

## Aberystwyth University

### *Perspective*

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## 1 **Dietary Biomarkers of Intake and Exposure: Exploration with Omics Approaches**

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70 certain genetic polymorphisms that are associated with health problems and develops medical  
71 foods to treat these problems. He has had grant funding from Balchem Company, a company  
72 that makes choline for diet supplements and animal feed. Dr. Zeisel is on advisory boards for  
73 Baxter, Proctor and Gamble, and Abbott, all companies with an interest in choline relative to  
74 their products. He received an honorarium for speaking from Nutraceuticals, a company that  
75 makes diet supplements.

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91

92 Abstract:

93 While conventional nutrition research has yielded biomarkers such as doubly labeled water for  
94 energy metabolism and 24-h urinary nitrogen for protein intake, a critical need exists for  
95 additional, equally robust biomarkers that allow for objective assessment of specific food intake  
96 and dietary exposure. Recent advances in high throughput mass spectrometry (MS) combined  
97 with improved metabolomics techniques and bioinformatic tools provide new opportunities for  
98 dietary biomarker development. In September 2018, the National Institutes of Health organized  
99 a 2-day workshop to engage nutrition and omics researchers and explore the potential of multi-  
100 omics approaches in nutritional biomarker research. The current perspective summarizes key  
101 gaps and challenges identified, as well as the recommendations from the workshop that could  
102 serve as a guide for scientists interested in dietary biomarkers research. Topics addressed  
103 included: study designs for biomarker development, analytical and bioinformatic considerations,  
104 and integration of dietary biomarkers with other omics techniques. Several clear needs were  
105 identified, including: larger controlled feeding studies, testing a variety of foods and dietary  
106 patterns across diverse populations, improved reporting standards to support study replication,  
107 more chemical standards covering a broader range of food constituents and human metabolites,  
108 standardized approaches for biomarker validation, comprehensive and accessible food  
109 composition databases, a common ontology for dietary biomarker literature and methodologic  
110 work on statistical procedures for intake biomarker discovery. Multidisciplinary research teams  
111 with appropriate expertise are critical to moving forward the field of dietary biomarkers and  
112 producing robust, reproducible biomarkers that can be used in public health and clinical  
113 research.

114 Key words: Dietary biomarkers, dietary intervention studies, diet, nutrition, metabolomics

115

116

117 Abbreviations used:

118 4DFR, 4-day Food Record; AUROC, Area Under the ROC curve; BFI, Biomarkers of Intake;

119 CIR, Carbon Isotope Ratio; CFS, controlled feeding studies; DBS, Dry blood spot, DLW, Doubly

120 Labelled Water; DPB, Dietary Pattern Biomarkers; FFQ, Food Frequency Questionnaires; FCIB,

121 Food Component Intake Biomarkers; FoodBall, Food Biomarker Alliance; FGF-21, fibroblast

122 growth factor-21; GC, Gas Chromatography; GC-C-IRMS, gas chromatography-combustion-

123 isotope ratio mass spectrometry; HILIC, Hydrophilic Interaction Chromatography; IRMS, Isotope

124 Ratio Mass Spectrometry; LC, Liquid Chromatography; Metlin, Metabolomics database; MoNA,

125 Mass bank of North America; MS, Mass Spectrometry; MWAS, Metabolome-wide association

126 studies; NIR, Nitrogen Isotope Ratio; NPAAS, Nutrition and Physical Activity Association Study;

127 NMR, Nuclear Magnetic Resonance spectroscopy; RP, Reverse Phase Chromatography; ROC,

128 Receiver Operating Characteristic Curve; SIR, Stable Isotope Ratio.

129

130

## 131 **Introduction**

132  
133           Prevailing dietary intake assessment methods [e.g., food frequency questionnaires  
134 (FFQ)] rely heavily on self-reported dietary recall and have a variety of systematic and random  
135 measurement errors. A systematic underreporting of dietary intake, especially of total calories  
136 and absolute amounts of macronutrients, in weight-loss trials has been well documented (1).  
137 This problem is further exacerbated by the increasing prevalence of ‘ready-to-eat meals’ in the  
138 Western diet, with incomplete ingredient lists and inability of the participants to complete the  
139 cumbersome and complicated dietary questionnaires. In addition, imperfect or incomplete food  
140 composition databases can lead to inaccuracies, when food intake data are converted to the  
141 corresponding nutrient intake data. Finally, differences in individual metabolism, due to genetics  
142 or the gut microbiome, add complexity to intake measurements. Ideally, self-reported dietary  
143 intake information should be independently validated against a biological or chemical marker  
144 that provides an accurate measure of the dietary intake and exposure. For example, candidate  
145 biomarkers such as alkyl resorcinols, for measuring wheat and rye intake, are beginning to be  
146 employed in epidemiologic studies (2). However, such objective markers of intake are limited to  
147 few nutrients and do not exist for most foods and dietary patterns.

148           Recent advances in high throughput mass spectrometry (MS) and nuclear magnetic  
149 resonance spectroscopy (NMR) combined with improved metabolomic, genomic and  
150 metagenomic techniques are now making it possible to identify new and improved dietary  
151 biomarkers. Several studies have demonstrated the feasibility of this combined multi-omic  
152 approach (3-5). In order to explore the potential of multi-omics approaches in dietary biomarker  
153 development, and to identify related challenges and approaches to address them, the National  
154 Institutes of Health (NIH) organized a workshop on “Omics Approaches to Nutritional  
155 Biomarkers” from *September 26-27, 2018 in N. Bethesda, MD*. This workshop engaged nutrition  
156 and omics researchers from the US, Canada, and several countries in Europe, all of whom

157 participated in scientific presentations and focused breakout sessions to discuss various  
158 aspects of dietary biomarker development.

159 **Table 1** presents a summary of the challenges and the resulting recommendations from  
160 the workshop. Each challenge is discussed in greater detail below. The recommendations are  
161 intended to serve as a guide for scientists wishing to identify, develop, validate or use dietary  
162 biomarkers in their research programs.

163

### 164 **Dietary Biomarker Definitions and Their Utility in Nutrition Research**

165 A dietary biomarker enables an objective measure of either dietary intake, its impact on  
166 host physiology and modify disease risk (6). Following a broader paradigm for biomarker utility,  
167 diet-related biomarkers are typically classified into 3 groups: 1) exposure biomarkers, 2)  
168 susceptibility markers and 3) outcome biomarkers. An exposure biomarker provides an  
169 objective measure of dietary intake of a particular food or nutrient (7). Susceptibility biomarker  
170 provides information about resilience or susceptibility to effects caused by food components,  
171 such as susceptibility to iron overload from meat consumption. In contrast, an outcome  
172 biomarker is used to assess how physiologic and clinical outcomes are affected by nutrient  
173 exposures (8). In addition to this “classical” set of biomarkers, several other dietary biomarker  
174 classification schemes have also emerged in the field of nutrition, depending on how a  
175 biomarker changes in relation to intake and length of exposure (6, 9).

176 No single classification scheme covers all the aspects of dietary biomarker functions and  
177 features (9). The same compound may be classified in different categories depending on the  
178 purpose of use. For example, total plasma homocysteine concentrations indicate folate status  
179 and serve both as a marker of nutrient status and a biomarker of treatment response in  
180 response to folate supplementation (10). Most of these biomarker classification schemes  
181 assume a unidirectional interaction, with specific dietary components impacting physiological  
182 systems. However, it is increasingly recognized that the relationship between dietary

183 components and physiological systems is bidirectional. In fact, dietary components impact the  
184 host's physiology which, in turn impacts how these dietary substances are metabolized (**Figure**  
185 **1**). Moreover, the food-host metabolic interaction is, embedded in a broader cultural and  
186 environmental system that influences the type and extent of food exposure and impact the  
187 metabolic end-products (i.e., biomarkers) detectable in human biospecimens.

188         The application of metabolomics allows a better characterization of this bidirectional  
189 relationship between diet and physiology enabling the measurement of both nutrient and non-  
190 nutrient metabolites that could serve as candidate biomarkers (11). However, non-nutrient  
191 markers are not well integrated in the current paradigm of biomarker classifications and no  
192 common biomarker ontology can address all these classifications. In recognition of these  
193 challenges, Gao et al. developed a detailed dietary biomarker classification framework that  
194 integrates both nutrient and non-nutrient markers from food components (9).

195         Under this new classification scheme, exposure biomarkers (which may be single  
196 biomarkers or combinations of multiple biomarkers) are further classified into food component  
197 intake biomarkers (FCIBs); biomarkers of food intake (BFIs) and dietary pattern biomarkers  
198 (DPBs). FCIBs are typically metabolites of chemicals present in different foods and include both  
199 nutrients and non-nutrients. BFIs, on the other hand are associated with a given food type or  
200 food group and mostly consist of non-nutrients, such as proline betaine for citrus fruit  
201 consumption. DPBs are used to distinguish between different dietary regimens such as  
202 Mediterranean, Western or Nordic diet patterns. DPBs can include both FCIB and BFI markers  
203 from a variety of foods found in a specific dietary pattern. This new type of diet-related  
204 biomarker classification scheme appears to offer both breadth and flexibility as it allows the  
205 same markers to be used for a variety of different purposes (9).

206         Currently only a few reliable intake biomarkers are known. These include 24-h urinary  
207 nitrogen for protein intake, doubly-labeled water (DLW) for total energy expenditure  
208 measurements and 24-h urinary sodium and potassium for sodium/potassium intake.

209 Unfortunately, methods such as DLW analysis are very expensive, while 24-h urinary nitrogen  
210 or urinary sodium and potassium measurements are too cumbersome for regular participant  
211 compliance, to be employed in large studies (12).

212 One approach that appears to be particularly promising for finding biomarkers of  
213 macronutrient intake is the use of isotope ratio mass spectrometry (IRMS) (13). Naturally  
214 occurring differences in the stable isotope ratio (SIR) of lighter elements, among foods such as  
215 carbon ( $^{13}\text{C}$  vs.  $^{12}\text{C}$ ; measured as  $\delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$  vs.  $^{14}\text{N}$  measured as  $\delta^{15}\text{N}$ ) are reliably  
216 incorporated into tissues and can be measured by IRMS. One of the advantages of this method  
217 is that SIRs are very stable and can be measured in a variety of biological specimens, including  
218 blood, hair and toenails. Biomarkers for macronutrient food components such as carbohydrates  
219 and protein have been explored using stable isotope ratio analysis (14-16).

220 Another approach is MS-based metabolomics, which is opening the door to measuring  
221 both micronutrient and non-nutrient biomarkers to reliably predict food intake. For example, the  
222 Phenol-Explorer database contains information more than 500 non-nutrient plant polyphenols  
223 that are specific for a particular foods or food groups (17). Using standard metabolomic methods  
224 and the Phenol-Explorer database to annotate polyphenol metabolites in urine, it was possible  
225 to measure over 80 polyphenol metabolites in 24-h urine samples and to identify good  
226 predictors of intake from some of their main food sources such as citrus fruit, coffee, tea and  
227 wine as estimated with 24-h dietary records (18). More recently, using a targeted assay for 34  
228 dietary polyphenols measured in urine, it was possible to study variations of urinary excretion  
229 according to geographical variations of the diet in four different countries enabling the  
230 identification of those phenolic compounds most strongly associated with intake of 110 plant-  
231 derived food groups (19). A recent study employed a non-nutrient biomarker alkylresorcinol  
232 metabolite in plasma for whole-grain consumption, to demonstrate its protective effect on the  
233 risk for ischemic stroke, demonstrating their potential clinical utility (2). These examples

234 illustrate that metabolomics when combined with the right kinds of databases, can be used to  
235 identify some useful dietary biomarkers.

236

## 237 **Approaches to Studying Biomarkers**

### 238 ***Study Designs***

239 Dietary intake biomarker development is best approached as an iterative process,  
240 involving a well-integrated methodologic strategy from biomarker discovery through validation.  
241 Biomarker development should also rely on sufficiently robust study designs to identify  
242 candidate biomarkers that subsequently can be successfully validated (20). While controlled  
243 feeding studies (CFS) are particularly informative for both biomarker discovery and validation,  
244 other study designs may be employed to capture the characteristics of dietary variation and  
245 identify candidate dietary biomarkers for a wide diversity of foods.

246 Cross-sectional studies are routinely used for initial dietary biomarker exploration for  
247 capturing the continuous distribution of dietary constituents in the habitual diets, including food  
248 groups or of dietary patterns (7). Key challenges of using cross-sectional studies to discover  
249 dietary biomarkers lie in the limitations of common dietary assessment instruments such as  
250 dietary recalls, food diaries, and FFQs (21). Additional challenges relate to measurement errors  
251 in dietary self-reporting (22), the inadequacies of food-composition tables, and the limited  
252 generalizability of diet-biomarker associations to other populations. Most reported candidate  
253 dietary biomarkers arise from foods that are routinely consumed and potentially more accurately  
254 recalled by participants (23, 24). In contrast, foods that are consumed infrequently are often  
255 difficult to capture with a recall or an FFQ and typically will result in the sporadic appearance of  
256 measurable biomarkers in blood or urine. In such cases, cross-sectional studies may be  
257 ineffective to identify such biomarkers, unless they have unusually long half-lives. These  
258 biomarkers may be less easily identified and would likely be among the more lipophilic

259 metabolites (7). Such biomarkers may not effectively replace traditional self-report dietary  
260 assessment methods of longer-term exposure, but their integration with dietary intake data may  
261 provide a more accurate assessment of exposure.

262 In contrast to cross-sectional studies, which are often used for dietary biomarker  
263 discovery, CFS are primarily used to evaluate the effects of diet on biological and physiological  
264 processes in humans. Nonetheless, feeding known amounts of specific foods or nutrients to  
265 study participants also provides an opportunity to evaluate biomarkers of dietary exposure (25).  
266 Typically, CFS use the same standardized menus for all participants, thereby reducing the  
267 variation in nutrient intake and the variance introduced by food type, as well as by the handling,  
268 storage, preparation, and processing of the food. These studies permit the testing of several  
269 factors such as the magnitude of consumption and duration of feeding (e.g., short-term vs. long-  
270 term), and can provide rich data on biomarker nutrkinetic and nutridynamic properties, similar to  
271 drug metabolism (26, 27). CFS also allow the assessment of metabolite variability due to host  
272 physiology, the type of intervention (e.g., dietary component, food, dietary pattern), the  
273 biomarker performance, as well as when and how often the samples should be collected.

274 In CFS, dietary constituents or foods may be administered at the same dose to all  
275 participants (28), various doses to provide a range of exposures (29), or doses based on body  
276 weight (30). These diets also depart from participants' habitual intake, and consequently need  
277 adequate duration for biomarker equilibration. An alternative to a set-menu CFS is a variable-  
278 menu CFS that preserves the normal variation in nutrient and food consumption at the individual  
279 level in the study population. This approach requires individualized menu plans for each  
280 participant that mimic their habitual food intake as estimated by using a 4-day food record  
281 (4DFR) and adjusted for energy requirements, on the basis of calibrated energy estimates and  
282 standard energy estimating equations (31).

283 To date, most CFS have been conducted for shorter durations, with small sample sizes  
284 and a limited capacity to capture interpersonal heterogeneity. In addition, these studies are

285 often expensive and laborious to conduct, thereby necessitating several methodological  
286 compromises (limiting the sample size, reducing the study duration, etc.) that may potentially  
287 affect the final study results. There is no clear consensus on the choice of feeding study designs  
288 or sample sizes needed for dietary biomarker development and validation. Recently there have  
289 been attempts to combine a variety of study designs such as crossover, controlled feeding and  
290 cross-sectional studies for biomarker explorations from discovery phase to testing them in free-  
291 living populations on habitual diets (32, 33). The final design depends, in large part, on the  
292 specific questions being addressed.

293         When substantial information is available on certain biomarkers, there may be no need  
294 to start from the beginning of the biomarker discovery process. Small, short-term feeding  
295 studies that yield candidate biomarkers may be followed by studies that characterize biomarker  
296 time and dose-response. Likewise, validation and testing of biomarker performance may be  
297 done in separate cohorts, and the process may be repeated with necessary corrections, until an  
298 optimal biomarker performance is achieved.

299         The replication of initial biomarker studies in different populations is often necessary to  
300 generalize the results, to accommodate population heterogeneity, and to properly account for  
301 food choice diversity and dietary patterns. Ideally, the first validation study should be conducted  
302 in a similar population to the initial discovery cohort, favoring repeated measures to minimize  
303 intra-person variation in biomarker measures. Existing large cohorts, such as the Women's  
304 Health Initiative, the Framingham Health Study, and the Nurses' Health Study can be leveraged  
305 for large validation studies. However, it is important to recognize the limitations of the dietary  
306 assessment methods and the bio-sampling protocols used in these types of studies.

307 Collaborative multi-center feeding studies using habitual diet feeding study designs such as the  
308 one employed by the Nutrition and Physical Activity Association Study (NPAAS) provide an  
309 excellent opportunity for recruiting diverse populations and exploring several nutritional factors  
310 and candidate biomarkers (34). Citizen science projects, such as the American Gut Project (35)

311 may also be useful to validate candidate biomarkers because they rapidly generate large  
312 sample sizes, involve broad national and international participation, and help to capture  
313 biomarkers of more prolonged exposure to a particular diet.

314         Criteria such as high sensitivity and high specificity for the dietary intake of interest are  
315 fundamental to good biomarkers that can be quantified in terms of the AUROC (area under the  
316 receiver operating characteristic curve). In addition, a potential food or diet intake biomarker  
317 should be able to explain a sizeable fraction of the feeding study variation in the given diet. The  
318 AUROCs allow setting a cut off value for a given biomarker, ranging from 0.5 with random  
319 association to 1.0 with strong association between the biomarker and dietary consumption and  
320 rely on the continuous performance of the biomarker, on a binary outcome (36). The specific cut  
321 point to be met in applying these criteria may depend on the context of the feeding study. It may  
322 also have to be adjusted to reflect the accuracy of estimated feeding study intake (e.g. accuracy  
323 of food composition databases), as well as the study duration and other aspects of the feeding  
324 study design. Investigators proposing novel biomarkers need to provide convincing evidence of  
325 a close correspondence between the actual intake and the biomarker estimated intake, rather  
326 than simply demonstrating a positive correlation between the two. While the AUROC are useful  
327 for dietary biomarker research; their utility highly depends on the availability of good gold  
328 standard markers with which, they can be compared for their classification (36).

329

### 330 ***Biologic sampling***

331         Regardless of the study design, careful consideration of the types of biological samples  
332 collected and analyzed is fundamental to ensuring meaningful outcomes. In terms of dietary  
333 biomarkers, urine appears to provide better metabolite coverage compared to plasma, due to  
334 the relative lack of interfering proteins and the fact that many dietary biomarkers are in higher  
335 concentrations in urine (11). Several factors impact the choice of biospecimen matrix for dietary  
336 biomarkers. Different specimens may yield different candidate biomarkers due to their unique

337 physiological origins or their different duration of exposure. Specimens such as saliva and sweat  
338 may provide insight on short-term dietary exposures, whereas red blood cells (RBCs) better  
339 capture medium-term exposure (11, 37, 38), while toenails (39) and hair appear to be promising  
340 matrices for long-term exposures (40, 41). Assessment of biomarkers in more than one matrix  
341 (both plasma and urine) will also provide information on the distribution and dynamic range of  
342 biomarkers in the system and their half-lives (11).

343 Archived biospecimens from well-conducted dietary interventions are potentially very  
344 useful resources for biomarker discovery and validation. However, precise information on the  
345 stability of certain dietary biomarkers upon storage, sample handling practices, especially for  
346 multi-site studies is important to determine potential confounders that contribute to metabolite  
347 variability. Currently, there are several large repositories for plasma and serum (42).

348 Unfortunately, there are not many cohort studies with repositories containing urine samples.  
349 While urine is often the preferred biomatrix for dietary biomarker studies, it is also important to  
350 remember that many useful dietary biomarkers have been identified in plasma, although sample  
351 collection requires trained phlebotomists (7). Indeed, the dietary metabolites with the strongest  
352 food correlations in population studies tend to replicate in both blood and urine and predict  
353 habitual diets (24). For this reason, it is still useful to expand dietary biomarker studies in blood-  
354 based (plasma) specimens. Overall, the lack of appropriately collected, publicly accessible  
355 repositories of specimens from intervention and cross-sectional studies represents a continuing  
356 impediment to dietary biomarker discovery and development. Certainly, encouraging the long-  
357 term storage of biospecimens from completed feeding studies will expedite the biomarker  
358 discovery and development process.

359 There is a critical need to standardize specimen collection and sample processing  
360 protocols to ensure greater reproducibility, comparability and generalizability across studies  
361 (43). For instance, Lloyd et al. conducted a systematic validation of biomarkers of habitual citrus  
362 fruit intake and demonstrated that both spot and overnight fasting urine samples provide a good

363 correlation with FFQ data (44). Garcia-Perez et al., explored the timing of urine collection and  
364 compared the quantification of biomarkers in spot urine versus 24-h urine samples (43).

365         Although repeated samples are highly desirable for accurate quantitative measurement,  
366 single samples may also be reasonably informative, especially for frequently consumed foods  
367 (45). This is especially true for biomarkers showing good reproducibility over time, which are  
368 well suited for prospective studies involving larger cohorts (45, 46). Furthermore, sample  
369 handling (standing time, storage temperature, and freeze/thaw cycles) affects many  
370 metabolites, which obviously affects the robustness of any identified metabolite biomarkers.  
371 However, if samples are handled consistently then biomarker-outcome associations (e.g., in  
372 nested case-control studies) can still perform well. Nonetheless, standardized sample handling  
373 practices should be encouraged.

374         The development of new sampling techniques is also becoming important to enable  
375 more efficient, cost-effective sample collection and better coverage in larger cohort studies. This  
376 is particularly important for studies with geographically isolated cohorts (47). For example, dried  
377 blood spots (DBS) are proving to be an inexpensive method for sample collection and storage  
378 and require minimal specialized equipment and offer several advantages including convenient  
379 transportation (48). Novel gastro-intestinal (GI) tract sampling methods are emerging which may  
380 identify novel dietary biomarkers related to intake and food microbial metabolism (49).  
381 Advances in wearable technology that can continuously monitor metabolites or allow intermittent  
382 sampling will likely complement and expedite the biomarker development process.

383

## 384 **Analytical and Statistical Considerations in Biomarker Development**

### 385 ***Analytical and Technological Issues***

386         High throughput, untargeted metabolomics approaches have revolutionized dietary  
387 biomarker development, allowing unbiased interrogation of both nutrients and non-nutrients.

388 Several analytical methods including NMR, MS combined with liquid chromatography (LC) and

389 gas chromatography (GC) have been used for dietary biomarker research. These methods differ  
390 in their sensitivity, sample processing requirements and metabolite coverage. NMR is a robust,  
391 relatively unbiased, inherently quantitative method that allows novel metabolite identification and  
392 requires little sample processing. However, NMR suffers from low sensitivity, enabling detection  
393 and/or quantification of 30-100 different, more abundant metabolites in a given biological  
394 sample. GC-MS is ideal for detecting a variety of nutrients (amino acids, sugars, organic acids,  
395 steroids, fatty acids and volatile metabolite analysis) and it is sufficiently sensitive to detect up to  
396 300 different chemicals in certain biomatrices. However, GC-MS requires extensive sample  
397 work-up and sample derivatization, making it more time consuming and more difficult to quantify  
398 compounds than NMR. High resolution LC-MS is a highly sensitive technique that allows the  
399 detection of up to 10,000 features and the identification of between 400-1500 different  
400 chemicals depending on the platform and methodology (targeted vs. untargeted). LC-MS is  
401 particularly suitable for detecting non-nutrient metabolites that occur in very low concentrations.  
402 As a result, it is gaining popularity as the preferred platform in both metabolomics and dietary  
403 biomarker studies. One of the limitations of LC-MS is that no single LC-system can cover all  
404 metabolite classes. Hydrophilic interaction chromatography (HILIC) typically must be used to  
405 separate more polar metabolites, reversed phase chromatography (RP) must be used to  
406 analyze neutral and nonpolar metabolites and chemical derivatization may be required to detect  
407 lower-abundance metabolites. While obtaining relative quantitation of compounds using LC-MS  
408 is straightforward and typical in metabolomics, determining the absolute concentration of  
409 compounds is more difficult and requires expensive isotopically labeled standards as well as  
410 multi-point calibration curves. Ideally, data from a variety of metabolomics platforms should be  
411 interrogated for discovering and/or quantifying candidate dietary biomarkers of specific dietary  
412 exposures.

413 Inter-laboratory reproducibility of untargeted LC-MS metabolomics data is another  
414 challenge and is heavily influenced by the instrument type and design. Nevertheless, Cajka et

415 al. have recently shown that nine different mass spectrometers can give rise to nearly identical  
416 results for identical biological samples, if detection saturation is avoided (50). Metabolite  
417 identification, coverage and reproducibility are also influenced by experimental conditions that  
418 include sample processing, storage, mode of detection, instrument run and choice of data  
419 reduction methods employed. No standardized and universally accepted protocols and pipelines  
420 exist for untargeted LC-MS-based metabolomics. Furthermore, LC-MS methodologies from  
421 individual labs are not freely shared among the community, further reducing confidence and  
422 reproducibility. The variability can be minimized by adopting appropriate quality control  
423 measures such as proper blanks, controls and standards in the experimental runs. There are  
424 now efforts within the metabolomics community to develop such standards and protocols to  
425 improve reliability and reduce variability within and across studies. It is also important to adopt  
426 the use of pooled reference samples (such as standard reference materials), to adjust for  
427 instrumental differences and batch variations over time. To improve the quality of data  
428 processing and metabolite identification, there is a critical need for sharing the raw data  
429 including quality control (QC) measures and blanks for data processing. Inter-lab comparison of  
430 assays, with appropriate standards, should be encouraged to ensure cross-validation of assays.

431

### 432 ***Data Analysis and Metabolite Identification for Biomarker Discovery***

433 While thousands of “features” can be detected on untargeted LC- MS-based  
434 metabolomics platforms, the actual identification of metabolites continues to be a major  
435 challenge. Raw data from MS instruments must undergo several processing steps before they  
436 can be statistically analyzed and compared. These steps involve the removal of adducts, peak  
437 identification and peak alignment, spectral deconvolution, compound identification (via matching  
438 to an MS/MS spectrum) and multivariate statistical analysis. There are many software tools  
439 including commercial packages that offer a wide range of excellent features but they all differ in  
440 their algorithms for picking MS peaks (51-53). As a result, there is only a 50-70% overlap

441 between the MS peaks detected by different packages, from the same raw data files using  
442 identical or near identical settings (54). Clearly, more standardization of the data analysis  
443 pipelines and peak picking algorithms is needed.

444         Compound identification which is the next step after spectral alignment and peak  
445 detection typically involves the comparison of MS/MS spectral features with well-curated MS  
446 databases. However, there is considerable diversity in the types of MS instruments and the  
447 types of MS spectra that can be collected on these instruments. As a result, it is often difficult to  
448 find a comprehensive MS database that fits with the type of MS spectra being collected other  
449 than the instrument-specific database provided by the vendor. Because the vendor-specific  
450 databases are often costly or do not cover the compounds of interest, there is a growing need  
451 for comprehensive, open-access MS databases that provide MS spectra for multiple platforms  
452 and which support broad metabolite identification activities. One such database is the  
453 MassBank of North America (MoNA) (55). MoNA is an open-access MS database that actively  
454 harvests and displays a large portion of the public MS/MS fragment spectral data for  
455 metabolites into a single, web-accessible resource containing over 130,000 experimental  
456 MS/MS spectral records from authentic compounds (including many food compounds) and  
457 nearly 140,000 predicted spectra generated for lipids.

458         Several other public databases also contain large, freely available collections of  
459 reference metabolite MS/MS spectral data covering multiple MS platforms (56-58). However,  
460 these databases are also populated with a large fraction (sometimes >80%) of predicted spectra  
461 commonly generated using programs such as MetFrag (59), CFM-ID (60), or Mass Frontier  
462 (61). While still useful, these predicted MS spectra are not as accurate nor as correct as  
463 experimentally collected MS spectra. Indeed, the dearth of experimental MS spectra collected  
464 for authentic compounds continues to be a major challenge in metabolite annotation. Recently  
465 an effort known as the Food Compound Exchange (FoodComEx) has been launched to help  
466 address this problem (62). This community-driven concept, which was sponsored by the Food

467 Biomarker Alliance (63) allows researchers from around the world to freely share metabolite  
468 standards and experimentally collected metabolite and food constituent spectra in a  
469 collaborative manner. Efforts such as FoodComEx should help create key community resources  
470 to expedite biomarker discovery (62). Clearly, more support is needed for these types of  
471 community-driven, bottom-up efforts.

472

### 473 ***Dietary Biomarker Discovery***

474       After the candidate metabolites have been identified (either through targeted or  
475 untargeted metabolomic approaches) the next challenge is to identify the most useful or  
476 important biomarkers from the collection of identified metabolites. Biomarker discovery and  
477 biomarker assessment are often aided by the availability of specialized statistical software  
478 packages. These packages typically use multivariate statistics, feature selection and/or machine  
479 learning to identify one or more compounds that maximize the sensitivity and/or  
480 specificity of the biomarker or biomarker panel for the dietary exposure on a receiver operating  
481 characteristic (ROC) curve. While, ROC curves mainly enable the stratification of individuals  
482 based on their consumption of dietary components, it may be difficult to identify individuals with  
483 sporadic consumption. Maximizing the AUROC by selecting the right chemical or the right  
484 combination of chemicals is often a central goal of biomarker identification or biomarker  
485 discovery. Several freely available web-servers and software packages such as MetaboAnalyst  
486 and Galaxy have emerged over the past decade to provide comprehensive web-based tools for  
487 not only routine metabolomic data analysis and functional interpretation, but also provide the  
488 tools for metabolomic-based biomarker discovery suites (64, 65). However, advanced statistical  
489 tools are essential for validating dietary biomarkers, for integrating multi-omics data in nutritional  
490 studies, for correcting measurement errors in self-reported dietary reports (66) and for  
491 generating disease-diet biomarker regression models (67). There is a clear need for greater  
492 standardization, including standardized reporting (or minimum reporting standards) for the

493 statistical analysis of nutritional metabolomics data. Similarly, standardized processes for  
494 evaluating study reliability, data normalization, handling multiple testing effects, performing  
495 study replications and cross validation or external validation are also needed. In this regard, it  
496 would be particularly useful for the community to have statistical code repositories to foster  
497 greater uniformity and greater levels of reproducibility.

498

### 499 **Dietary Biomarker Validation**

500 LC-MS-based dietary biomarker discovery can provide hundreds of candidate  
501 biomarkers, but these biomarkers need to be thoroughly validated to be meaningfully employed  
502 in large cohort studies. The goal of validation is to ensure that newly discovered biomarkers can  
503 reliably and reproducibly predict dietary intake of food components. Biomarker validation  
504 requires analytical and biological testing of the performance of the biomarkers. It also requires  
505 an assessment of their specificity to food components, and their robustness in larger cohorts.  
506 While several concepts exist regarding the validation of biomarkers, there are no universally  
507 accepted validation criteria for dietary intake biomarkers. Dragsted et al. have recently devised  
508 an 8-step validation process that systematically assesses candidate biomarker plausibility,  
509 dose-response, time-response, robustness, reliability, stability, analytical performance, and  
510 reproducibility (20). Each criterion is important for establishing overall biomarker validity, but  
511 may be evaluated in different order, depending on the status of the candidate dietary biomarker.

512 Standardized dietary biomarker validation criteria allow the grading of markers based on  
513 their performance. Biomarker plausibility evaluates the credibility of the association between the  
514 biomarker and its food components. Plausibility can be based on a variety of sources of  
515 evidence, including research literature or *in silico* analysis of predictable biomarkers from  
516 compounds in the existing food composition databases and/or experimental data from  
517 metabolomics. Biomarker kinetics (including dose-response and temporal response to a single  
518 acute exposure) can be used to determine the suitability of the biomarker over heterogeneous

519 food intake distributions, and variable biomarker half-lives. Time-related response to multiple  
520 exposures (e.g., medium or longer-term feeding studies) may yield information on the  
521 distribution pattern of the biomarker across biological tissues (RBCs, hair, nails, etc.). The  
522 robustness and reliability criteria are used to determine how the biomarker behaves in a mixed  
523 meal or as part of a normal diet in the real world among diverse populations (i.e.,  
524 generalizability) and how it performs in comparison with other known biomarkers or other gold  
525 standards. Analytical performance criteria are used to determine the biomarker performance in  
526 both qualitative and quantitative terms, using known chemical standards ensuring a higher level  
527 of confidence in the biomarker performance. Cross-validation of the biomarker across  
528 laboratories confirms the reproducibility of the biomarker against food intake and completes the  
529 entire validation process.

530         This 8-step view of biomarker validation covers the entire spectrum of biomarker  
531 development from discovery through validation employing similar strategies and analytical  
532 platforms, as they move from one step to another, depending on the purpose of the biomarkers.  
533 When there is substantial information available on a biomarker or a set of biomarkers, some of  
534 these validation steps may be eliminated to make more significant strides towards validation.  
535 Initial studies can employ small-scale acute feeding studies, followed by other studies enabling  
536 characterization of other elements such as dose and temporal response relationships or the  
537 testing of the candidate biomarker performance in separate cohorts. Wider acceptance and  
538 adoption of such a systematic approach by the research community will expedite dietary  
539 biomarker research, bridge the gaps between discovery and validation, and turn biomarker  
540 development into a tractable process.

541

#### 542 **Areas Where More Data Are Needed**

543         The paucity of validated dietary intake biomarkers represents a fundamental challenge  
544 for food and nutrition research and it highlights the need to acquire more data about the

545 chemical compounds found in food and their fate after ingestion. Some are metabolically inert,  
546 and the biomarker compound found in blood, urine or feces is identical to the compound found  
547 in a specific food (i.e., proline betaine for citrus consumption). In other cases, the consumed  
548 nutrients or non-nutrients are metabolically transformed by endogenous processes or the gut  
549 microbiota. This leads to chemical byproducts that are very different from the ones originally  
550 ingested in the food (e.g., microbial product equol from daidzein, after soy consumption).  
551 Therefore, to develop a large set of robust, specific and fully validated food-specific biomarkers,  
552 it will be necessary to do two things: 1) acquire more data about the chemical constituents found  
553 in food (the “food metabolome”) and 2) acquire more data about the way that these chemical  
554 constituents are biologically transformed in the human body.

555           More than 150 food composition databases exist; however, most of these databases  
556 contain a relatively small number (10-100) of non-unique compounds for a vast number of  
557 foods. For instance, the USDA nutrient composition database (68) contains chemical data for  
558 nearly 250,000 different foods, but it only lists an average of 50 chemical compounds in each  
559 food item. While this information is useful for general nutritional assessment, it is not useful for  
560 identifying potential food-specific biomarkers.

561           More recently, a small number of on-line, electronic databases have emerged with more  
562 detailed chemical composition data for a smaller number of “raw” or mildly processed foods  
563 (**Table 2**). However, their utility in nutrition research community is limited by their lack of  
564 visibility and their lack of standardization or integration with each other. Another issue relates to  
565 the fact that these databases are still relatively incomplete. Most raw foods contain >10,000  
566 different compounds, yet the average compound coverage in even the most comprehensive  
567 food composition database is <1000 compounds per food item. Indeed, untargeted analyses of  
568 hundreds of different foods by the Dorrestein lab at University of California at San Diego  
569 (UCSD) has found that <5% of the detected MS peaks in any given food item can be assigned,  
570 using these databases (69, 70). This highlights an even more serious problem with today's food

571 composition databases; that is, they do not have sufficient authentic reference NMR or MS/MS  
572 spectra to permit broad and accurate compound identification. The availability of more authentic  
573 reference spectra would permit identification of more food-specific compounds in both foods and  
574 in human biofluids or excreta. Fewer than 1000 food-derived compounds have had their NMR or  
575 MS/MS spectra experimentally collected and deposited into food-specific databases (71). The  
576 lack of authentic chemical standards for food constituents and the lack of authentic referential  
577 spectra are the two most serious data-related issues hampering the identification, discovery or  
578 validation of food-specific biomarkers.

579         To find better food-specific biomarkers, it is important to know more about the way that  
580 these chemical constituents are biologically transformed *in vivo*. The fact that gut microbial  
581 activity influences the presence/abundance of certain food-specific metabolites adds another  
582 layer of complexity to food-specific biomarker identification. Indeed, the inter-individual variation  
583 due to differences in host genetics and the gut microbiome suggests that some degree of  
584 personalization may be required to properly interpret a number of food-specific biomarkers.

585         While steady progress is being made to identify food-specific, liver-specific, and other  
586 tissue- and microbially-derived biomarkers, many challenges still exist. Just as with food  
587 constituents mentioned above, there is a profound shortage of authentic chemical standards  
588 and authentic reference NMR or MS/MS spectra for these important compounds. Fewer than  
589 200 these compounds appear to exist in chemical or spectral libraries, yet they probably number  
590 in the tens of thousands in human biofluids or excreta (71).

591         Because bio-transformed compounds are difficult to isolate and expensive to synthesize  
592 via classical organic synthetic chemistry, there are two emerging approaches to address these  
593 problems. One approach is to enzymatically synthesize these compounds, while the other  
594 approach is to computationally generate them (*in silico* metabolomics). The biosynthetic  
595 approach involves adding purified precursors to an artificial gut (72), to homogenized fecal  
596 material (73) or to isolated liver microsomes (74) and allowing the selected biomatrix to perform

597 the work. The limitation of this approach is that substantial effort is required to purify the  
598 products from each biomatrix and to collect the required MS/MS or NMR spectra. Furthermore,  
599 as highlighted earlier, there are relatively few precursor molecules (<1000) available to feed  
600 such a biosynthetic pipeline. So, while the experimental approach will likely generate many  
601 novel and authentic compounds, it is unlikely to generate enough compounds to cover >10-20%  
602 of the desired chemical space.

603 The *in-silico* approach involves using computational approaches to generate metabolite  
604 structure by modeling biotransformation reactions (phase I, phase II and microbial reactions) on  
605 a known set of food constituent precursors. There are several commercial programs that  
606 effectively model these biotransformation processes, as well as a new freeware tool such as  
607 BioTransformer (75). Once the compound structures are computationally generated, it is  
608 possible to identify them in real samples by matching the observed MS/MS spectra using tools  
609 such as CSI-FingerID (76), molecular networking approaches via GNPS (57) or through the  
610 comparison of observed MS/MS spectra with predicted MS/MS spectra via CFM-ID (60). The  
611 advantages of this *in silico* approach are that it is fast, inexpensive and not limited by the  
612 availability of physical compounds. The disadvantages are that the predictions are not  
613 sufficiently accurate, and no authentic compounds or authentic spectra are generated.

614 Biomarker measurement should be sensitive enough to capture dietary exposure  
615 information and should fall within the dynamic range of measurable limits commonly found in a  
616 population. However, dynamic ranges for most biomarkers are not currently known. In addition,  
617 from the personalized nutrition and health perspective, ranges may differ depending on  
618 physiological status and vary among adults and children. Capturing this variation is important to  
619 understand response versus non-response to a dietary exposure. To ensure sensitivity,  
620 concentration ranges (for different age groups) for each biomarker should be well defined (77,  
621 78). Developing and establishing reference ranges across different populations, including  
622 children and adults, for a variety of dietary markers is helpful before planning larger studies.

623 Another area where more data are needed concerns the half-life of putative dietary  
624 biomarkers. How fast a dietary compound is absorbed and how long it stays in the system  
625 before elimination can impact the timing of sampling and the utility of the biomarker. For  
626 example, food components with faster absorption, and elimination kinetics have a very narrow  
627 window for sampling (e.g., proline-betaine for citrus fruits) (44). Similarly, some biomarkers from  
628 microbial metabolism (e.g., urolithin) can only be detected 30-45 h, after the intake of  
629 ellagitannin (79, 80). Metabolites with very short half-lives may not be sensitive and contribute to  
630 measurement errors and may not render as useful biomarkers. Depending on the objectives of  
631 the biomarkers, it is desirable to choose biomarkers with sufficiently longer half-lives such as  
632 lipophilic metabolites, to minimize intra-individual variation (7). Comprehensive knowledge of the  
633 half-life of metabolites will certainly enhance biomarker identification approaches and expedite  
634 biomarker development. To this end, high-throughput methodologies for evaluating half-lives of  
635 metabolites (i.e. biomarkers) are needed to help advance the field.

636

### 637 **Integration of Dietary Biomarkers with Other Omics Techniques**

638 Dietary biomarkers are primarily small molecules derived from either the food itself or  
639 from the digestion and biotransformation of specific food-derived compounds. However, the  
640 abundance and the type of potential dietary biomarkers can be significantly altered by  
641 physiological parameters, which can contribute to significant inter-individual variability.

642 Gut microbial metabolism also plays a vital role, in determining which circulating  
643 metabolites may be present. This has become apparent in relation to several classes of  
644 phytochemicals, including the *Brassica*-derived glucosinolates and flavonoids present in a  
645 variety of plant foods (81, 82). A well-known example is the bacterial conversion of the soy  
646 isoflavone daidzein to equol, which due to inter-individual differences in gut microbial community  
647 composition only occurs in a subset of individuals, upon soy consumption (83, 84). The ability to  
648 characterize the gut microbiome and its functional capacity (via 16S rRNA gene sequencing and

649 metagenomics, respectively) has helped to explain the variation in production of some putative  
650 dietary biomarkers. However, the fact that so many compounds (both endogenous and food-  
651 derived) are affected by microbial metabolism suggests that these effects must be considered,  
652 in selecting reliable dietary biomarkers (85).

653 Host genetics also has an important role in determining both the type and abundance of  
654 certain dietary biomarkers. Of note is the impact of single nucleotide polymorphisms (SNPs) on  
655 both nutrient metabolism and dietary preferences impacting the metabolites that can be  
656 detected and potential biomarkers. MWAS (metabolome-wide association studies) or mGWAS  
657 (genome-wide association studies with metabolomics) have linked metabolite levels to many  
658 human SNPs (86-88). So far, these studies have identified thousands of SNPs and thousands  
659 of metabolites that appear to co-vary. Some of these SNPs are known to account for up to 60%  
660 of the variability of circulating levels of certain metabolites (89).

661 Given the significant effects of genetics on metabolite levels, it is essential that anyone  
662 conducting dietary biomarker studies carefully consider genetic data when selecting or  
663 identifying potential dietary biomarkers. The dietary biomarker community has two options: 1)  
664 use previously collected MWAS data to exclude certain metabolites as potential dietary  
665 biomarkers (due to their strong genetic control) or 2) use genetic/SNP data to adjust or  
666 recalibrate dietary biomarker data to work for specific individuals. Both approaches are feasible;  
667 however, over the short term, it is likely that the use of pre-existing MWAS data to exclude or  
668 disqualify proposed dietary biomarkers will be the easiest and most cost-effective approach.

669 A number of interesting applications of genetics to dietary biomarkers are also starting to  
670 emerge. Some of the most fascinating ones may lie with the impact of SNPs on dietary  
671 preferences. Individuals who have adverse reactions to certain foods are unlikely to consume  
672 them and therefore should not have nutrient markers for those foods. On the other hand,  
673 individuals who have cravings for certain foods will likely have an abundance of markers for

674 those foods. Several examples of how SNPs affect dietary preferences (and therefore dietary  
675 biomarker levels) are described in **Table 3**.

676 Overall, the existing evidence strongly suggests that genomics, microbiome analysis,  
677 and metagenomics can play a role in the detection, identification, validation and quantification of  
678 many known and putative dietary biomarkers. Therefore, the use of other omics (i.e. non-  
679 metabolomic) techniques in dietary biomarker analysis can serve to complement the  
680 metabolomic information that is normally collected for dietary biomarker studies.

681

### 682 **Pathways to Precision Nutrition**

683 Simply stated, precision nutrition is the nutritional analog of precision medicine. More  
684 specifically, it is nutrition or dietary guidance designed to optimize health, facilitate disease  
685 prevention and enhance therapeutic benefit through molecular (metabolomic, genomic,  
686 proteomic, metagenomic) profiling at the level of the individual. Precision nutrition approaches  
687 require a keen understanding of how genetic-metabotype-diet interactions affect dietary  
688 biomarker levels and determine nutrient status. There are classical examples wherein genetic  
689 variation (i.e., SNPs) influences metabolic differences by influencing dietary requirements and  
690 responses to different diets. For example; dietary choline deficiency produces liver or muscle  
691 dysfunction in most men and postmenopausal women. Fortunately, the majority of  
692 premenopausal women are actually protected against choline deficiency, because of the  
693 hormonal induction of phosphatidyl ethanolamine-N-methyltransferase (*PEMT*), an enzyme that  
694 enables endogenous synthesis of choline (90). However, a SNP in *PEMT* (*rs12325817*)  
695 prevents induction by estrogen, making a subset of these women susceptible to choline  
696 deficiency illustrates how polymorphisms in enzymes, in critical metabolic pathways can impair  
697 nutrient metabolism (91). Unfortunately, these kinds of diet-related SNPs can only be confirmed  
698 by challenging individuals with differential diet regimens (low and high).

699 From this example of differential choline metabolism, it is apparent that precision  
700 nutrition approaches require data on both dietary intake and SNPs. Yet, to date, there are no  
701 catalogs of SNPs that can inform dietitians or other clinicians about specific nutrient  
702 requirements that might serve as the basis for practicing precision nutrition. Therefore, there is a  
703 critical need for catalogs of gene signatures that alter the metabolism of nutrients. These SNPs  
704 need to be confirmed for whether they can predict changes in a biomarker's relationship to an  
705 individual's nutrient status.

706 Systematic integration of SNP data together with the broader metabotype based  
707 biomarkers will certainly advance precision nutrition efforts. The metabotype based  
708 personalized nutrition approach uses a broader metabolic phenotype that characterizes  
709 biological diversity between and within individuals. For example, comprehensive metabolite  
710 and/or lipidomic profiles may provide insights in relation to the response or not to dietary  
711 challenges.

712 Precision nutrition efforts are also emerging through integrated studies of the  
713 microbiome and metabolome. In particular, Zeevi et al. (92) showed how a machine-learning  
714 algorithm that integrates metabolomic data, dietary habits, physiological measurements,  
715 physical activity, and gut microbiota can predict personalized postprandial glycemic response to  
716 complex (regular) meals. This result was further validated in a separate cohort of 100 test  
717 subjects, and then again in a blinded randomized controlled dietary intervention of 26  
718 individuals. Implementing these molecularly-informed custom diets led to significantly lower  
719 postprandial glycemic responses and consistent alterations in the gut microbiota of these test  
720 subjects. This is an excellent example of a well conducted, carefully validated biomarker study.  
721 It also demonstrates the remarkable potential of precision nutrition and shows how customized  
722 dietary guidance can be computationally designed to optimize health and enhance therapeutic  
723 benefit through comprehensive, multi-omic molecular profiling.

724

## 725 **Conclusion**

726 It is evident that there are gaps and challenges to establishing nutrient or food-specific  
727 biomarkers. There are also some compelling ideas and novel resources that are starting to  
728 emerge that may help to address these challenges. Clearly, more human feeding studies, with  
729 well-chosen designs, are needed to fuel the dietary intake biomarker development process.

730 In addition, it is important to appreciate that dietary biomarker development is a  
731 multidisciplinary enterprise and benefits from engaging several collaborative efforts. Fostering  
732 collaborations among analytical or natural product chemists, omics (metabolomics, genomics,  
733 proteomics, metagenomics) specialists, physicians, dietitians and nutritionists, statisticians,  
734 epidemiologists and bioinformaticians is critical for advancing the field. Chemists are needed to  
735 measure, synthesize or isolate the appropriate chemical standards, and to collect the relevant  
736 referential spectra. Omics specialists are needed to perform large-scale omics studies to  
737 discover or validate the appropriate biomarkers. Physicians, dietitians, nutritionists, and  
738 epidemiologists are needed to design the diets or dietary interventions, assemble the cohorts,  
739 collect the samples and acquire the meta-data. Statisticians need to be involved at various  
740 levels of biomarker development to help with biomarker discovery and validation, to assist with  
741 data modeling, and account for measurement error. Bioinformaticians are needed to consolidate  
742 or integrate the data, to develop data exchange standards, to create ontologies and bring some  
743 order to this very diverse array of data types. Finally, dedicated study participants are needed to  
744 generate the specimens.

745 Interestingly, such a multi-faceted collaboration aimed at discovering food-based  
746 biomarkers has recently been undertaken by several countries in the European Union (and  
747 Canada) under the Joint Programming Initiative, a Healthy Diet for a Healthy Life (JPI-HDHL).  
748 Over the past 4 years the initiative, called FoodBAII or the Food Biomarker Alliance, has  
749 generated a wealth of data on dietary biomarkers (63). In particular, FoodBAII brought chemists,  
750 omics (metabolomics, transcriptomics, genomics) scientists, dietitians, clinicians, statisticians

751 and bioinformaticians together to work, collaborate and create much-needed resources. The  
752 result has been a number of useful tools, databases, chemical libraries, white papers, guidelines  
753 and other resources that are starting to form the basis, for using dietary biomarkers in nutritional  
754 epidemiology (63). This effort has stimulated a keen interest by many other scientific groups and  
755 communities around the world to extend and expand these promising ideas and resources.  
756 More support for these kinds of concerted and coordinated activities is essential to advance  
757 dietary biomarker research and to establish precision nutrition as an integral part of the drive  
758 towards precision health.  
759

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763

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765           PM has been the primary lead for organizing the workshop and JWL and DSW chaired  
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767 manuscript and for the final content. Other authors have actively participated in planning the  
768 workshop, presenting their work and sharing their opinions on dietary biomarker development.  
769 All the authors have read and approved the final version of the manuscript.

770

## References

1. Freedman LS, Commins JM, Moler JE, Arab L, Baer DJ, Kipnis V, Midthune D, Moshfegh AJ, Neuhaus ML, Prentice RL, et al. Pooled results from 5 validation studies of dietary self-report instruments using recovery biomarkers for energy and protein intake. *Am J Epidemiol* 2014;180(2):172-88. doi: 10.1093/aje/kwu116.
2. Sun T, Zhang Y, Huang H, Wang X, Zhou L, Li S, Huang S, Xie C, Wen Y, Zhu Y, et al. Plasma alkylresorcinol metabolite, a biomarker of whole-grain wheat and rye intake, and risk of ischemic stroke: a case-control study. *Am J Clin Nutr* 2019;109(2):1-7. doi: 10.1093/ajcn/nqy323.
3. Brennan L. The nutritional metabolomics crossroads: how to ensure success for dietary biomarkers. *Am J Clin Nutr* 2017;105(2):293-4. doi: 10.3945/ajcn.116.150847.
4. Cheung W, Keski-Rahkonen P, Assi N, Ferrari P, Freisling H, Rinaldi S, Slimani N, Zamora-Ros R, Rundle M, Frost G, et al. A metabolomic study of biomarkers of meat and fish intake. *Am J Clin Nutr* 2017;105(3):600-8. doi: 10.3945/ajcn.116.146639.
5. Guasch-Ferre M, Bhupathiraju SN, Hu FB. Use of Metabolomics in Improving Assessment of Dietary Intake. *Clin Chem* 2018;64(1):82-98. doi: 10.1373/clinchem.2017.272344.
6. Corella D, Ordovas JM. Biomarkers: background, classification and guidelines for applications in nutritional epidemiology. *Nutr Hosp* 2015;31 Suppl 3:177-88. doi: 10.3305/nh.2015.31.sup3.8765.
7. Scalbert A, Rothwell JA, Keski-Rahkonen P, Neveu V. The food metabolome and dietary biomarkers. . 1st Edition ed. Boca Raton: CRC Press, 2017.
8. Biesalski HK, Dragsted LO, Elmadfa I, Grossklaus R, Muller M, Schrenk D, Walter P, Weber P. Bioactive compounds: definition and assessment of activity. *Nutrition* 2009;25(11-12):1202-5. doi: 10.1016/j.nut.2009.04.023.
9. Gao Q, Pratico G, Scalbert A, Vergeres G, Kolehmainen M, Manach C, Brennan L, Afman LA, Wishart DS, Andres-Lacueva C, et al. A scheme for a flexible classification of dietary and health biomarkers. *Genes Nutr* 2017;12:34. doi: 10.1186/s12263-017-0587-x.
10. Sobczynska-Malefora A, Harrington DJ. Laboratory assessment of folate (vitamin B9) status. *J Clin Pathol* 2018;71(11):949-56. doi: 10.1136/jclinpath-2018-205048.
11. Scalbert A, Brennan L, Manach C, Andres-Lacueva C, Dragsted LO, Draper J, Rappaport SM, van der Hooft JJ, Wishart DS. The food metabolome: a window over dietary exposure. *Am J Clin Nutr* 2014;99(6):1286-308. doi: 10.3945/ajcn.113.076133.
12. Thomas DM, Watts K, Friedman S, Schoeller DA. Modelling the metabolism: allometric relationships between total daily energy expenditure, body mass, and height. *Eur J Clin Nutr* 2018. doi: 10.1038/s41430-018-0230-y.
13. O'Brien DM. Stable Isotope Ratios as Biomarkers of Diet for Health Research. *Annu Rev Nutr* 2015;35:565-94. doi: 10.1146/annurev-nutr-071714-034511.
14. Choy K, Nash SH, Kristal AR, Hopkins S, Boyer BB, O'Brien DM. The carbon isotope ratio of alanine in red blood cells is a new candidate biomarker of sugar-sweetened beverage intake. *J Nutr* 2013;143(6):878-84. doi: 10.3945/jn.112.172999.

15. Yeung EH, Saudek CD, Jahren AH, Kao WH, Islas M, Kraft R, Coresh J, Anderson CA. Evaluation of a novel isotope biomarker for dietary consumption of sweets. *Am J Epidemiol* 2010;172(9):1045-52. doi: 10.1093/aje/kwq247.
16. Yun HY, Lampe JW, Tinker LF, Neuhouser ML, Beresford SAA, Niles KR, Mossavar-Rahmani Y, Snetselaar LG, Van Horn L, Prentice RL, et al. Serum Nitrogen and Carbon Stable Isotope Ratios Meet Biomarker Criteria for Fish and Animal Protein Intake in a Controlled Feeding Study of a Women's Health Initiative Cohort. *J Nutr* 2018;148(12):1931-7. doi: 10.1093/jn/nxy168.
17. Neveu V, Perez-Jimenez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford)* 2010;2010:bap024. doi: 10.1093/database/bap024.
18. Edmands WM, Ferrari P, Rothwell JA, Rinaldi S, Slimani N, Barupal DK, Biessy C, Jenab M, Clavel-Chapelon F, Fagherazzi G, et al. Polyphenol metabolome in human urine and its association with intake of polyphenol-rich foods across European countries. *Am J Clin Nutr* 2015;102(4):905-13. doi: 10.3945/ajcn.114.101881.
19. Zamora-Ros R, Achaintre D, Rothwell JA, Rinaldi S, Assi N, Ferrari P, Leitzmann M, Boutron-Ruault MC, Fagherazzi G, Auffret A, et al. Urinary excretions of 34 dietary polyphenols and their associations with lifestyle factors in the EPIC cohort study. *Sci Rep* 2016;6:26905. doi: 10.1038/srep26905.
20. Dragsted LO, Gao Q, Scalbert A, Vergeres G, Kolehmainen M, Manach C, Brennan L, Afman LA, Wishart DS, Andres Lacueva C, et al. Validation of biomarkers of food intake-critical assessment of candidate biomarkers. *Genes Nutr* 2018;13:14. doi: 10.1186/s12263-018-0603-9.
21. Illner AK, Freisling H, Boeing H, Huybrechts I, Crispim SP, Slimani N. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. *Int J Epidemiol* 2012;41(4):1187-203. doi: 10.1093/ije/dys105.
22. Neuhouser ML, Tinker L, Shaw PA, Schoeller D, Bingham SA, Horn LV, Beresford SA, Caan B, Thomson C, Satterfield S, et al. Use of recovery biomarkers to calibrate nutrient consumption self-reports in the Women's Health Initiative. *Am J Epidemiol* 2008;167(10):1247-59. doi: 10.1093/aje/kwn026.
23. Lloyd AJ, Beckmann M, Halder S, Seal C, Brandt K, Draper J. Data-driven strategy for the discovery of potential urinary biomarkers of habitual dietary exposure. *Am J Clin Nutr* 2013;97(2):377-89. doi: 10.3945/ajcn.112.048033.
24. Playdon MC, Sampson JN, Cross AJ, Sinha R, Guertin KA, Moy KA, Rothman N, Irwin ML, Mayne ST, Stolzenberg-Solomon R, et al. Comparing metabolite profiles of habitual diet in serum and urine. *Am J Clin Nutr* 2016;104(3):776-89. doi: 10.3945/ajcn.116.135301.
25. Tasevska N, Runswick SA, Welch AA, McTaggart A, Bingham SA. Urinary sugars biomarker relates better to extrinsic than to intrinsic sugars intake in a metabolic study with volunteers consuming their normal diet. *Eur J Clin Nutr* 2009;63(5):653-9. doi: 10.1038/ejcn.2008.21.
26. Serrano JC, Jove M, Gonzalo H, Pamplona R, Portero-Otin M. Nutridynamics: mechanism(s) of action of bioactive compounds and their effects. *Int J Food Sci Nutr* 2015;66 Suppl 1:S22-30.

27. van Duynhoven JPM, van Velzena EJJ, Westerhuis JA, Foltz M, Jacobs DM, Smilde AK. Nutrikinetics: Concept, technologies, applications, perspectives. *Trends Food Sci Tech* 2012;26(1):4-13. doi: 10.1016/j.tifs.2012.01.004.
28. Lampe JW, King IB, Li S, Grate MT, Barale KV, Chen C, Feng Z, Potter JD. Brassica vegetables increase and apiaceous vegetables decrease cytochrome P450 1A2 activity in humans: changes in caffeine metabolite ratios in response to controlled vegetable diets. *Carcinogenesis* 2000;21(6):1157-62.
29. McEvoy CT, Wallace IR, Hamill LL, Hunter SJ, Neville CE, Patterson CC, Woodside JV, Young IS, McKinley MC. Increasing Fruit and Vegetable Intake Has No Dose-Response Effect on Conventional Cardiovascular Risk Factors in Overweight Adults at High Risk of Developing Cardiovascular Disease. *J Nutr* 2015;145(7):1464-71. doi: 10.3945/jn.115.213090.
30. Navarro SL, Schwarz Y, Song X, Wang CY, Chen C, Trudo SP, Kristal AR, Kratz M, Eaton DL, Lampe JW. Cruciferous vegetables have variable effects on biomarkers of systemic inflammation in a randomized controlled trial in healthy young adults. *J Nutr* 2014;144(11):1850-7. doi: 10.3945/jn.114.197434.
31. Lampe JW, Huang Y, Neuhouser ML, Tinker LF, Song X, Schoeller DA, Kim S, Raftery D, Di C, Zheng C, et al. Dietary biomarker evaluation in a controlled feeding study in women from the Women's Health Initiative cohort. *Am J Clin Nutr* 2017;105(2):466-75. doi: 10.3945/ajcn.116.144840.
32. Heinzmann SS, Brown IJ, Chan Q, Bictash M, Dumas ME, Kochhar S, Stamler J, Holmes E, Elliott P, Nicholson JK. Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption. *Am J Clin Nutr* 2010;92(2):436-43. doi: 10.3945/ajcn.2010.29672.
33. Munger LH, Trimigno A, Picone G, Freiburghaus C, Pimentel G, Burton KJ, Pralong FP, Vionnet N, Capozzi F, Badertscher R, et al. Identification of Urinary Food Intake Biomarkers for Milk, Cheese, and Soy-Based Drink by Untargeted GC-MS and NMR in Healthy Humans. *J Proteome Res* 2017;16(9):3321-35. doi: 10.1021/acs.jproteome.7b00319.
34. Tasevska N, Midthune D, Tinker LF, Potischman N, Lampe JW, Neuhouser ML, Beasley JM, Van Horn L, Prentice RL, Kipnis V. Use of a urinary sugars biomarker to assess measurement error in self-reported sugars intake in the nutrition and physical activity assessment study (NPAAS). *Cancer Epidemiol Biomarkers Prev* 2014;23(12):2874-83. doi: 10.1158/1055-9965.EPI-14-0594.
35. Knight R, Leach J. Internet: <http://americangut.org/>.
36. Raghavan R, Ashour FS, Bailey R. A Review of Cutoffs for Nutritional Biomarkers. *Adv Nutr* 2016;7(1):112-20. doi: 10.3945/an.115.009951.
37. Burri BJ, Neidlinger TR, Clifford AJ. Serum carotenoid depletion follows first-order kinetics in healthy adult women fed naturally low carotenoid diets. *J Nutr* 2001;131(8):2096-100. doi: 10.1093/jn/131.8.2096.
38. Davidson EA, Pickens CA, Fenton JI. Increasing dietary EPA and DHA influence estimated fatty acid desaturase activity in systemic organs which is reflected in the red blood cell in mice. *Int J Food Sci Nutr* 2018;69(2):183-91. doi: 10.1080/09637486.2017.1348494.
39. Filippini T, Ferrari A, Michalke B, Grill P, Vescovi L, Salvia C, Malagoli C, Malavolti M, Sieri S, Krogh V, et al. Toenail selenium as an indicator of environmental exposure:

- A cross-sectional study. *Mol Med Rep* 2017;15(5):3405-12. doi: 10.3892/mmr.2017.6388.
40. Le Marchand L, Yonemori K, White KK, Franke AA, Wilkens LR, Turesky RJ. Dose validation of PhIP hair level as a biomarker of heterocyclic aromatic amines exposure: a feeding study. *Carcinogenesis* 2016;37(7):685-91. doi: 10.1093/carcin/bgw049.
  41. Valenzuela LO, O'Grady SP, Enright LE, Murtaugh M, Sweeney C, Ehleringer JR. Evaluation of childhood nutrition by dietary survey and stable isotope analyses of hair and breath. *Am J Hum Biol* 2018;30(3):e23103. doi: 10.1002/ajhb.23103.
  42. Prentice RL, Willett WC, Greenwald P, Alberts D, Bernstein L, Boyd NF, Byers T, Clinton SK, Fraser G, Freedman L, et al. Nutrition and physical activity and chronic disease prevention: research strategies and recommendations. *J Natl Cancer Inst* 2004;96(17):1276-87. doi: 10.1093/jnci/djh240.
  43. Garcia-Perez I, Posma JM, Chambers ES, Nicholson JK, J CM, Beckmann M, Draper J, Holmes E, Frost G. An Analytical Pipeline for Quantitative Characterization of Dietary Intake: Application To Assess Grape Intake. *J Agric Food Chem* 2016;64(11):2423-31. doi: 10.1021/acs.jafc.5b05878.
  44. Lloyd AJ, Beckmann M, Fave G, Mathers JC, Draper J. Proline betaine and its biotransformation products in fasting urine samples are potential biomarkers of habitual citrus fruit consumption. *Br J Nutr* 2011;106(6):812-24. doi: 10.1017/S0007114511001164.
  45. Kotsopoulos J, Tworoger SS, Campos H, Chung FL, Clevenger CV, Franke AA, Mantzoros CS, Ricchiuti V, Willett WC, Hankinson SE, et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. *Cancer Epidemiol Biomarkers Prev* 2010;19(4):938-46. doi: 10.1158/1055-9965.EPI-09-1318.
  46. Townsend MK, Clish CB, Kraft P, Wu C, Souza AL, Deik AA, Tworoger SS, Wolpin BM. Reproducibility of metabolomic profiles among men and women in 2 large cohort studies. *Clin Chem* 2013;59(11):1657-67. doi: 10.1373/clinchem.2012.199133.
  47. Fave G, Beckmann M, Lloyd AJ, Zhou S, Harold G, Lin W, Tailliant K, Xie L, Draper J, Mathers JC. Development and validation of a standardized protocol to monitor human dietary exposure by metabolite fingerprinting of urine samples. *Metabolomics* 2011;7(4):469-84. doi: 10.1007/s11306-011-0289-0.
  48. Jacob M, Malkawi A, Albast N, Al Bougha S, Lopata A, Dasouki M, Abdel Rahman AM. A targeted metabolomics approach for clinical diagnosis of inborn errors of metabolism. *Anal Chim Acta* 2018;1025:141-53. doi: 10.1016/j.aca.2018.03.058.
  49. Elliott DRF, Walker AW, O'Donovan M, Parkhill J, Fitzgerald RC. A non-endoscopic device to sample the oesophageal microbiota: a case-control study. *Lancet Gastroenterol Hepatol* 2017;2(1):32-42. doi: 10.1016/S2468-1253(16)30086-3.
  50. Cajka T, Smilowitz JT, Fiehn O. Validating Quantitative Untargeted Lipidomics Across Nine Liquid Chromatography-High-Resolution Mass Spectrometry Platforms. *Anal Chem* 2017;89(22):12360-8. doi: 10.1021/acs.analchem.7b03404.
  51. Domingo-Almenara X, Montenegro-Burke JR, Ivanisevic J, Thomas A, Sidibe J, Teav T, Guijas C, Aisporna AE, Rinehart D, Hoang L, et al. XCMS-MRM and METLIN-MRM: a cloud library and public resource for targeted analysis of small molecules. *Nat Methods* 2018;15(9):681-4. doi: 10.1038/s41592-018-0110-3.

52. Pluskal T, Castillo S, Villar-Briones A, Oresic M. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* 2010;11:395. doi: 10.1186/1471-2105-11-395.
53. Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, Kanazawa M, VanderGheynst J, Fiehn O, Arita M. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods* 2015;12(6):523-6. doi: 10.1038/nmeth.3393.
54. Rafiei A, Sleno L. Comparison of peak-picking workflows for untargeted liquid chromatography/high-resolution mass spectrometry metabolomics data analysis. *Rapid Commun Mass Spectrom* 2015;29(1):119-27. doi: 10.1002/rcm.7094.
55. Fiehn O. Mass Bank of North America <http://mona.fiehnlab.ucdavis.edu/>.
56. Tautenhahn R, Cho K, Uritboonthai W, Zhu Z, Patti GJ, Siuzdak G. An accelerated workflow for untargeted metabolomics using the METLIN database. *Nat Biotechnol* 2012;30(9):826-8. doi: 10.1038/nbt.2348.
57. Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, Nguyen DD, Watrous J, Kapono CA, Luzzatto-Knaan T, et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nat Biotechnol* 2016;34(8):828-37. doi: 10.1038/nbt.3597.
58. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vazquez-Fresno R, Sajed T, Johnson D, Li C, Karu N, et al. HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res* 2018;46(D1):D608-D17. doi: 10.1093/nar/gkx1089.
59. Ruttkies C, Schymanski EL, Wolf S, Hollender J, Neumann S. MetFrag relaunched: incorporating strategies beyond in silico fragmentation. *J Cheminform* 2016;8:3. doi: 10.1186/s13321-016-0115-9.
60. Allen F, Pon A, Wilson M, Greiner R, Wishart D. CFM-ID: a web server for annotation, spectrum prediction and metabolite identification from tandem mass spectra. *Nucleic Acids Res* 2014;42(Web Server issue):W94-9. doi: 10.1093/nar/gku436.
61. Mass Frontier 8.0 Spectral Interpretation Software.
62. Food Compound Exchange <http://foodcomex.org/>.
63. FoodBALL FBA. Internet: <http://foodmetabolome.org/>.
64. Chong J, Xia J. MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data. *Bioinformatics* 2018;34(24):4313-4. doi: 10.1093/bioinformatics/bty528.
65. Kirpich AS, Ibarra M, Moskalenko O, Fear JM, Gerken J, Mi X, Ashrafi A, Morse AM, McIntyre LM. SECIMTools: a suite of metabolomics data analysis tools. *BMC Bioinformatics* 2018;19(1):151. doi: 10.1186/s12859-018-2134-1.
66. Prentice RL, Huang Y, Tinker LF, Beresford SA, Lampe JW, Neuhaus ML. Statistical Aspects of the Use of Biomarkers in Nutritional Epidemiology Research. *Stat Biosci* 2009;1(1):112-23. doi: 10.1007/s12561-009-9003-4.
67. Prentice RL, Pettinger M, Neuhaus ML, Tinker LF, Huang Y, Zheng C, Manson JE, Mossavar-Rahmani Y, Anderson GL, Lampe JW. Application of blood concentration biomarkers in nutritional epidemiology: example of carotenoid and tocopherol intake in relation to chronic disease risk. *Am J Clin Nutr* (Accepted).
68. Gebhardt SEC, R.L. Howe, J.C. Haytowitz, D.B. Pehrsson, P.R. Lemar, L.E. Holcomb, G.T. Stup, M.A. Thomas, R.G. Exler, J. Showell, B.A. Holden, J.M. USDA national nutrient database for standard reference, release 19. Home Page., 2006.

69. da Silva RR, Dorrestein PC, Quinn RA. Illuminating the dark matter in metabolomics. *Proc Natl Acad Sci U S A* 2015;112(41):12549-50. doi: 10.1073/pnas.1516878112.
70. Dorrestein PC. Personal Communication.
71. Wishart DS. FooDB: The food composition database <http://foodb.ca> 2018.
72. Alander M, De Smet I, Nollet L, Verstraete W, von Wright A, Mattila-Sandholm T. The effect of probiotic strains on the microbiota of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). *Int J Food Microbiol* 1999;46(1):71-9.
73. Dodd D, Spitzer MH, Van Treuren W, Merrill BD, Hryckowian AJ, Higginbottom SK, Le A, Cowan TM, Nolan GP, Fischbach MA, et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature* 2017;551(7682):648-52. doi: 10.1038/nature24661.
74. Zuniga A, Li L. Ultra-high performance liquid chromatography tandem mass spectrometry for comprehensive analysis of urinary acylcarnitines. *Anal Chim Acta* 2011;689(1):77-84. doi: 10.1016/j.aca.2011.01.018.
75. Djoumbou-Feunang Y, Fiamoncini J, Gil-de-la-Fuente A, Greiner R, Manach C, Wishart DS. BioTransformer: a comprehensive computational tool for small molecule metabolism prediction and metabolite identification. *J Cheminform* 2019;11(1):2. doi: 10.1186/s13321-018-0324-5.
76. Duhrkop K, Shen H, Meusel M, Rousu J, Bocker S. Searching molecular structure databases with tandem mass spectra using CSI:FingerID. *Proc Natl Acad Sci U S A* 2015;112(41):12580-5. doi: 10.1073/pnas.1509788112.
77. Achaintre D, Bulete A, Cren-Olive C, Li L, Rinaldi S, Scalbert A. Differential Isotope Labeling of 38 Dietary Polyphenols and Their Quantification in Urine by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry. *Anal Chem* 2016;88(5):2637-44. doi: 10.1021/acs.analchem.5b03609.
78. Achaintre D, Gicquiau A, Li L, Rinaldi S, Scalbert A. Quantification of 38 dietary polyphenols in plasma by differential isotope labelling and liquid chromatography electrospray ionization tandem mass spectrometry. *J Chromatogr A* 2018;1558:50-8. doi: 10.1016/j.chroma.2018.05.017.
79. Cerda B, Periago P, Espin JC, Tomas-Barberan FA. Identification of urolithin a as a metabolite produced by human colon microflora from ellagic acid and related compounds. *J Agric Food Chem* 2005;53(14):5571-6. doi: 10.1021/jf050384i.
80. Piwowarski JP, Granica S, Stefanska J, Kiss AK. Differences in Metabolism of Ellagitannins by Human Gut Microbiota ex Vivo Cultures. *J Nat Prod* 2016;79(12):3022-30. doi: 10.1021/acs.jnatprod.6b00602.
81. Cassidy A, Minihane AM. The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. *Am J Clin Nutr* 2017;105(1):10-22. doi: 10.3945/ajcn.116.136051.
82. Narbad A, Rossiter JT. Gut Glucosinolate Metabolism and Isothiocyanate Production. *Mol Nutr Food Res* 2018;62(18):e1700991. doi: 10.1002/mnfr.201700991.
83. Atkinson C, Berman S, Humbert O, Lampe JW. In vitro incubation of human feces with daidzein and antibiotics suggests interindividual differences in the bacteria responsible for equol production. *J Nutr* 2004;134(3):596-9. doi: 10.1093/jn/134.3.596.
84. Decroos K, Vanhemmens S, Cattoir S, Boon N, Verstraete W. Isolation and characterisation of an equol-producing mixed microbial culture from a human faecal

- sample and its activity under gastrointestinal conditions. *Arch Microbiol* 2005;183(1):45-55. doi: 10.1007/s00203-004-0747-4.
85. Hullar MA, Lancaster SM, Li F, Tseng E, Beer K, Atkinson C, Wahala K, Copeland WK, Randolph TW, Newton KM, et al. Enterolignan-producing phenotypes are associated with increased gut microbial diversity and altered composition in premenopausal women in the United States. *Cancer Epidemiol Biomarkers Prev* 2015;24(3):546-54. doi: 10.1158/1055-9965.EPI-14-0262.
  86. Kastenmuller G, Raffler J, Gieger C, Suhre K. Genetics of human metabolism: an update. *Hum Mol Genet* 2015;24(R1):R93-R101. doi: 10.1093/hmg/ddv263.
  87. Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang TP, et al. An atlas of genetic influences on human blood metabolites. *Nat Genet* 2014;46(6):543-50. doi: 10.1038/ng.2982.
  88. Yet I, Menni C, Shin SY, Mangino M, Soranzo N, Adamski J, Suhre K, Spector TD, Kastenmuller G, Bell JT. Genetic Influences on Metabolite Levels: A Comparison across Metabolomic Platforms. *PLoS One* 2016;11(4):e0153672. doi: 10.1371/journal.pone.0153672.
  89. Rhee EP, Yang Q, Yu B, Liu X, Cheng S, Deik A, Pierce KA, Bullock K, Ho JE, Levy D, et al. An exome array study of the plasma metabolome. *Nat Commun* 2016;7:12360. doi: 10.1038/ncomms12360.
  90. Zeisel SH. Nutritional genomics: defining the dietary requirement and effects of choline. *J Nutr* 2011;141(3):531-4. doi: 10.3945/jn.110.130369.
  91. Resseguie ME, da Costa KA, Galanko JA, Patel M, Davis IJ, Zeisel SH. Aberrant estrogen regulation of PEMT results in choline deficiency-associated liver dysfunction. *J Biol Chem* 2011;286(2):1649-58. doi: 10.1074/jbc.M110.106922.
  92. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M, et al. Personalized Nutrition by Prediction of Glycemic Responses. *Cell* 2015;163(5):1079-94. doi: 10.1016/j.cell.2015.11.001.
  93. Rothwell JA, Urpi-Sarda M, Boto-Ordonez M, Llorach R, Farran-Codina A, Barupal DK, Neveu V, Manach C, Andres-Lacueva C, Scalbert A. Systematic analysis of the polyphenol metabolome using the Phenol-Explorer database. *Mol Nutr Food Res* 2016;60(1):203-11. doi: 10.1002/mnfr.201500435.
  94. Manach C. Phytohub <http://phytohub.eu/>. INRA 2016.
  95. Neveu V, Moussy A, Rouaix H, Wedekind R, Pon A, Knox C, Wishart DS, Scalbert A. Exposome-Explorer: a manually-curated database on biomarkers of exposure to dietary and environmental factors. *Nucleic Acids Res* 2017;45(D1):D979-D84. doi: 10.1093/nar/gkw980.
  96. Ingram CJ, Mulcare CA, Itan Y, Thomas MG, Swallow DM. Lactose digestion and the evolutionary genetics of lactase persistence. *Hum Genet* 2009;124(6):579-91. doi: 10.1007/s00439-008-0593-6.
  97. Smith CE, Coltell O, Sorli JV, Estruch R, Martinez-Gonzalez MA, Salas-Salvado J, Fito M, Aros F, Dashti HS, Lai CQ, et al. Associations of the MCM6-rs3754686 proxy for milk intake in Mediterranean and American populations with cardiovascular biomarkers, disease and mortality: Mendelian randomization. *Sci Rep* 2016;6:33188. doi: 10.1038/srep33188.

98. Li D, Zhao H, Gelernter J. Strong protective effect of the aldehyde dehydrogenase gene (ALDH2) 504lys (\*2) allele against alcoholism and alcohol-induced medical diseases in Asians. *Hum Genet* 2012;131(5):725-37. doi: 10.1007/s00439-011-1116-4.
99. Gelernter J, Kranzler HR, Sherva R, Almasy L, Koesterer R, Smith AH, Anton R, Preuss UW, Ridinger M, Rujescu D, et al. Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. *Mol Psychiatry* 2014;19(1):41-9. doi: 10.1038/mp.2013.145.
100. Li D, Zhao H, Gelernter J. Further clarification of the contribution of the ADH1C gene to vulnerability of alcoholism and selected liver diseases. *Hum Genet* 2012;131(8):1361-74. doi: 10.1007/s00439-012-1163-5.
101. Schumann G, Liu C, O'Reilly P, Gao H, Song P, Xu B, Ruggeri B, Amin N, Jia T, Preis S, et al. KLB is associated with alcohol drinking, and its gene product beta-Klotho is necessary for FGF21 regulation of alcohol preference. *Proc Natl Acad Sci U S A* 2016;113(50):14372-7. doi: 10.1073/pnas.1611243113.
102. Calancie L, Keyserling TC, Taillie LS, Robasky K, Patterson C, Ammerman AS, Schisler JC. TAS2R38 Predisposition to Bitter Taste Associated with Differential Changes in Vegetable Intake in Response to a Community-Based Dietary Intervention. *G3 (Bethesda)* 2018;8(6):2107-19. doi: 10.1534/g3.118.300547.
103. Mikolajczyk-Stecyna J, Malinowska AM, Chmurzynska A. TAS2R38 and CA6 genetic polymorphisms, frequency of bitter food intake, and blood biomarkers among elderly woman. *Appetite* 2017;116:57-64. doi: 10.1016/j.appet.2017.04.029.
104. Eriksson N, Wu S, Do CB, Kiefer AK, Tung JY, Mountain JL, Hinds DA, Francke U. A genetic variant near olfactory receptor genes influences cilantro preference. *Flavour* 2012;1(22).
105. Coffee, Caffeine Genetics C, Cornelis MC, Byrne EM, Esko T, Nalls MA, Ganna A, Paynter N, Monda KL, Amin N, et al. Genome-wide meta-analysis identifies six novel loci associated with habitual coffee consumption. *Mol Psychiatry* 2015;20(5):647-56. doi: 10.1038/mp.2014.107.
106. von Holstein-Rathlou S, BonDurant LD, Peltekian L, Naber MC, Yin TC, Claflin KE, Urizar AI, Madsen AN, Ratner C, Holst B, et al. FGF21 Mediates Endocrine Control of Simple Sugar Intake and Sweet Taste Preference by the Liver. *Cell Metab* 2016;23(2):335-43. doi: 10.1016/j.cmet.2015.12.003.
107. Merino J, Dashti HS, Li SX, Sarnowski C, Justice AE, Graff M, Papoutsakis C, Smith CE, Dedoussis GV, Lemaitre RN, et al. Genome-wide meta-analysis of macronutrient intake of 91,114 European ancestry participants from the cohorts for heart and aging research in genomic epidemiology consortium. *Mol Psychiatry* 2018. doi: 10.1038/s41380-018-0079-4.
108. Tanaka T, Ngwa JS, van Rooij FJ, Zillikens MC, Wojczynski MK, Frazier-Wood AC, Houston DK, Kanoni S, Lemaitre RN, Luan J, et al. Genome-wide meta-analysis of observational studies shows common genetic variants associated with macronutrient intake. *Am J Clin Nutr* 2013;97(6):1395-402. doi: 10.3945/ajcn.112.052183.

<b>Table: 1 Strategies and approaches for advancing dietary biomarker development</b>	
<b>Challenges</b>	<b>Recommendations/ Resources needed</b>
<b>Define Dietary Biomarkers and Their Utility in Nutrition Research</b>	
Multiple dietary biomarker definitions in use	Adopt a universally accepted biomarker classification scheme with a well-developed ontology for use by the nutritional epidemiology and dietary biomarker community
Lack of publicly available comprehensive databases on dietary biomarkers	Develop or expand well-curated, publicly available international databases on dietary biomarkers such as Exposome-Explorer and Phenol-Explorer for prioritization of candidate biomarkers
Lack of comprehensive food composition databases	Develop and maintain comprehensive food composition databases
<b>Approaches to Studying Biomarkers</b>	
Studies are often conducted with no clear regard for human heterogeneity	Capture information on host factors (e.g., genetics, gut microbiome, behavioral and cultural practices) that may help to explain heterogeneity in dietary biomarker measures.
Current feeding studies are “siloeed” and often single studies conducted for shorter duration, involving smaller sample sizes	Conduct larger CFS, testing a variety of foods and dietary patterns across diverse populations to identify universal candidate biomarkers
Shortage of appropriately collected specimen repositories for dietary biomarker development	Collect a variety of biospecimens (e.g., fecal samples, blood cells, saliva, toenails, hair) as part of feeding studies, to discover and validate both short- and long-term dietary biomarkers
	Leverage existing biospecimen repositories from feeding studies and prospective cohorts, to validate dietary biomarkers
	Encourage long-term storage of biospecimens from completed feeding studies for dietary biomarker development studies
Lack of standardized specimen collection and processing protocols for omics analysis	Implement well-standardized specimen collection and processing protocols to ensure reproducibility, comparability and generalizability across studies
Cumbersome sampling procedures and lack of integration of advanced devices for sample collection	Develop new sampling techniques for efficient collection and wider acceptance and improved adherence in large studies (e.g., dried blood spots) and adopt wearables and smart phone devices that allow for continuous metabolite monitoring
<b>Analytical and Statistical Considerations of Biomarker Development</b>	
Metabolite coverage and reproducibility	Encourage sharing of spectral data and chemical databases of biologically feasible structures of metabolites
	Support internationally coordinated efforts for providing resources on food constituent libraries and biomarker data from various labs

	Facilitate distribution of relevant metabolite standards (e.g., FoodComEx)
Shortage of strategies to evaluate variation within and between laboratories	Develop standardized approaches for evaluating laboratory variation and normalizing for drift and differences across labs
Shortage of statistical methodologies for handling measurement error and applying to dietary exposure assessment	Conduct methodologic work on statistical procedures for intake biomarker discovery and disease application
Sharing sensitive metadata across labs is difficult	Establish secure portals accessible via cloud computing and portability environments for sharing metadata
Lack of minimum reporting standards for statistical analytic pipeline/workflow for nutritional metabolomics studies	Establish minimum reporting standards to support study replication.
<b>Dietary Biomarker Discovery and Validation</b>	
Dietary biomarker development is lengthy with no clear validation criteria	Adopt a universal dietary biomarker validation strategy that is accepted by the nutrition research community
Untargeted metabolomics produces multiple metabolites with no quantitative measures	Develop targeted and quantitative assays for validation studies, after initial biomarker identification
<b>Areas Where More Data Are Needed</b>	
Lack of comprehensive food composition databases	Create and maintain truly comprehensive food composition databases, by expanding existing databases, such as FooDB, in terms of chemical coverage and breadth of human food intake
	Integrate more fully the various food composition databases using shared links, common identifiers and common ontologies
	Extend food composition databases to archive experimentally acquired or accurately predicted referential MS/MS and/or NMR spectra data to facilitate food or dietary biomarker identification
Lack of concerted efforts and community resources necessary for dietary biomarker development	Support international efforts to prepare, acquire or synthesize authentic food-specific compounds and their MS/MS and/or NMR spectra and enable access via open-source databases (e.g., GNPS, MoNA, FooDB, HMDB, the Metabolomics Workbench and MetaboLights)
	Support international efforts to prepare, acquire or synthesize authentic gut-derived, liver-derived or similarly bio-transformed food compounds and their MS/MS and/or NMR spectra. Facilitate access, via open-source databases such as GNPS, MoNA, FooDB, HMDB, the Metabolomics Workbench and MetaboLights.
	Improve algorithms and open-access software to more accurately predict metabolic bio-transformation products

	(mimicking liver, microbial or promiscuous bio-transformations) to facilitate <i>in silico</i> metabolomics
	Improve algorithms and open-source software to more accurately predict MS/MS spectra (at multiple collision energies and on different platforms), NMR spectra, collisional cross-section data (for IMS data) and GC or HPLC retention times of small molecules
Specificity is a challenge for dietary biomarker development	Use combinations of biomarkers from either single study or pooled data from several feeding studies to increase marker specificity
	Develop reference ranges for biomarkers across different populations and age ranges (children vs adults)
<b>Integration of Dietary Biomarkers with Other Omics Techniques</b>	
Neither genomics nor metabolomics tools alone provide complete understanding of how dietary components are metabolized	Integrate other omics methods in dietary biomarker analysis with a view to understanding the impact of individual variation and personalized responses
	Identify and further explore the effect of SNPs on dietary biomarker measures
	Improve tools (databases, software, statistical methods) to facilitate the integration of genomics, metagenomics, proteomics and metabolomics data in nutritional studies.
Lack of systematically collected catalogues of SNPs	Continuously update databases or catalogs of SNPs, genes and gene signatures that alter the metabolism, presence or abundance of known and potential dietary biomarkers
<b>Other critical elements</b>	
Lack of concerted efforts for biomarker development	Foster collaboration among multidisciplinary researchers
	Encourage public-private partnerships for collecting and sharing the data on dietary biomarkers that would not be otherwise freely available
	Train early career scientists in dietary biomarker development
Lack of common ontology for dietary biomarker literature	Support standard ontology efforts through development of newer and broader algorithms for electronically mining the literature
	Convene taskforces for developing common data elements for dietary biomarker research

Database	Description	Unique features	Reference
Phenol-Explorer	First comprehensive database on polyphenol content in foods	Plant polyphenol metabolites with 375 biotransformation products	(93)
PhytoHub	Plant based metabolite database on phytochemicals present in foods commonly ingested with human diets	Plant metabolites with 578 biotransformation products	(94)
HMDB	Human Metabolome Database on small molecule metabolites found in the human body	A variety of endogenous metabolites with >1000 biotransformation products; data on 3056 metabolites linked to 2192 SNPs with 6777 specific metabolite-SNP interactions; data on 2901 metabolites that vary with physiology and data on 5498 metabolites that vary with pathophysiological conditions	(58)
Exposome-Explorer	Biomarkers of exposure to environmental risk factors for diseases	Data on 145 dietary biomarkers including their concentrations in various populations, type of biospecimens analyzed, the analytical techniques used, their reproducibility over time and correlations with food intake	(95)
FooDB	Database on food constituents, chemistry and biology	Data and referential MS and NMR spectra on >26,000 food chemicals found in >720 raw or lightly processed foods	(71)
GNPS	Global Natural Product Social Molecular Networking- database of raw, processed or identified tandem mass (MS/MS) spectrometry data	Food-specific data and includes MS-MS spectra from a large (>3500) number of different foods	(57)

<b>Table 3 – Genetics, SNPs and Food Preferences</b>			
<b>Gene Name</b>	<b>SNP ID</b>	<b>Effect</b>	<b>Reference</b>
<i>MCM6</i> (intron)	rs182549	Lactose intolerance	(96)
<i>MCM6</i> (intron)	rs4988235	Lactose intolerance	(96)
<i>MCM6</i>	rs3754686	Proxy for milk intake	(97)
<i>ALDH2</i>	rs671	Alcohol intolerance	(98)
<i>ADH1B</i>	rs1229984	Alcohol aversion	(99)
<i>ADH1C</i>	rs698	Alcohol dependence	(100)
<i>KLB</i>	rs11940694	Increased alcohol consumption	(101)
<i>TAS2R38</i>	rs713598	Brassica vegetable & coffee aversion	(102)
<i>TAS2R38</i>	rs1726866	Brassica vegetable & coffee aversion	(102)
<i>TAS2R38</i>	rs10246939	Brassica vegetable & coffee aversion	(102, 103)
<i>OR10A2</i>	rs72921001	Cilantro/coriander aversion	(104)
<i>CYP1A1</i>	rs2472297	Increased coffee consumption	(105)
<i>CYP1A1</i>	rs2470893	Increased coffee consumption	(105)
<i>AHR</i>	rs6968865	Increased coffee consumption	(105)
<i>FGF21</i>	rs838133	Sweet tooth (candy preference)	(106)
<i>FGF21</i>	rs838133	Increased carbohydrate and lower fat consumption	(107)
<i>FGF21</i>	rs838145	Increased carbohydrate and lower fat consumption	(108)
<i>RARB</i>	rs7619139	Increased carbohydrate consumption	(107)
<i>DRAM1</i>	rs77694286	Increased protein consumption	(107)
<i>FTO</i>	rs1421085	Increased protein consumption	(107)

## Legend

Figure:1 Bidirectional interaction between dietary components and physiological systems embedded in food consumption driven by food environments and further influenced by cultural, and lifestyle factors. Consumption of nutrients such as fatty acids, amino acids, vitamins, trace elements and bioactive compounds has an impact on host physiology, affecting both the health status and susceptibility to disease. Metabolism of dietary components is also influenced by the genetic make of an individual. In addition, dietary components may directly impact gut microbiota composition and function, which may exacerbate metabolic and physiological outcomes and further influencing disease susceptibility. Host physiology and altered susceptibility to disease in turn impact how these dietary substances are metabolized.