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Advances in Nutritional Metabolomics

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Abstract

Metabolomics is maturing as an experimental approach in nutrition science, and it is a useful analysis for revealing systems biology outcomes associated with changes in diet. A major goal of this review is to present the rapidly evolving body of scientific literature that seeks to reveal connections between an individual's metabolic profile and experimentally manipulated or naturally varied dietary intakes. Metabolite profiles in tissue, serum, urine, or stool reflect changes in metabolic pathways that respond to dietary intervention which makes them accessible samples for revealing metabolic effects of diet. Three broadly defined areas of investigation related to dietary-metabolomic strategies include: (1) describing the metabolite variation within and between dietary exposures or interventions; (2) characterizing the metabolic response to dietary interventions with respect to time; and (3) assessing individual variation in baseline nutritional health and/or disease status. An overview of metabolites that were responsive to dietary interventions as reported from original research in human or animal studies is provided and illustrates the breadth of metabolites affected by dietary intervention. Advantages and drawbacks for assessing metabolic changes are discussed in relation to types of metabolite analysis platforms. A combination of targeted and non-targeted global profiling studies as a component of future dietary intervention trials will increase our understanding of nutrition in a systems context.

Keywords

Nutrition; metabolomics; dietary intervention; metabolism; analytical platforms

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CONFLICT OF INTEREST
The authors confirm that this article content has no conflicts of interest.

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INTRODUCTION

Nutritional metabolomics has emerged as a high-throughput and sensitive approach to identify and characterize biochemical pathways that underlie complex relationships between dietary exposures and chronic diseases with altered metabolic phenotypes [1–3]. The ability to identify novel correlations between dietary patterns and health, or between consumption of specific foods and disease-related outcomes has presented challenges due to individual variability in complex metabolic pathways and digestion, as well as novel metabolite identification [4, 5]. Food-omics refers to the metabolite profiling of foods prior to consumption [6, 7], while nutritional metabolomics has emerged with two major goals: (1) to determine the effects of dietary compounds on host metabolism after consumption for a defined period of time, and (2) to identify dietary intake or phytochemical dose-dependent associated metabolite biomarkers.

The number of nutritional metabolomics studies has substantially increased in the last decade, as evidenced by the number of original research articles cited in Pubmed Central with the terms ‘metabolome’ and ‘diet’ (Fig. 1). The solid line illustrates the number of reports from human studies, the dashed line represents papers reporting metabolite data across animal models (*e. g.* rodents, pigs, dogs etc.), and the dotted line shows review articles published in this field. Fig. (1) demonstrates an increasing trend in nutritional metabolomics investigations and future studies will benefit from a critical synthesis of this data prior to conducting new experiments. For example, dietary exposure studies are designed to improve our understanding of disease-fighting and health-promoting properties of medical foods, phytochemicals, food ingredients, food-associated toxicants, and dietary patterns. The results of such studies can guide future dietary intervention studies that seek to examine mechanistic relationships between metabolic changes and healthy organs or diseased tissues. Unfortunately, the nutritional research community lacks a synthesis of original nutritional metabolome data across trial designs, species, biological samples and analytical platforms. Previous review articles have largely emphasized the opportunities, limitations and importance of diet-metabolome research. In this review, we summarize nutritional metabolome data from the literature. As further discussed below, the literature reveals a breadth of metabolites influenced in dietary intervention studies. Thus, a renewed focus for nutritional metabolome investigations should include both targeted (biased to a select group of metabolites) and non-targeted (analysis of all detectable metabolites) studies. We discuss the application of metabolomics to nutritional investigations with a focus on experimental design and biological interpretation unique to food components, including the influence of the gut microbiome.

OVERVIEW OF NUTRITIONAL METABOLOMICS LITERATURE

A review of the literature was performed for nutritional metabolomics studies targeted toward metabolites detected in urine, blood, and stool (Tables 1–3, respectively). Diet responsive metabolites included nucleotides, sterols, lipids, carbohydrates, and amino acids. This collection of metabolites was derived from animal and human studies, which spanned a variety of dietary exposures and represented both acute and long-term changes. Bolded

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metabolites in all three tables indicate those metabolites that were classified as ‘diet modifiable’ in three or more distinct, independent studies.

All of the metabolites reported in the tables were overlaid onto the Meta Cyc human metabolism pathway map (Fig. 2). This metabolic mapping overview visualizes the extent of metabolism that is influenced by the diet, suggesting that narrowing analytical focus to a short list of target metabolites could prevent detection of important, sensitive, and subtle metabolic reactions that lie outside the scope of the targeted list.

EXPERIMENTAL DESIGN IN NUTRITIONAL METABOLOMICS

While some may have portrayed omics experiments as descriptive, ‘fishing expeditions’, a proper experimental design allows for both hypothesis-driven research, as well as generating new hypotheses. Experimental design is particularly essential for nutritional metabolomics research given the thousands of monitored metabolites and the biological variation inherent to clinical studies [8]. As an example, Xu *et al.* recently reported the results of a study using ^{1}H NMR spectroscopy to assess the effects of diet (lactovegetarian or omnivorous) and gender (male or female) on the urinary metabolome [9]. It was found that the most influential low molecular weight metabolites responsible for the differences between the diet groups were N-acetyl glycoprotein (NAG), succinate, citrate, trimethylamine-N-oxide (TMAO), taurine, glycine, hippurate, phenylalanine, methylhistidine and formate. The study was sufficiently powered and enabled the distinction between diet and gender effects on the metabolome. Often, however, metabolite studies are underpowered. In such a case, while metabolite variation would have been noted, the data would not have been able to support the influence of diet and gender found in the study [9]. Below, we discuss important considerations in experimental design for nutritional metabolomics studies including study size, study controls, time-points, sample matrix, individual metabolite variation, and the influence of the gut microbiome.

Study Size

The minimum number of biological replicates for statistically significant evaluation of a metabolomic dataset is determined based on pilot data and/or previously reported variation. Typically, the number of replicates required for a given statistical power should be estimated based on the variance and magnitude of the hypothesized response variable. In the metabolomics setting, the parameters (fold-change and variance) are dependent on both the metabolite and instrument platform. Thus, the number of replications is generally driven by study limitations such as subject availability and experimental costs, though increased numbers of biological replication will always provide higher confidence and statistical power. Biological replication should not be confused with analytical replication, in which a given extract of a sample is measured several times on the same instrument. Analytical replication is performed to address variation induced by the metabolite detection platform, and involves the collection of duplicate or triplicate data for each biological sample. Biological replication requires sampling from several subjects (recommended absolute minimum n=5 replicates), followed by independent metabolite extraction and data acquisition of those samples. These two sources of variation are independent, and analytical

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(instrumental or measurement) variation is typically considerably smaller than biological variation [10].

Study Controls

The scientific discovery of meaningful and reproducible metabolome changes in response to dietary interventions requires consistent, detectable metabolite levels, and also a clear understanding of variation in a baseline metabolite profile. Statistical analyses can articulate a change in a single metabolite over a personal baseline, which may be unique for each individual. The identification of statistically significant changes in a dietary treatment group relative to a control group can be more complex [11, 12].

Dietary intervention studies can be inherently difficult to control when compared to drug treatment trials because there is not a true placebo. Nutrients and phytochemicals have been shown to have less functional relevance when isolated for use as single agents, and rather exist as a complex network of essential and nonessential components [13]. In lieu of a true placebo, control groups are commonly comprised of non-intervention participants that follow existing dietary recommendations and guidelines. A recent example comes from a parallel intervention trial with 5 dietary intervention groups where study participants were randomly assigned to a 6-month low-fat diet that differed by various combinations of low/ high glycemic index and low/high protein [14]. The control group did not follow any specific glycemic index recommendations. The goal of this trial was to evaluate the impact of dietary protein and glycemic index on weight (re) gain in a large number of families that suffer from obesity or overweight [15], yet the control participant metabolite profiles were excluded from the metabolomic investigation due to variation in glycemic load of the diet [14]. Thus, the design of this study exemplifies the problem of introducing bias when estimating the intervention's true "metabolite effect" because the natural "non-intervention" metabolomic variation was not characterized. Although the study revealed that urinary hippurate was associated with dietary fiber intake at a group level in this population, we also know that this urine metabolite may be changed to some extent in a control population as a microbial byproduct and result of flavonoid metabolism (Table 1) [16].

Time-points

For nutritional studies, metabolite variation is assessed at multiple timepoints to consider the time-dependent nature of responses to a nutritional intervention. While the dynamics of the response are the most obvious motivation for monitoring several timepoints, additional sampling provides an opportunity to visualize trends and increase confidence in the results, particularly when the effect observed may be subtle. Furthermore, the results are more robust when proper study participant controls are included at each time point [17, 18]. Terminology regarding study duration is an essential distinction for reporting diet-affected metabolites, and is referred to herein as a short-term (acute, transient response) or a long-term response (chronic metabolic phenotype). Examples of short-term experimental designs have consisted of metabolite assessments after a few hours to 28 days [19, 20]. Long-term trial designs include samples collected for analysis after one month to years post-intervention [21, 22]. These long-term trials represent metabolite assessments that incorporate the complex interactions between diet-derived phytochemicals and nutrients,

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hormone flux, gut microbiota and temporal and spatial metabolite variations [23]. Limited evidence exists for assessing multiple timepoints in the same trial, restricting the ability to perform comparisons that allow classification of metabolites as either short-term or long-term responders [24, 25].

Sample Matrix

Blood plasma, serum, urine, stool, saliva, muscle, and liver metabolomes may all reflect different aspects of dietary intakes and responses [17, 26–29]. Thus, the rationale for selecting a particular biofluid to extract for analysis will be essential for biological interpretation of the observed metabolome. For example, if the goal of the study is to examine bioavailability, serum is traditionally assessed given the transmission of metabolites from food to gut to liver to blood. However, urine and/or stool may be more appropriate to evaluate degradation or detoxification pathways. Furthermore, as discussed in more detail below, the fecal metabolome might offer otherwise elusive insight into the response of the microbiome to nutritional interventions [19, 29–34].

The benefit of including multiple biofluids is illustrated in a recent study that showed that cluster analysis of blood and urine identified 3 distinct dietary patterns on the basis of the energy contribution assessment of different food groups in 160 individuals [35]. The combination of three-day diet records, plasma fatty acid profiles and ^{1}H NMR spectra of urine metabolites were used to evaluate associations with the intake of specific food groups. Specifically, there were fatty acid profiles across percentiles of red-meat intake that showed significant differences in plasma oleic acid concentrations, and increased urinary O-acetylcarnitine content in the red-meat diet group. Oleic acid has been typically associated with olive oil intake, yet showed a stronger relationship as the primary monounsaturated fatty acid in beef [36]. The vegetarian cluster group showed increased urinary glycine and phenylacetyl glutamine [35]. Thus the analysis of both plasma and urine allowed for more comprehensive and robust diet-metabolite relationships.

Individual Variation

Metabolomics studies in mammals have advanced our understanding of inter- and intra-individual metabolite variation [37–39]. Nutritional metabolomics study design is further complicated by the diverse and dynamic biochemical makeup of cells and tissues, and because the metabolite profile is only a snapshot of metabolism at a given time [11]. Metabolite fluctuations occur due to many factors, including but not limited to, diet history and environmental exposures, presence and severity of infections or chronic diseases, and genetics [39]. The integrated nature of these factors contributes to individual metabolite variation that is inherently difficult to control for.

Crossover studies (*e. g.* run-in diets prior to an intervention [40] or wash-out periods [41]) are common trial design strategies to reduce baseline variation in study participants prior to beginning a dietary intervention; though the optimal amount of time for the normalization period is not well understood. A run-in diet may initiate a short-term, transient metabolic response and confound the biological mechanisms under investigation. Furthermore, the variation by which individuals are uniquely affected by a run-in diet is not well understood.

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These study parameters merit methodical, focused research to better characterize fluctuations in metabolic status during different life stages [40].

Influence of Gut Microbiome

Gut and serum metabolism is also affected by variation in gut microbiome composition [42]. The gut microbiome and its relation to the diet are important to evaluate in dietary intervention trials with metabolomic endpoints. A recent comparison between germ-free mice colonized by human baby flora and conventional mice demonstrated the complexity of diet modifiable microbiome/metabolome covariation. In this study, the effect of the intestinal microbiome on plasma metabolites revealed that more than 10% of the plasma metabolome is directly dependent upon the microbiome [43]. Some examples for microbial dependent compounds in plasma include phenylalanine metabolism (*e. g.* cinammic acid), glycine conjugated compounds that can lead to the formation of hippuric acid (Table 2), and other plasma metabolites derived from gut anaerobes (*e. g.* phenyl-propionylgylcine). The gut microbiome also directly affects the host's ability to metabolize lipids, carbohydrates and proteins, and can carry out a number of phase II detoxification mechanisms [43]. Additionally, there is evidence that gut microbes metabolize nonnutritive phytochemicals [44, 45]. For example, Wang *et al.* recently showed that levels of three microbiome-dependent diet-derived metabolites, choline, trimethylamine N-oxide, and betaine, could predict risk for cardiovascular disease in mice [23].

Long term diet patterns and geographical location also correlate with unique gut microbiomes [46–48], supporting an association between gut microbial function and the nutritionally modulated metabolome [49, 50]. Stool is a relevant biological sample for microbial metabolic assessment (Table 3), whereby coprastanol, the microbial derived metabolite of cholesterol, was decreased in excreted stool during an 8 week study observing cholesterol metabolism in humans when calcium phosphate was supplemented [51]. Using NMR, significant amounts of amino acids and fatty acids were also detected in fecal water from people consuming a vegetarian diet [29]. However, an important limitation of fecal analysis is the inability to detect metabolites that have increased intestinal bioavailability, and thus are actually absorbed by the host colonic epithelium. New analytical methods are under development for the quantitative analysis of tissue microbial metabolites and this represents an emerging, integral part of global metabolomics platforms [52], and was recently reviewed in [53].

TECHNICAL CONSIDERATIONS IN NUTRITIONAL METABOLOMICS

In general, there are two approaches to a metabolomics experiment: targeted or non-targeted. A targeted approach involves the directed analysis of a pre-determined panel of metabolites relevant to the hypothesis of the study. The advantage of this type of approach is that it can be optimized for the detection of specific molecules, which enables increased sensitivity and absolute quantitation. It is limiting in scope, however, in that it requires *a priori* knowledge of the metabolites of interest. Alternatively, a non-targeted approach is performed in a broad and unbiased manner to enable the detection of many metabolites. The results of a non-targeted approach tend to be hypothesis-generating and drive the next set of experiments to

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validate the findings. The advantage of a non-targeted approach is the potential for novel discoveries. However, a substantial disadvantage of this approach is the challenge of metabolite annotation [54]. It is possible to combine a targeted and non-targeted experimental design to enable unbiased profiling while simultaneously monitoring a set of known metabolites within the data.

Ultimately, the choice of experimental design will depend on the goal of the study, such that metabolite profiling data can be hypothesis generating with a non-targeted approach and may enable the identification of novel metabolic pathways in response to a dietary change. However, if the goal is to assess a specific metabolic pathway or set of molecules with known mechanisms of action, than a targeted metabolite profiling approach could more appropriate as it can be optimized for selectivity and sensitivity for these targets.

Sample Preparation

The preparation methods may vary based on sample type, target metabolites, and analytical platform. While the stated goal of metabolomics is to profile the entire metabolome, this is technically impossible using a single analytical platform or sample extraction procedure. As a result, having a list of metabolites of interest is important when designing an appropriate sample extraction method. The sample extraction method should provide reproducible recovery of target compounds for profiling purposes, and additionally provide complete recovery for absolute quantitation. Sample integrity (frozen until extraction) and sample homogeneity (to ensure a representative subsample and efficient extraction) are critical factors to consider. Specifically, a method that incorporates solvents capable of solubilizing the target compounds is generally accomplished through adjustment of the solvent polarity, ranging from highly polar (water, often pH adjusted or buffered), through moderate polarity (water-methanol mixtures) to non-polar solvents such as chloroform-methanol mixtures designed for lipid extraction. When the goal is a broad, global profile, selecting a solvent of moderate polarity will provide a representative sample compatible with multiple instrument platforms. The limitation is that the method is not optimized for any metabolite, limiting the quantitative accuracy (when quantitation is desirable). It is advised to reference the literature and replicate methods used in previous studies when the optimal extraction methodology is unclear.

Analytical Platform

Nutritional metabolomics has been performed using either mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR). Mass spectrometry generally excels in sensitivity and breadth of coverage, while NMR offers more readily interpreted structural information. The largest portion of metabolomics experiments utilizes mass spectrometry coupled to a chromatography system. The chromatographic platforms most frequently used are liquid chromatography (LC), gas chromatography (GC), or capillary electrophoresis (CE), with LC being the most frequently used and CE the least. Each separation method has both advantages and limitations. The ability to couple chromatography to MS is the most significant reason MS is used more frequently than NMR for metabolomics experiments. GC-MS is typically used to monitor small polar metabolites, such as monosaccharides, amino acids, organic acids, nucleosides, and also routinely detects more non-polar

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compounds such as fatty acids and sterols. Its major limitation is the requirement for volatility, which is achieved through chemical derivatization of small molecule extract. Extensive mass spectral libraries aid in the identification of compounds detected. The various LC-MS platforms eliminate the need for derivatization and are not limited to small molecules, as volatility is not a limiting criterion for detection. Mass spectral libraries for LC-MSMS are also developing rapidly, though the growth curve lags behind that for GC-MS spectra. NMR offers the advantage of simple sample prep and data collection, as well as being a useful tool for structure identification. Its major limitation is the lack of chromatographic separation: sample complexity is more limiting with NMR than chromatographically coupled MS systems. The lists of metabolites identified from nutritional metabolomics studies are provided according to the biological sample detected and alongside the actual platform utilized (Tables 1–3).

Metabolic

Flux—The flux of metabolites through metabolic pathways can be measured with the use of stable isotopes. Isotope-based metabolic flux analysis has traditionally provided fundamental knowledge on cooperating actions in a complex network of genes, transcripts, proteins, and metabolites. A detailed description of studies that use isotope labeling to inform metabolic flux from dietary exposures is beyond the scope of this review, yet metabolomics analysis of samples collected from these trials may provide invaluable and innovative advances to the field of nutritional, non-targeted metabolomics and may provide unique mechanistic insights [56].

EXAMPLE NUTRITIONAL METABOLOMICS DATASET

To illustrate the challenges associated with time-point effects on metabolites, a pilot dataset from an ongoing dietary intervention trial (conducted under approved protocols from the Colorado State University Research Integrity and Compliance Review Office and University of Colorado Health Institutional Review Boards) is shown in (Fig. 3). In this case, a single individual's data revealed dramatic changes in the global metabolite profile after 2 and 4 weeks of substantially increased navy bean intake. Fecal metabolites were extracted using an aqueous-methanol solvent (n = 3 replicates per timepoint), and a non-targeted GC-MS profiling technique was applied as previously described [57]. Fecal metabolite profiles were assessed by principal component analysis (PCA), and the model explained 81% of the variation (Fig. 3A). The PC scores indicated fecal metabolite profiles changed due to the dietary intervention. PC1 explained variation observed at the 2-week time-point, as baseline and 4-week samples had similar x-axis coordinates (approximately −200 units). The 2-week time-point can be interpreted to represent a transient effect, whereby some of the metabolites that increased or decreased at 2-weeks did not differ between baseline and 4-week samples. This transient effect explained most of the variation in the model (49% out of 81% visualized on this plot). Conversely, PC2 explained variation associated with a steady change in metabolite content over time, as the baseline, 2-week, and 4-week clusters were equidistant across the y-axis. Four metabolites associated with PC1 and PC2, are shown as examples of transient and response phase effects associated with each component (Fig. 3B). Z-scores (a test statistic indicating the number of standard deviations a sample group

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differed from baseline) for glutamate and cholate indicate the metabolite content changed after 2-weeks, and then reverted closer to base line at 4-weeks. On the other hand, glucose and coprostanol steadily changed compared to baseline over the course of the 4-week diet. Note that all metabolites varied at 2-weeks, but the different trends over time may infer distinct types of metabolite-responses. In the case of glucose and coprostanol (the latter a strictly gut microbe associated metabolite), the changes would eventually reach a new steady state level, whereas the glutamate/cholate-like trend is not in a consistent direction. The temporal response in this dietary intervention can be divided into three stages:

1. **Baseline phase:** The baseline metabolite profile is intended to be an accurate representation of the biochemical makeup of a given biological sample prior to intervention.
2. **Transient phase:** The transient metabolite profile consists of small molecules that may differ *via* natural variation among foods in the regular diet. The transient effect may be distinct across diet studies and among individuals. This is commonly referred to as an acclimation or adjustment period to a new food or dietary supplement.
3. **Response phase:** The metabolite profile response phase represents small molecules that change with a trend that is distinct from baseline and control samples. Response metabolites vary due to the consistent intake of new dietary components. This phase can also be considered a new steady-state level following a dietary intervention.

Fig. (4). shows that the baseline, transient, and response phases are reflected in the PCA. Individual metabolite identifications allow for determination of affected metabolic pathways-networks following a dietary intervention. Investigations into specific biochemical pathways may be difficult to interpret when there is large variation in baseline metabolite profiles of one experimental group. Thus, for pathway-focused analyses derived from omic-level data, a common first step is to combine multivariate and univariate statistical tests to determine metabolites that vary. One can perform ANOVA on principal components to identify components that vary according to the response phase, and subsequently select metabolites that contribute to the response variation by conducting outlier-like tests for each principal component's loadings. Biased multivariate methods such as partial least squares-discriminant analysis can achieve similar results, but may fail to describe trends in the transient phase if the analysis is initially biased towards the response phase. In (Fig. 3B), one may focus interpretation around the biochemical relationship between coprostanol and glucose, which both exhibited significant loadings for the PC2 described in (Fig. 3A). Thus, this example dataset illustrates that multivariate statistics can be used to identify sets of metabolites that vary according to baseline, transient, or response phases and that trends for each set of metabolites can be independently assessed in the context of biochemical pathways.

SUMMARY

Nutritional metabolomics investigations have utilized both targeted and non-targeted experimental approaches in a variety of non-invasively collected biological samples.

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Additional studies are needed to gain a better understanding of dietary-associated changes in small molecules detected in organs and tissues. This review focused on evaluation and detection of short and long-term diet responsive metabolites reported in the literature from blood, urine, and stool, as well as specific experimental considerations in the design of nutritional metabolomics studies.

Advances in our knowledge of the host metabolome response across dietary exposure or intervention trials alongside careful consideration of experimental design have provided compelling data for future dietary-focused studies. Given the emerging role and evidence that gut microbiota influences host metabolism, cross-omic or trans-omics data integration will be instrumental for over all results interpretation [50, 58].

The full potential of metabolomics to the nutritional science, dietetics, and public health nutrition community has become better realized over the past few years, and will continue to advance by the availability of digital repositories of raw and/or annotated data sets useful for mechanistic and metabolic pathway analysis and inform the field of nutrition on diet-modifiable metabolic networks.

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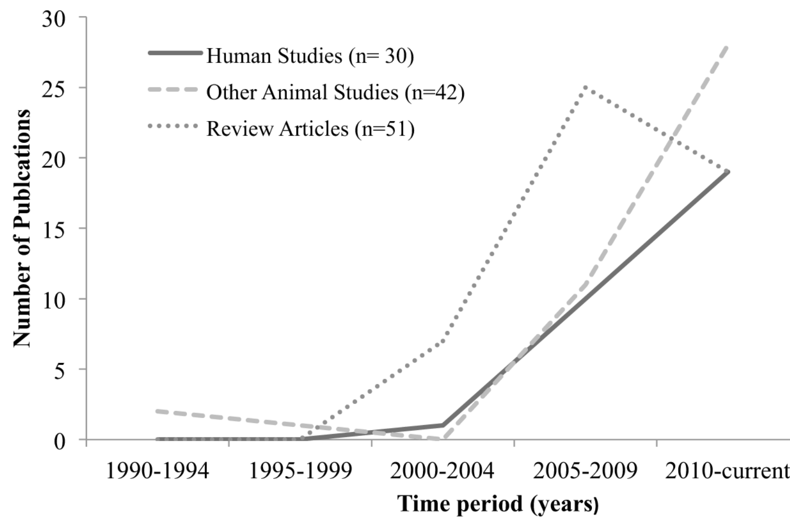

Fig. 1. Number of publications on "metabolomics and diet"* cited in PubMed Central (n= 123)

The following search criteria was used in PubMed Central: 'metabolome AND diet' and 'fecal metabolites AND diet'. Review articles also included summaries from conferences and opinion articles.

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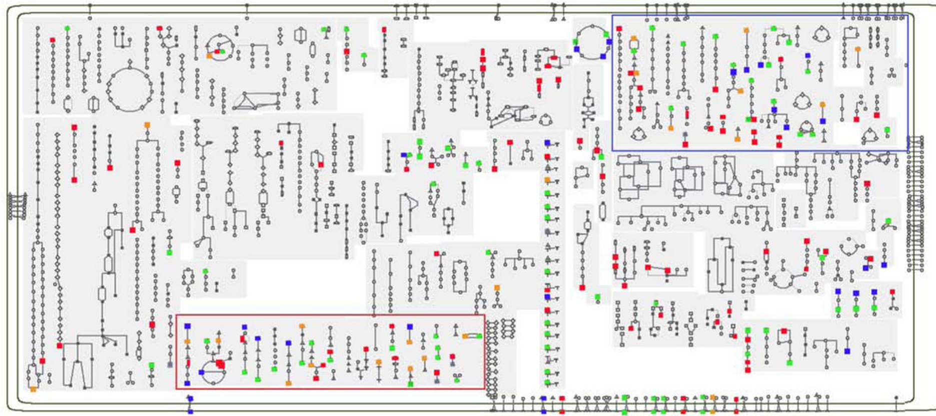

Fig. 2.

The metabolites listed in the tables were overlaid onto the core metabolic map offered at Meta Cyc for *Homo sapiens*. Red points represent diet-responsive metabolites in serum, blue points in urine, and orange points in fecal samples. Green points represent metabolites that were found to be diet-responsive in two or more biofluids. Particularly rich coverage is provided in amino acid metabolism (red box) and catabolism (blue box).

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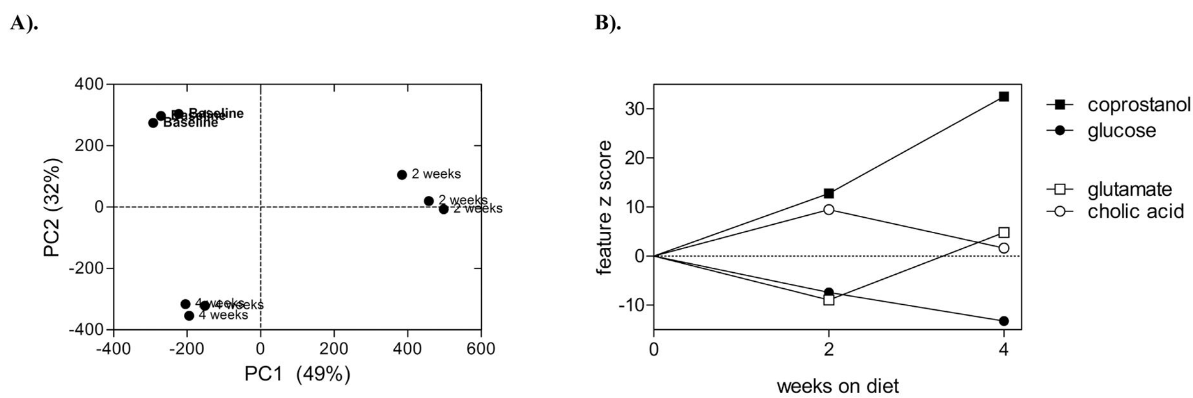

Fig. 3.

A pilot example of fecal metabolite profiling results from a healthy adult that participated in a dietary navy bean intervention trial for 28 days. (**A**) Principal component analysis scores plot for three timepoints: baseline, 2-weeks, and 4-weeks. (**B**) Selected metabolites that change in a transient or steady pattern at each timepoint examined.

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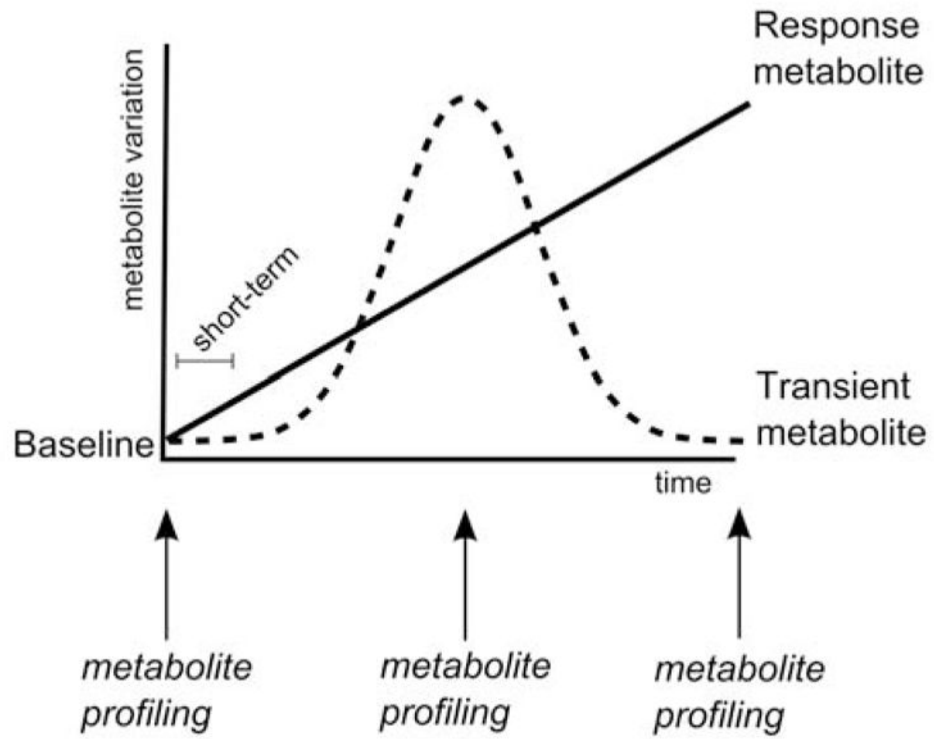

Fig. 4.
Model for interpretation of three distinct effect phases that may occur during a dietary intervention study, and that can be observed *via* metabolomic profiling.

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Table 1

Dietary Modulation of the Urine Metabolome

Sample	Metabolites Identified by Platform			
	GC-MS	LC-MS	NMR	Other*
Urine	**Hippurate**	**Trimethylamine-N-oxide**	Proline	Niacin
	4-hydroxphenylacetic acid	Phenylalanine	Betaine	Proline
	Tartrate	**Histidine**	4- hydroxyhippurate	Betaine
	Ethanol	**Citrate**	Fumarate	Hesperidin
	Mannitol	Acetaminophen	Lactate	Narirutin
	3-methyl-oxovalerate	Acetate	Glucose	
	Nitrogen	Choline	**Glycine**	
	Creatinine	**Phenylacetylglutamine**	Methylamine	
	Succinate	**Taurine**	Phenylacetylglycine	
	Putrescine	**Methionine**	Formate	
	Threonine	Urocanate	**Branched Chain Amino Acids**	
	3-hydroxyisovalerate	Sucrose	P-cresolsulfate	
	Arginine	Cis-aconitate	Trigonelline	
	Acetone	Methylhistidine	Theobromine	
	N (1) -methyl-2-pyridone-5-carboxylamide (PYR)	Dimethylsulfone	Caffeine	
		Tyrosine	Prolinebetaine	
	Ascorbate derivatives	Hydroxynicotnic acid	Hesperidin	
	4-cresylsulfate	Urolithin B glucuronide	Narirutin	
	S-methyl-l-cysteine sulfoxide	Urolithin A	β-aminoisobutyrate	
		Urolithin B	Oxodecanoic acid	
		2-oxoglutarate	Acylcarnitines	
		Fumarate	**Creatinine**	
		Hippurate	**Trimethylamine-N-oxide**	
		Lactate	**Hippurate**	
		Creatinine	**Phemylacetylglutamine**	
		Succinate	Acetone	
References	[59, 60]	[9, 59, 61, 62]	[11, 14, 17, 22, 24, 63–66]	[67–69]

*Other Platforms used in Identifying Metabolites: ICP-OES: Inductively Coupled Plasma Spectroscopy, DAD-MS/MS: Diode Array Detector Mass Spectrometry, FIEI-MS: Flow Injection Electrospray-ionisation.

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Table 2

Dietary Modulation of the Blood Metabolome

Sample	Metabolites Identified by Platform			
	GC-MS	**LC-MS**	**NMR**	**Other***
Blood	Betaine	Butyrylcarnitine	B-hydroxybutyrate	Caffeic Acid
	N-dimethylglycine	L-tryptophan	Lactate	Sulphonatemethylepicatechin
	Dimethyl sulfone	**Choline**	Acetate	
	Stearic Acid	Cysteine	Betaine/trimethylamine-N-oxide	
	Oleic Acid	**Glycine**	Glycine betaine	
	LysoPC 14:0	**Methionine**	β-glucose	
	LysoPC 18:0	Serine	α-glucose	
	LysoPC 18:1	Urate	inosine/adenosine and nucleotides	
	LysoPC 18:2	Phenylalanine	Campesterol	
	LysoPC 20:2	Alanine	DHA	
	L-Carnitine	**Histidine**	Cholestenol	
	L-Valine	**Branched-chain amino acids**	Sphingosine moiety	
	D-Pipecolic acid	Isobutyrate	Nervonic acid	
	L-Tyrosine	Palmitic acid	Erythrosphingosine	
	L-Leucine	EPA	Threosphingosine	
	Propionylcarnitine	Cholic acid	3-*O*-methylsphingosine	
	SIRT1	Saccharic acid	5-*O*-methylsphingosine	
	11-dehydro thromboxane B (2)	Sucrose	*N*-methylalanine	
	3-hydroxybutanoic acid	γ-linoleic acid	palmitoleic acid	
	Sulfoglycolithocholic acid	Phenylacetylglutamine	3-hydroxy-3-methylglutaric acid	
	Threitol	Tryptophan	Idonic acid	
	Hydrocinnamic acid	**Taurine**	Lactobionic acid	
	α-ketoglutaric acid	Citric Acid	3-phenyllactic acid	
	Docosapentaenoic acid	L-proline	Glycerol-3-galactoside	
	O-acetylcarnitine	α-tocopherol	Raffinose	
	Proline	Betasitosterol	Actetoacetate	
	Tyrosine	Urolithin A glucuronide	Glucose	
	Carnitine	*Niacine*	**Creatinine**	
	SIRT1	Biuret	Triacylglycerol	
		Creatine	Glycerol	
		Oleic Acid	Ascorbic acid	
		Hippuric Acid	urolithin C (trihydroxydibenzopyranone)	
		Adenine	Acetone	
		LysoPC		
		Uric Acid		
		Creatinine		
		Leucine		

Sample	Metabolites Identified by Platform			
	GC-MS	LC-MS	NMR	Other*
		Putrescine		
References	[2, 38, 59, 70–72]	[38, 40, 59, 61, 73–76]	[11, 20, 21]	[1, 67, 71]

*Other Platforms used in Identifying Metabolites: ICP-OES: Inductively Coupled Plasma Spectroscopy, DAD-MS/MS: Diode Array Detector Mass Spectrometry, FIEI-MS: Flow Injection Electrospray-ionisation.

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Table 3

Dietary Modulation of the Fecal Metabolome

Sample	Metabolites Identified by Platform			
	GC-MS	LC-MS	NMR	Other
Stool	Galactonic Acid	9-octadecenoic Acid	Propanoic Acid	Cholic Acid
	E. rectale	Lithocholic acid	Fumaric Acid	Deoxycholic Acid
	Coprostanol	Deoxycholic acid	**Glycine**	Lithocholic Acid
	Cholestanone	β-muricholic acid	Homocysteine	Hyodeoxycholic Acid
	Deoxycholic acid	Chenodeoxycholic acid	Enterobacteriaceae	
	Betahyocholic acid	Benzaldehyde	Cholic acid	
	Lithocholic acid	Urolithin A Cytotoxic	Hyodeoxycholic acid	
	Cholic acid	haem metabolite (haem factor)	Valerate	
	Benzoic acid		Isovalerate	
	Acetophenones		Isobutyrate	
	Cholesterol		Lactate	
	Cholestanol		Benzene	
	Coprostanone		Acetic Acid	
	Cholestenone		Butonic Acid	
			Glutamic Acid	
			Alanine	
References	[51, 77]	[29, 78]	[33, 79]	[33, 67, 79–81]

*Other Platforms used in Identifying Metabolites: ICP-OES: Inductively Coupled Plasma Spectroscopy, DAD-MS/MS: Diode Array Detector Mass Spectrometry, FIEI-MS: Flow Injection Electrospray-ionisation.