradation of plant polymers (e.g., dietary fiber) via different metabolic pathways; long-term dietary habits have been associated with these groupings<sup>(10)</sup>. Through the metabolism of dietary constituents, the gut microbiome can influence the magnitude and flux of metabolites to which the host is exposed and some of the variation in what have been identified as genotype-dependent metabolic phenotypes actually may be due to the composition and activity of the gut microbiome<sup>(11,12)</sup>. Indeed, of the genotype-dependent metabolic phenotypes identified by Suhre *et al.*<sup>(6)</sup>, an altered microbiome has been associated with cardiovascular disease<sup>(13)</sup>, type 2 diabetes<sup>(14)</sup>, some cancers<sup>(15,16)</sup>, and Crohn's disease<sup>(17)</sup>. However, the relationships between the gut microbiome, diet, and metabolic phenotypes need to be addressed in rigorous experimental settings using approaches that integrate metabolomics, host genomics and the gut microbiome.

## How can we use controlled feeding studies and other dietary interventions to develop phenotype profiles?

Controlled feeding studies in healthy humans have been used for over a century to establish the quantitative requirements and confirm essentiality of nutrients in humans. Typically, these studies had small sample sizes, were intensively controlled, and often focused on restriction and refeeding of specific nutrients or nutrient sources. They were used to evaluate the acute effects of food deprivation, show experimentally the effects of dietary restrictions on development of deficiency diseases, establish specific amino acid requirements, and describe vitamin metabolism<sup>(18)</sup>. Consequently, they were crucial in determining recommended daily dietary allowances. Controlled interventions and defined background diets have also been useful for testing response to varying doses of a dietary constituent<sup>(19)</sup> and for testing and monitoring biomarkers of disease susceptibility and dietary exposure<sup>(20)</sup>. More recently, dietary interventions have been used to test the effects of particular dietary patterns<sup>(21)</sup> and to test genotype-phenotype interactions<sup>(22)</sup>.

Controlled feeding studies, particularly with randomized crossover designs where each person serves as their own control, are a useful venue in which to test genotype-diet interactions as well as genotype-phenotype interactions. In the latter case, the relationship between genotype and phenotype can sometimes be better characterized on the background of the same dietary exposures (i.e., a controlled diet)<sup>(22)</sup>. Participant screening protocols for recruitment into controlled feeding studies also can be set up to enrich *a priori* for particular genotypes or phenotypes so as to provide more equal distributions of sample sizes in subgroups, particularly if the prevalence of a particular variant is low, and to increase statistical power to compare these subgroups.

Controlled feeding studies also provide a useful approach in which to characterise host-gut microbial interactions and to determining gut microbial community response to diet. In the context of controlled dietary interventions, gut bacterial community composition has been shown to differ significantly when participants consume different diets<sup>(23,24)</sup>, although the overall response of the gut bacterial community is often unique for each individual<sup>(24,25)</sup>. Most studies have tested effects of fermentable complex carbohydrates (e.g., dietary fibers, resistant starch)(Table 1). Network analysis of the gut microbial community reveals niche specialization based on a metabolic interconnection between different bacteria that are often specialized in one enzymatic transformation in the pathway of dietary metabolism<sup>(10,26-28)</sup>. The type of carbohydrate ingested often influences the prevalence of certain groups of gut bacteria and the subsequent composition of the microbial metabolic end products to which the host is exposed (e.g., short chain fatty acids; Table 1). Differences in gut microbial metabolism of various phytochemicals also contribute to gut bacterial metabolic phenotypes that influence dietary exposures<sup>(29)</sup>. Being able to test for the effects of these phenotypes in the context of nutrition interventions is important, since some subgroups may be more responsive to the intervention than others. For example, Niculescu *et al*<sup>(30)</sup> reported differential lymphocyte gene expression by bacterial metabolic phenotype in postmenopausal women receiving an isoflavone supplement; a greater increase in estrogen-responsive genes was observed in women who carried the bacteria capable of converting the soy isoflavone daidzein to equol.

“Omics”—transcriptomics, proteomics, and metabolomics—approaches have been hypothesised to revolutionise our understanding of the interactions of the various systems that are often studied in isolation and have the potential to revolutionise many aspects of our study of nutrition and health promotion. Despite the excitement, at this stage, the technologies still require rigorous evaluation and validation. Controlled feeding studies are a useful approach in which to validate and test the robustness of these omics approaches with

the goal of ultimately being able to use them to evaluate the effects of totality of diet on totality of response in humans. In addition, they provide important details on the behavior of proteins, transcripts and metabolites under controlled conditions.

Several studies have used the construct of controlled feeding interventions to test effects of diet on omics measures (Table 2). The majority of these have utilized metabolomics to characterize response to phytochemical-containing foods—fruits, vegetables, tea, nuts—compared to a control in healthy individuals. Many of the metabolites identified typically correspond to dietary biomarkers of the intervention foods consumed (e.g., proline betaine after consumption of citrus fruits). Although many studies also yield a handful of endogenous metabolites that differ in abundance between the interventions, these compounds are often generally reported as differences in metabolite profiles owing to a lack of adequate pathway analysis tools. Thus, it is often unclear whether differences in metabolite profiles are indicative of perturbations in specific pathways or molecular targets in response to the dietary intervention, or are unrelated compounds identified by chance. Some investigators have explored pathways manually. For example, Solanky *et al*<sup>(31)</sup> found that soy consumption was associated with osmolyte fluctuations and differences in energy metabolism. Work in our lab (unpublished data) suggests potential differences in energy utilization from glucose to fat between a diet devoid of fruits and vegetables compared to a diet high in crucifers, citrus and soy. These examples provide provocative views of other mechanisms through which plant foods may promote health; however, even with manual analyses, the interpretation is still broad, speculative, and incomplete.

Other investigations have employed alternative omics technologies to study response to diet and have evaluated other endpoints beyond differences in metabolite profiles. Brauer *et al*<sup>(32)</sup>, interrogated the proteome in response to two weeks of a diet high in cruciferous vegetables, and assessed whether response differed by glutathione *S*-transferase (*GSTM1*) genotype. GST enzymes metabolise a variety of exogenous compounds, including isothiocyanates from cruciferous vegetables, and the *GSTM1* variants resulting in a complete lack of gene product are common<sup>(22)</sup>. Twenty-four distinct peaks were associated with cruciferous vegetable consumption compared to a fruit- and vegetable-free diet, two of which were identified that changed in a *GSTM1*-genotype-dependent manner. Another study provides an example of a novel use of omics to link metabolic phenotypes with dietary preferences. Taking a targeted approach, Rezzi *et al*<sup>(33)</sup> used lipidomics to determine metabolites associated with chocolate “desiring” or chocolate “indifferent” preferences among individuals consuming 50 g/d chocolate or bread as a placebo. Heinzmann *et al*<sup>(34)</sup> used metabolomics to study the stability of phenotypic response to diet through sequential dietary challenges. They found that inter-individual differences were often greater than differences within an individual in response to dietary modulation, providing evidence that individuals each have a unique metabolic phenotype. Moreover, intra-individual differences between consecutive dietary challenges were linked to differences in excretion of microbial co-metabolites suggesting flexibility in gut microbiome function in response to dietary modulation. As the authors point out, these differences illustrate the importance of assessing response to diet in the context of a crossover rather than parallel study design in order to move toward personalised nutrition. As a whole, these controlled feeding studies illustrate the potential for omics technology in characterizing individual nutritional phenotypes, but make evident the challenges (i.e., compound identification, pathway analysis) that still exist.

## How can we most effectively integrate omics data so as to be able to apply them toward personalised nutrition?

Given that cellular functions are carried out via orchestrated activities of multiplex components of biological systems, data from different omics platforms can shed light on

cellular activities at different levels. Methods that integrate omics data from different molecular profiling studies, e.g. data from transcriptomics, proteomics or metabolomics studies, have the potential to provide new insight into how different components of biological systems interact with each other and form the basis of an individual's health. Here, we provide an overview of available methods of data integration from multiple omics platforms, provide examples of each of different approaches, and discuss their advantages and limitations.

Current methods for integrative analysis of omics data from multiple data platforms can be broadly grouped into three categories. The first class of models, which we refer to as *concordance analysis* methods, studies concordance/correlation between two omics data sets, e.g., comparing the gene expression levels and proteomics datasets on the same set of subjects. The objective of such an approach is to identify genes/proteins/metabolites with an orchestrated activity in a given biological setting. To this end, methods of multivariate analysis, including different variations of Principal Component Analysis (PCA), Partial Least Squares (PLS), Self-Organizing Maps (SOM), as well as methods of network visualization and analysis, have been used to assess the associations among multiple data sets. For instance, Hirai *et al*<sup>(35)</sup> applied PCA as well as SOM to discover relationships between transcriptome and metabolome in *Arabidopsis*. In another study, Hirai *et al*<sup>(36)</sup> analyzed the network of gene-to-gene and gene-to-metabolite associations. More recently, Cao *et al*<sup>(37)</sup> proposed a sparse PLS procedure for comparative analysis of data from two omics platforms and applied their method to data from cDNA and Affymetrix chips in NCI60 cancer cell lines.

Concordance analysis methods provide interesting information about components of biological systems that interact with each other in a given setting. Moreover, such analyses can lend themselves to better classificatory models based on a combination of biomarkers from different platforms. However, these approaches often provide limited new insight into the underlying biological mechanisms as omics data from different platforms often show low levels of correlation due to complex mappings of genes to proteins and metabolites, and various post-transcriptional events<sup>(38)</sup>. Further, the underlying assumption in the majority of these methods is that omics measurements are obtained on the same set of individuals, or more formally, share a common dimension. Van Deun *et al*<sup>(39)</sup> reviewed these different approaches for analysis of multiple omics data, in the setting where the data sets share a common set of features.

The second class of integrative models, which we refer to as *sequential integration*, includes methods that incorporate multiple sets of omics data in order to discover new biomarkers or delineate biological mechanisms of complex phenotypes. It uses multiple omics data sets, in a sequential manner, to further narrow down, or expand, the set of biomarkers. Sequential integration methods can exploit different methods of data analysis, from simple differential expression analysis to gene-set enrichment analysis or analysis of networks. In examples of such an approach, Putluri *et al*<sup>(40)</sup> first identified the set of differentially active metabolites, and then used meta genomic data to identify pathways associated with prostate cancer progression. In another study, Putluri *et al*<sup>(41)</sup> coupled this approach with a concordance analysis based on metabolomics flux measurements to delineate pathways and biomarkers associated with bladder cancer. More recently, Imielinski *et al*<sup>(42)</sup> used gene set enrichment analysis coupled with the knowledge of biological networks and compared two sequential approaches, called “gene-centric” and “protein-centric,” in a study of molecular bases of breast cancer. In each of these approaches, the authors first evaluated the enrichment of biological pathways based on one source of data (transcriptomic or proteomic) and then filtered the set of identified pathways based on the second source of data. The authors also compared the results of these methods with a concordance-based approach, where the

pathways were identified based on gene and protein pairs that demonstrated orchestrated levels of activity.

Sequential integration methods offer an opportunity to gain new insight based on multiple sources of omics data. Moreover, these methods do not require the omics measurements to be necessarily observed for the same set of individuals. Finally, unlike methods of concordance analysis, which cannot be directly extended to analysis of more than two sets of omics data, sequential integration methods offer the flexibility of analyzing multiple omics datasets. However, the power of these methods is clearly limited by the ability of the omics data chosen for the first stage of analysis to capture important biological mechanisms. As the study by Imielinski *et al*<sup>(42)</sup> indicates, the results of the analysis can vary depending on the omics platform used for the first stage of analysis. This sensitivity to the order of analysis can potentially hinder the applications of sequential integration methods, and additional studies are needed to determine whether data-driven criteria can be developed to assess the optimal order of analysis in these methods.

The final group of omics integration techniques, which we refer to as *concurrent integration* methods, includes emerging approaches that attempt to address some of the shortcomings of the above two sets of approaches. Similar to sequential integration methods, concurrent integration methods try to exploit the information content of multiple sets of omics data. However, these methods often include measures of activity of biological pathways, or their components, based on multiple omics data. This is often achieved by defining a combined score for the activity of each pathway based on activities of its members measured by different omics datasets. Poisson *et al*<sup>(43)</sup> compared the performance a number of methods for combining data from multiple omics platforms, by considering different summary measures defined based on individual test statistics, with methods based on a single omics data source and show that the integrative approaches can improve the power of the analysis. In a recent study, Jauhainen *et al*<sup>(44)</sup> proposed a multivariate approach, using a mixed linear model, to assess the association of transcriptomics and metabolomics measurements with cancer progression. The proposed model requires measurements to be observed on the same set of samples, but offers the potential for discovering novel biological mechanisms, as well as biomarker identification. On the other hand, Shojaie *et al.* (Shojaie A, Panzitt K, Putluri N, Putluri V, Samanta S, Vareed SK, Basu S, Ittmann M, Michailidis G, Palapattu G and Sreekumar A. A Network-Based Integrative Approach to Study the Role of Metabolic Pathways in Prostate Cancer Progression, 2012) propose a network-based method, based on the NetGSA method<sup>(45)</sup>, for integrating multiple sources of omics data, which can be applied to data from different samples. This procedure does not lend itself directly to selection of biomarkers, and follow-up analyses are needed to determine which components of the selected pathways should be used as biomarkers.

Concurrent integrative methods have also been proposed for gaining insight into biological mechanisms in the cell. An example of such an approach includes the proposal of Shojaie *et al.* (Shojaie A., Jauhainen A, Kallitsis M, and Michailidis G. Inferring Regulatory Networks by Combining Perturbation Screens and Steady State Gene Expression Profiles, 2012) to integrate perturbation screens and steady-state gene expression profiles for discovering causal genetic regulatory mechanisms. In this study, the authors compare their proposed integrative approach with state-of-the-art methods based on a single source of omics data, and show superior estimates of regulatory networks can be obtained that by combining multiple omics data. Table 3 summarises the different classes of integration methods.

Novel biomedical technologies continue to improve the quality of the omics data, as well as to reduce the cost of obtaining such data. In nutrition studies, biological experiments now generate multiple sources of omics data including transcriptomic, proteomic, metabolomic

and gut microbial community measurements. The main challenge is now integrating such measurements in a systematic way, in order to provide a holistic view of biological systems. As more and more measurements become available, the complexity of the analysis, i.e. the number of variables in statistical models, increases. This poses additional challenges for design of trials, and necessitates the use of advanced statistical models appropriate for analysis of high dimensional problems. A potential solution for this challenge is to incorporate available biological knowledge, including information on biological pathways and genetic, protein interaction and metabolic networks. Incorporating biological information can both reduce the dimensionality of the problem, and also improve the power and reproducibility of analysis methods.

## A Way Forward to Personalised Nutrition

There is still a lot of effort needed to establish a robust health phenotype framework on which to develop personalised dietary recommendations. The improving omic technologies and the ability to integrate various omics platforms in a systematic fashion will facilitate providing a holistic view of cellular functions related to healthy phenotypes; however, the characterisation of the contribution of diet to the biochemical and metabolic parameters associated with healthy phenotypes would benefit from systematic evaluation under controlled conditions in well-described groups of individuals. Controlled human feeding studies are a useful experimental setting in which to conduct this work. Nonetheless, these types of studies are expensive and funding multiple, new large-scale dietary interventions that capture a variety of dietary patterns and intakes is likely to be prohibitive.

An efficient and effective way to develop some of the necessary omics databases under experimental conditions may be to take a collaborative approach, leveraging existing samples from previously conducted human interventions. Stored samples from controlled feeding studies are stashed away in freezers around the globe and in many cases are well characterized and ideal for further omic analysis. Statistical techniques for integrating multiple omics data from a common platform but different study populations, i.e., meta-analysis techniques, already exist; they improve statistical power by integrating samples from multiple related studies<sup>(46-54)</sup> and also allow for testing of reproducibility of results across studies. Looking toward future studies, the adoption of standardized sample and metadata collection protocols would allow for easier pooling of data across studies.

Overall, the careful collection and integration of omics data from controlled dietary interventions may provide us with the data necessary to successfully move toward a goal of more personalised dietary recommendations. Nonetheless, even with the generation of expansive, integrated datasets that allow for in-depth characterisation of health phenotypes, several factors need to be considered if personalised nutrition is to move toward being a part of routine health practice. Adherence to dietary recommendations for chronic disease prevention at the population level, such as those of national and international associations (e.g., US Department of Agriculture, World Cancer Research Fund, American Heart Association, etc) is associated with lower risk of chronic disease; for example, greater adherence to the 2005 US Dietary Guidelines was inversely associated with risk of coronary heart disease, stroke, diabetes, and total cancer.<sup>(55)</sup> In theory, tailored recommendations may be an improvement over general, population-based dietary recommendations; however, whether more extensive phenotyping, beyond current approaches, is cost-effective in promoting health and preventing disease will need to be determined. Further, in practice, finding individualised approaches that facilitate and maintain desired dietary behavior on the heels of a personalised diet prescription for health will likely remain an ongoing challenge for nutrition practitioners.



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Table 1

Summary of human dietary intervention studies of response of the gut microbiome to diet.

Dietary intervention, reference and year	Sample size/ population	Dietary intervention/ dose of food agent	Treatment duration	Platform/biological sample/ key outcomes
<b>Dietary intervention in normal weight individuals</b>				
Hooda <i>et al</i> <sup>(56)</sup> , 2012	n=20 M; Healthy adults	Randomized controlled crossover, TRTS- no fiber, polydextrose (PDX; 21g/d);soluble corn fiber(SCF;21 g/d)	21 d	Pyrosequencing of V4 region of 16S rRNA gene/faeces. PCA showed gut microbial community shifts with fiber interventions.
Ross <i>et al</i> <sup>(57)</sup> , 2011	N=17; 11 F, 6 M	Randomized controlled crossover, Whole grain /(WG; 150/d) vs. refined grain (RG)	2-wk	Bacterial enumeration using FISH; <i>C. leptum</i> group increased in WG diet along with stool frequency.
Costabile <i>et al</i> <sup>(58)</sup> , 2008	n=31; 16 F, 15 M	Randomized controlled crossover, 48g/d breakfast cereal of either 100% whole grain (WG) (11.8g DF/100g; chosen after a pre-screening for bifidogenicity) or wheat bran (WB) (27g DF/100g) ad-lib diet; energy composition was not the same; WG contained higher content of non-sugar carbohydrate	two 3-wk periods	Bacterial group enumeration using FISH showed changes in several groups with both WG and WB diets and differences between diets. No change in faecal SCFA. Increase in fasting plasma ferulic acid with WB.
Finley <i>et al</i> <sup>(59)</sup> , 2007	n=40 pre-metabolic syndrome; n=40 controls; 20	1/2 cup (130g) of pinto bean purees. chicken-noodle soup	4-wk equilibration and 12-wk intervention	Faecal bacterial species enumeration using FISH. Breath methane measured. No effect of bean consumption, except <i>E. limosum</i> levels decreased by 50%.
Smith <i>et al</i> <sup>(60)</sup> , 2006	F, 20 M n=18 M	Single-blind randomized crossover, self-managed with addition of seven experimental foods (bread, muffin, brownie, choc milk drink, muesli, pasta, mashed potatoes) with or without lupin kernel fiber (LKF); LKF diet provided 17-30g additional fiber/d; Mean fiber intake was 23g/d on control and 45g/d on LKF; LKF diet was sig. lower in starch	28-d ;3-d pooled faecal collection at end of each period	FISH of 16S rRNA genes/faeces with probes for total bacteria and specific groups. No difference in total bacteria, but changes in certain groups in response to treatment
Johnson <i>et al</i> <sup>(61)</sup> , 2006	n=38 M	Single-blind crossover, same as Smith <i>et al</i> <sup>(60)</sup> above	28-d; 3-d pooled faecal collection at end of each period	SCFA and bacterial enzyme activity in faeces. LKF altered bowel function parameters and decreased faecal pH. Faecal SCFA increased and $\beta$ -glucuronidase activity decreased. No difference in faecal ammonia concentration.
Tuohy <i>et al</i> <sup>(25)</sup> , 2001	n=31; 17F, 14M	20g FOS + 10g partially hydrolyzed guar gum (PHGG)/d vs. placebo	crossover, 3-wk feeding periods; two consecutive d stool sample mix	FISH of 16S rRNA genes. Increase in <i>Bifidobacterium</i> spp., but no difference in total bacteria between diets or other spp. enumerated. No change in faecal pH.

Dietary intervention, reference and year	Sample size/ population	Dietary intervention/ dose of food agent	Treatment duration	Platform/biological sample/ key outcomes
Hylla <i>et al</i> <sup>(62)</sup> , 1998	n=12; 5 F, 7 M	Randomized controlled crossover, high vs. low resistant starch (RS): amylo maize starch in bread, pasta, cake and biscuits [Hylon VII, Natl Starch]	4-wk	Changes in breath hydrogen and several faecal parameters associated with bacterial activity (e.g., pH, certain SCFA, $\beta$ -glucosidase, secondary bile acids).
<b>Dietary intervention in overweight/obese individuals</b>				
Weickert <i>et al</i> <sup>(63)</sup> , 2011	n=69; 43 F, 26 M	Randomized controlled crossover, High Cereal Fiber (HCF) 43g/d, moderately high cereal/fiber/ protein diets MIX 23% protein of energy-intake, cereal fiber 26g/d	18 wk	FISH/Flow cytometry of faecal bacteria. No effect of diet on bacterial groups.
Russell <i>et al</i> <sup>(23)</sup> , 2011	n=17 M; obese	Randomized controlled crossover. Maintenance diet (85 g protein, 116 g fat, and 360 g carbohydrate/d) high-protein and moderate-carbohydrate (HPMC; 139 g protein, 82 g fat, and 181 g carbohydrate/d) diet and a high-protein and low-carbohydrate (HPLC; 137 g protein, 143 g fat, and 22 g carbohydrate/d)	7 d maintenance followed by 14-d intervention	FISH of 16S rRNA genes/GC-MS analysis of faecal water content/faeces. HPMC and HPLC diets resulted in increased proportions of branched-chain fatty acids and concentrations of phenylacetic acid and N-nitroso compounds. HPLC diet decreased proportion of butyrate in faecal SCFA concentrations, concomitant with reduction in Roseburia/Eubacterium rectale bacteria, and reduced fiber-derived, antioxidant phenolic acids.
Duncan <i>et al</i> <sup>(64)</sup> , 2007	n=19 M	Randomized controlled crossover, high (399g carbs, 52%), medium (HPMC; 164g/d, 35%) or low carb (HPLC; 24g/d, 4%) ad-lib diet for 4 wks; nonstarch polysaccharide levels in maintenance: 28g, HPMC 12g, HPLC 6g	3-d maintenance/4-wk experimental diet	Bacterial enumeration using FISH showed shifts in certain groups of bacteria. Faecal SCFA and ammonia lower during experimental periods than maintenance and proportions of some SCFA differed by diet.
Ley <i>et al</i> <sup>(65)</sup> , 2006	n=12; obese adults	Reduced fat ~30% of calories from fat or 25% of calories from carbohydrate; fiber was 10-15g/d	1 y	Pyrosequencing of 16S rRNA gene. Ratio of Bacteroidetes to Firmicutes decreased over time associated with weight loss. No difference between diets

Abbreviations: F, female; FISH, fluorescent in-situ hybridization of 16S rRNA genes; M, male; PCA, Principal Components Analysis; SCFA, short-chain fatty acids.

Table 2

Summary of human dietary intervention studies using metabolomic and proteomic platforms.

Dietary intervention, reference and year	Sample size/ population	Dietary intervention/ dose of food agent	Treatment duration	Platform/biological sample/ key outcomes
<b>Fruits and vegetables</b>				
May D, Navarro SL, Ruczinski I, Hogan J, Ogata Y, Schwarz Y, Levy L, Holzman T, McIntosh MW, Lampe JW (2012) Metabolomic Profiling of Urine in Response to a Randomized, Controlled Feeding Study of Select Fruits and Vegetables, and Application to an Observational Study	n=10; 5 F, 5 M; Healthy adults	Randomized controlled crossover, mixture of cruciferous vegetables, citrus fruits and soy (F&V) compared to fruit and vegetable-free diet (basal); 5 g/kg BW	2 wk	Metabolomics/8 h fasting urine ; more abundant in the F&V: markers of dietary intervention (e.g., crucifers, citrus and soy metabolites), fatty acids and niacin; more abundant in basal: riboflavin, several acylcarnitines, and amino acid metabolites; differences in energy utilization between diet treatments.
Van Dorsten <i>et al</i> <sup>(66)</sup> , 2012	n=58 (29 in each treatment arm); 25 F, 33 M Hypertensive adults	Randomized double-blind placebo-controlled double-crossover, capsules containing a polyphenol rich mix of either red wine and red grape juice extracts (800 mg) or only red grape extract (800 mg), and placebo	4 wk	Metabolomics/urine; 18 phenolic acids elevated after either polyphenol treatment including syringic acid, 3- and 4-hydroxyhippuric acid and 4-hydroxymandelic acid.
Brauer <i>et al</i> <sup>(32)</sup> , 2011	n=36; 17 F, 19 M; n=42; 17 F, 25 M; Healthy adults recruited based on <i>GSTM1</i> genotype (present or null)	2 separate randomized controlled crossover trials of mixed vegetables: 1) 436 g cruciferous; 90 g allium; and 270 g apiaceous; 2) 7 g/kg BW cruciferous; 14 g/kg BW cruciferous; 7 g/kg BW cruciferous + 4 g/kg BW apiaceous; both compared to fruit and vegetable-free diet (basal)	6 d; 2 wk	Proteomics/8 h fasting serum; 24 distinct peaks associated with cruciferous vegetables; 20 associated with <i>GSTM1</i> genotype; joint study analysis showed 6 peaks changed in genotype-dependent manner; 2 identified as TTR and ZAG.
Heinzmann <i>et al</i> <sup>(67)</sup> , 2010	n=8; 7 F, 1 M Healthy adults	Standardized mixed fruit meal (apple, orange, grapes and grapefruit; no dosage provided)	3 d	Metabolomics/urine; excretion of proline betaine, tartaric acid, hippuric acid and benzoic acid was increased compared to baseline.
Walsh <i>et al</i> <sup>(68)</sup> , 2007	n=21; 12 F, 9 M Healthy adults	Non-controlled crossover, 2 d habitual diet; 2 d low phytochemical diet and 2 d high phytochemical diet (100 mL × 4 apple, carrot, and strawberry drinks)	2 d	Metabolomics/fasting urine; higher excretion of hippurate and lower excretion of creatinine and methylhistidine discriminated the high phytochemical and habitual diets from the low phytochemical diet.
<b>Other plant foods</b>				
Tulipani <i>et al</i> <sup>(69)</sup> , 2011	n=42; Adults with metabolic syndrome	Randomized parallel intervention, mixed nuts, 30 g d and control	12 wk	Metabolomics/urine; 20 potential markers of nut intake including fatty acid, phase II, microbially-derived phenolic, and serotonin metabolites.

Dietary intervention, reference and year	Sample size/ population	Dietary intervention/ dose of food agent	Treatment duration	Platform/biological sample/ key outcomes
Llorach <i>et al</i> <sup>(70)</sup> , 2010	n=24; Healthy adults	Randomized blind placebo-controlled, encapsulated almond skin extract, 3.5 g	Single dose of 10 capsules	Metabolomics/urine; 34 metabolites of almond skin including flavonoids, hydroxyphenylvalerolactone, 4-hydroxy-5-(phenyl)-valeric acid, hydroxyphenylpropionic acid, hydroxyphenylacetic acid and other phenolic acid conjugates.
van Dorsten <i>et al</i> <sup>(71)</sup> , 2006	n=17 M; Healthy adults	Randomized crossover, black tea (6 g/d), green tea (6 g/d) or caffeine (control)	2 d	Metabolomics/urine; green and black tea increased urinary excretion of hippuric acid and 1,3-dihydroxyphenyl-2-O-sulfate; greater increase in several citric acid cycle intermediates with green tea.
Solanky <i>et al</i> <sup>(31)</sup> , 2005	n=9 F; Pre-menopausal	Controlled, miso (50 g/d; n=6) or soy protein (60 g/d; n=3)	4 wk	Metabolomics/urine; increased TMAO, methylamine, dimethyl amine, choline, creatine, glutamine (soy only) and glutamate (soy only), and decreased creatinine, hippurate, benzoate, citrate (miso only) and lactate (miso only).
<b>Miscellaneous</b>				
Rasmussen <i>et al</i> <sup>(72)</sup> , 2012	n=77; 44 F, 33 M; Overweight, adults	Randomized, high (23-28% of energy; n=42) or low (10-15% of energy; n=35) protein diet	6 mo	Metabolomics/urinary; creatine increased with a high protein diet; citric acid increased with the low protein diet.
Moazzami <i>et al</i> <sup>(73)</sup> , 2011	n=17 M; Prostate cancer patients	Randomized controlled crossover, whole grain rye, rye bran and refined white wheat product, control (485 g/d)	6 wk	Metabolomics/fasting plasma; metabolites increased after rye bran included 3-hydroxybutyric acid, acetone, betaine, N,N-dimethylglycine, and dimethyl sulfone.
Heinzmann <i>et al</i> <sup>(34)</sup> , 2011	n=7; 6 F, 1 M Healthy adults	Controlled, various dietary challenges including mixed fruit (apple, orange, grapes and grapefruit), fish, wine and grapes, beef and fish	7 d	Metabolomics/urine; inter-individual metabolic differences were greater than effects of any single dietary challenge; differences to dietary challenges differed between individuals.
Zivkovic <i>et al</i> <sup>(74)</sup> , 2009	n=3; 1 F, 2 M; Healthy adults	Standardized test beverages, 40% kcal needs; 230 g lactose-free milk, 227 g low-fat yogurt, 30 g 100% whey protein powder, 118 g banana, 22 g flax seed oil	3 single test beverages	Targeted lipidomics/plasma; serum fatty acid differences were greater among individuals than within; 3 metabolites discriminated individuals in ApoB fraction: TG16:1n7, TG18:2n6, and PC18:3n3.
Llorach <i>et al</i> <sup>(75)</sup> , 2009	n=10; 5 F, 5 M; Healthy adults	Randomized crossover, 40 g cocoa powder with water or 250 mL milk, or 250 mL milk alone	Single test beverages	Metabolomics/urine; 27 cocoa phytochemical metabolites identified after both cocoa-containing beverages.
Bertram <i>et al</i> <sup>(76)</sup> , 2007	n=28; 8 y-old boys	Randomized, 53 g/d protein from lowfat milk or low-fat meat	7 d	Metabolomics/urine and serum; urinary hippurate excretion was decreased with milk; urinary creatinine, histidine and urea was increased with meat.
Rezzi <i>et al</i> <sup>(33)</sup> , 2007	n=75 M; Healthy adults	Controlled crossover, chocolate (50 g) or bread (placebo)	Single feedings	Lipidomics/urine and plasma; metabolic phenotypes associated with chocolate desiring or chocolate indifferent preferences.
Stella <i>et al</i> <sup>(77)</sup> , 2006	n=12 M; Healthy adults	Randomized crossover, vegetarian (420 g/d), low-meat (60 g/d), or high-meat (420 g/d)	15 d	Metabolomics/urine; urinary excretion of carnitine, creatinine, taurine, TMAO, methyhistidine was increased with highmeat; p-hydroxyphenylacetate increased after vegetarian diet.



Abbreviations: BW, body weight; F, female; *GSTM1*, glutathione *S*-transferase M1; M, male; SMCSO, *S*-methyl-L-cysteine sulfoxide; TMAO, trimethylamine-N-oxide; TTR, transthyretin; ZAG zinc  $\alpha$ -2-glycoprotein.

**Table 3**

Summary of methods for integrative analysis of multiple omics datasets.

Integration Approach	Reference	Methodology/Tools	Omics Data
<i>1) Concordance Analysis</i>			
	Hirai <i>et al</i> <sup>(35)</sup> , 2004	PCA, SOM	Transcriptome and metabolome in <i>Arabidopsis</i>
	Hirai <i>et al</i> <sup>(36)</sup> , 2005	network analysis	Transcriptome and metabolome in <i>Arabidopsis</i>
	Le Cao <i>et al</i> <sup>(37)</sup> , 2009	sparse PLS	cDNA and mRNA in NCI60 cancer cell lines
	Van Deun <i>et al</i> <sup>(39)</sup> , 2009	multiple methods	Comparative analysis of integration methods assuming data on the same subjects
<i>2) Sequential Integration</i>			
	Putluri <i>et al</i> <sup>(40)</sup> , 2011	DE, OCM	Metabolomics, meta-genomics in Prostate cancer
	Putluri <i>et al</i> <sup>(41)</sup> , 2011	DE, OCM, CA, PLS	Metabolomics abundance & flux data, meta-genomics in Bladder cancer
	Imielinski <i>et al</i> <sup>(42)</sup> , 2012	GSEA, network analysis	Transcriptomics, proteomics in Breast cancer
<i>3) Concurrent Integration</i>			
	Poisson <i>et al</i> <sup>(43)</sup> , 2011	DE, p-value weighting, GSEA	Transcriptomics, metabolomics
	Jauhainen <i>et al</i> <sup>(44)</sup> , 2012	sparse mixed linear model	Transcriptomics and metabolomics in cancer
	Shojaie A, Panzitt K, Putluri N, Putluri V, Samanta S, Vareed SK, Basu S, Ittmann M, Michailidis G, Palpattu G, Sreekumar A (2012) A Network-Based Integrative Approach to Study the Role of Metabolic Pathways in Prostate Cancer Progression	NetGSA, GSEA, rank-based integration	Transcriptomics and metabolomics in Prostate cancer

Abbreviations: DE, differential analysis; GSEA, gene set enrichment analysis; CA, correlation analysis; PCA, principal component analysis; SOM, selforganizing maps; PLS, partial least squares; OCM Oncomine concept mapping; NetGSA, network-based gene set analysis.