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## Symposium 2: Use of biomarkers in dietary assessment and dietary exposure

# Combining traditional dietary assessment methods with novel metabolomics techniques: present efforts by the Food Biomarker Alliance

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FFQ, food diaries and 24 h recall methods represent the most commonly used dietary assessment tools in human studies on nutrition and health, but food intake biomarkers are assumed to provide a more objective reflection of intake. Unfortunately, very few of these biomarkers are sufficiently validated. This review provides an overview of food intake biomarker research and highlights present research efforts of the Joint Programming Initiative 'A Healthy Diet for a Healthy Life' (JPI-HDHL) Food Biomarkers Alliance (FoodBAll). In order to identify novel food intake biomarkers, the focus is on new food metabolomics techniques that allow the quantification of up to thousands of metabolites simultaneously, which may be applied in intervention and observational studies. As biomarkers are often influenced by various other factors than the food under investigation, FoodBAll developed a food intake biomarker quality and validity score aiming to assist the systematic evaluation of novel biomarkers. Moreover, to evaluate the applicability of nutritional biomarkers, studies are presently also focusing on associations between food intake biomarkers and diet-related disease risk. In order to be successful in these metabolomics studies, knowledge about available electronic metabolomics resources is necessary and further developments of these resources are essential. Ultimately, present efforts in this research area aim to advance quality control of traditional dietary assessment methods, advance compliance evaluation in nutritional intervention studies, and increase the significance of observational studies by investigating associations between nutrition and health.

**Dietary assessment: Food intake biomarkers: Food metabolome: Metabolomics**

## Traditional dietary assessment techniques

For more than 100 years, nutritional research has played an important role for our understanding of diet–health relations in the human body. Nutrition researchers have contributed substantially to meaningful ways of improving health of individuals as well as populations. Valid and reproducible dietary assessment has been the cornerstone of this work. Food diaries, 24 h recalls and FFQ are commonly used self-report dietary assessment methods in human studies of nutrition in relation to health[1]. Multiple days, i.e. 7 d, weighed food records, the so-called gold standard dietary assessment method, are often used in controlled dietary studies[2]. However, dietary records are very time consuming and labour intensive for both participants and researchers, and they are not very accurate[3,4]. Therefore, they are not commonly used in large studies. As an alternative, 24 h recalls, i.e. a structured interview aiming to collect detailed information about all food items consumed during the preceding 24 h, are more frequently used in research settings. A major drawback of 24 h recalls is that 2–3 recalls are often not sufficient to capture the day-to-day variation of a variety of nutrients and foods such as vitamin A, vitamin C, cholesterol and fish[1]. Therefore, the use of 24 h recalls is often considered to be too time- and labour-intensive for use in large studies[5]. For larger studies, self-administered FFQ are most often the method of choice and are generally used to evaluate food consumption during the past 1–12 months. In contrast to food records and recalls, FFQ require an intensive preparation before the method is applied in actual studies, but the administration and processing of a validated FFQ can be done rather efficiently for several thousand participants at once. However, the large supply of available foods, inaccurate estimation of portion sizes, socially desirable answers and errors in food composition tables also make FFQ prone to measurement error[6–9]. A common concern with self-reported dietary intake is the objectivity and accuracy of self-reported data.

## Nutritional biomarkers

The limitations of the present dietary measurement tools have motivated many researchers to search for nutritional biomarkers as a complementary or alternative measure of dietary intake. Objective biological food intake and nutrient intake biomarkers may provide valuable information beyond self-reported food intake data and are particularly valuable when food composition data are not available or limited. Unfortunately, there are still very few well-validated nutritional intake biomarkers, with the exception of urinary nitrogen for protein, potassium and sodium[10]; carotenoids for vegetables and fruits[11,12]; and *n*-3 fatty acids for oily fish[13]. These examples of the well-validated food intake biomarkers are all natural food constituents. Researchers also explore the value of nutritional biomarkers reflecting food additives, nutritional biomarkers that are the result of the digestion or absorption of foods, and endogenous metabolites that are affected by the consumption of certain foods and as such may act as nutritional biomarkers. Previous research has for instance highlighted significant associations between fish consumption and biomarkers of mercury, arsenic and polychlorinated biphenyl[14,15]. Moreover, the Dutch National Institute for Public Health and the Environment and the University of Ghent are presently focusing part of their research on mycotoxins, a food contaminant where dietary exposure represents the main exposure[16]. During the past 3 years, two draft calculators have been developed

to study associations between dietary mycotoxin intakes and urinary mycotoxin concentrations. Furthermore, these two research centres are conducting an intervention study in which volunteers are exposed to a single dose of a rapidly eliminated mycotoxin (i.e. deoxynivalenol), after which urine is collected quantitatively at different time intervals up to 24 h after administration.

## New metabolomics techniques

Recent developments in analytical chemistry suggest that much more useful food intake biomarkers can and should be identified. New metabolomics techniques provide a unique opportunity. Using metabolomics techniques, it is possible to measure up to thousands of metabolites at once providing valuable information on the food metabolome (i.e. essential and non-essential chemicals derived from foods after digestion and subsequent metabolism by the tissues and the microbiota)[17], using plasma[18], serum[19], erythrocytes and leucocytes[20], urine[21], saliva[22], faeces[23], cerebrospinal fluid[24], as well as hair[25]. Metabolomics techniques do not only allow identification of numerous biomarkers at once, but also to conduct studies where we may account more precisely for food–food interactions. Moreover, these new techniques provide the opportunity to more easily use combinations of biomarkers to predict food intakes[26]. Commonly used platforms for food metabolomics analyses are NMR spectroscopy[27], liquid chromatography–MS and GC–MS[28,29]. Advantages of NMR include its high reproducibility, short sample preparation time and small interlaboratory variability[30,31]. Conversely, NMR only allows identification of a limited number of metabolites as compared to liquid chromatography–MS and GC–MS[30,32]. MS techniques allow identification of many more metabolites, but tissue samples require more preparation and the overall throughput is lower[30]. Given the pros and cons of the different platforms, a multiplatform approach is preferred to identify a broad range of metabolites[33,34].

## Between- and within-person variability in food intake biomarkers

During identification of novel food intake biomarkers, one needs to be aware that metabolite concentrations may be influenced by genetic factors, different rates of digestion, absorption, metabolism, and excretion[35], smoking[36], body composition[37], lifestyle[38], drug use[39] and geographical location[40]. To illustrate, random plasma $^{13}C$ concentrations have been shown to be highly correlated with cane sugar/high fructose maize syrup consumption of the preceding meal ($R^2 = 0{\cdot}90$)[41]. However, these concentrations only reflect very recent intakes that are probably not detected when studying fasting concentrations. Additionally, although red meat consumption has been significantly associated with creatinine[42], creatinine is also a product of muscle catabolism. Red wine consumption has been associated with various resveratrol metabolites[43]. However, also in this example, between-person variability should be considered due to for instance the production of resveratrol metabolites by colonic microflora[44]. Ideally, a robust food intake biomarker is not markedly influenced by such factors, but in any case such aspects need to be taken into account in the validation process of food intake biomarkers. However, a general validation system for food intake biomarkers is still lacking and better guidance for biomarker validation is needed. During the past years, a system to score food intake biomarker quality and validity has been developed, allowing researchers to evaluate potentially interesting food intake biomarkers for standard analytical quality control along with criteria related to biomarker kinetics (dose–response, time–response), metabolic and other host factor effects, food matrices, and specificity for the actual foods (Q Gao, G Praticò, A Scalbert *et al.*, unpublished results). In addition to the aforementioned effort, the University Medical Centre Groningen and Dutch National Institute for Public Health and the Environment are investigating to what extent food intake biomarkers are biased by within-person variation, and if so, whether correction for within-person variation improves the estimate of the proportion of people with less than adequate or excessive intakes.

## The Food Biomarkers Alliance

It is clear that food metabolomics is a complex discipline requiring the expertise and facilities in nutrition, metabolomics, epidemiology, clinical science, analytical chemistry, molecular biology, food sciences, bioinformatics and statistics. Therefore, a large group of experts in food metabolomics decided to join forces in the Food Biomarkers Alliance (FoodBAll), which is a Joint Project Initiative 'A Healthy Diet for a Healthy Life' (JPI-HDHL). This is a nationally funded collaboration between twenty-four partners from thirteen countries (Belgium, Canada, Denmark, Finland, France, Germany, Ireland, Italy, Norway, Spain, Sweden, Switzerland and the Netherlands), aiming to identify and validate food intake biomarkers of commonly consumed foods in Europe that can improve food intake assessments. FoodBAll started its funded activities in December 2014 and will continue for 3 years up to mid-2018 (http://foodmetabolome.org/)[45]. FoodBAll aims to systematically explore and validate food intake biomarkers to provide a better assessment of the food intake in different European regions. In order to identify and validate new food intake biomarkers, the FoodBAll team decided to focus on the following aspects of food intake biomarker research: (1) discover novel food intake biomarker by means of extensive systematic literature reviews, observational studies and acute intervention studies; (2) develop a validation scoring system for food intake biomarkers and its application to new biomarkers; (3) explore and validate alternative/less-invasive food intake biomarker sampling techniques; (4) develop tools for identification of food intake biomarkers and online resources to facilitate sharing of food intake

biomarker data and resources with the (scientific) community; (5) explore biological health effects of food intake biomarkers; (6) describe the value of food intake biomarkers for authorities and stakeholders. In order to align all the work from many different disciplines, a food intake biomarker was defined as being a specific measurement in a biological specimen accurately reflecting the intake, selective and dose-dependent, of a food constituent or food. In contrast to a food intake biomarker, a nutritional biomarker relates to nutrients and can be any biological indicator of nutritional status linked either to intake, metabolism or effect of a nutrient.

## Present actions on novel food intake biomarker discovery

### *Present scientific literature*

During the past decades already numerous studies have been conducted that used dietary biomarkers or studied potentially new dietary biomarkers[20]. A French-Canadian research collaboration extracted and organised the available data for all the nutritional biomarkers used so far in scientific studies in the Exposome-Explorer database[46]. Compounds and nutrients included in the database are for instance kaempferol, isorhamnetin, *m*-coumaric acid and phloretin for apples; naringenin for grapefruit; carotenoids, lycopene and lutein for tomato; 5-heneicosylresorcinol, 5-tricosylresorcinol and alkylresorcinols for whole-grain wheat; daidzein, genistein, isoflavones and *O*-desmethylangolensin for soya products; and iodine, margaric acid, pentadecylic acid and phytanic acid for dairy products[17,46]. For a full overview of the identified metabolites presented in the Exposome-Explorer database, please visit the website (http://exposome-explorer.iarc.fr)[46]. The FoodBAll research team is also presently reviewing the state of the art of the present food intake markers to expand the Exposome-Explorer database. All major food groups are addressed, including alcoholic beverages, food of animal origin, fruit and vegetables, cereals and wholegrain, fats and oils, legumes, non-alcoholic beverages, confectionary, and spices and herbs. Most of the single foods within these groups are also reviewed.

### *Application of new food metabolomics techniques in observational studies*

Future studies to expand the present scientific literature are warranted. Observational studies collecting information on dietary intake and biological samples studies may provide valuable data to identify new (long-term) food intake biomarkers[17]. A commonly used approach in these types of studies is to rank participants according to their food intakes[47,48] and explore associations with metabolites identified by the metabolomics platform of choice. As an example, Madrid-Gambin *et al.* recently applied urinary NMR in an observational dataset of the PREDIMED study to describe the fingerprinting of dietary pulses, where dietary pulse intakes were assessed with an FFQ and classified as non-pulse and habitual pulse consumers[48]. This approach revealed differences for sixteen metabolites coming from choline pathways, protein-related compounds and energy metabolism, and hence provided leads for potentially novel food intake biomarkers for pulse intake[48]. An extensive overview of similar studies available in the present literature can be found in a recent review by Manach *et al.*[49]. The FoodBAll consortium researchers aim to expand the literature on this matter by conducting similar studies using plasma and urine biobanks of cross-sectional and longitudinal cohorts with extensive dietary intake data. Altogether these databases include 197 000 individuals, men and women of all ages, from the general population to selected populations such as obese subjects and former cancer patients. A limitation of this approach is that high correlations may exist between the various foods consumed. Hence, associations may appear that are not the result of the food analysed, but of a highly correlated other food[17].

### *Application of new food metabolomics techniques in (acute) intervention studies*

Controlled acute dietary intervention studies are assumed to be less sensitive to confounding by correlated foods and therefore provide an important additional source of information in the field of food intake biomarker discovery. Gibbons and Brennan recently published an extensive overview of intervention studies using a metabolomics approach[50]. In addition, the same research group recently successfully predicted citrus intakes in the Irish National Adult Nutrition Survey by urinary proline betaine using calibration curves obtained from an acute intervention study[51]. A German research group also just published a randomised crossover intervention study on the effect of dietary non-fermentable and fermentable fibre (i.e. cellulose and inulin), and propionate (i.e. major product of the fermentation of fibres) on odd-chain fatty acid concentrations, showing significant effects of inulin and propionate, but not cellulose[52]. To contribute to this research area, seven study centres are presently conducting well-defined standardised short-term intervention studies. All study centres follow the same design, exploring potential food intake biomarkers of fourteen foods, including sugar-sweetened beverage, apple, tomato, banana, milk, cheese, bread, meat/meat products, red meat and white meat, potato, carrot, peas, lentils, beans and chickpeas (Fig. 1). Participants are exposed to a test meal after which urine and blood samples are collected over a time-course up to 24 h with the option to continue to 48 h. Collected samples are analysed by a multiplatform approach, including untargeted liquid chromatography–MS, GC–MS and NMR. The first results of these studies have just been published, which pointed towards lactose, galactose, and galactonate as potential biomarkers of milk intake, urinary 3-phenyllactic acid of cheese intake, and pinitol and trigonelline of soya drinks[53]. Moreover, FoodBAll researchers are conducting metabolomics analyses using previously collected data from intervention studies. FoodBAll has access to fifty-four human intervention studies with biobanks of more than 14 000 men

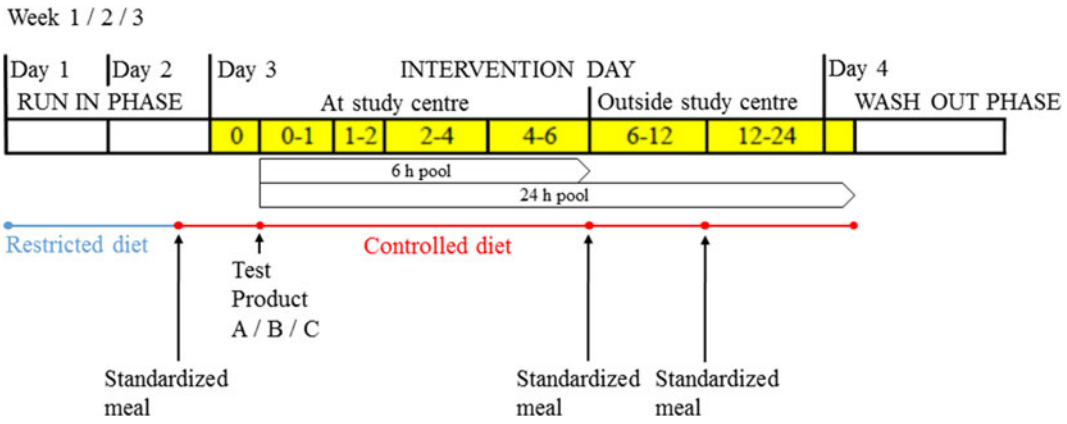


**Fig. 1.** (Colour online) Design of the randomised, controlled, crossover studies as conducted as part of the Food Biomarkers Alliance (FoodBAll) project (adapted from Munger *et al.*[53]). Test products are administered in random order and urine samples are collected before and after ingestion of test product during defined intervals (yellow blocks) up to 24 h; 6 and 24 h pools are later prepared in the laboratory.

and women from age 8 to 95 years, including up to twenty different foods (e.g. dairy, meat, apples, cocoa, Nordic diet) and more than fifteen different nutrients or compounds (e.g. vitamin C, vitamin $B_{12}$ and polyphenols). Although intervention studies are assumed to be less sensitive to detect food–metabolite associations confounded by other correlated foods, the highly controlled character of these studies may result in identification of metabolites that are not specific enough for the food under investigation when explored in free-living human subjects (i.e. population-based studies)[17].

### *Novel alternative/less-invasive food intake biomarker sampling techniques*

Despite many potential advantages of food intake biomarkers, they may also be costly and more invasive than self-reported dietary assessment methods. To date, venous blood sampling is the most commonly used approach to collect biological samples from human volunteers. This is a time-consuming process that requires well-trained personnel. Recent technological developments allow us to conduct food intake biomarker analyses with high sensitivity requiring very small blood volumes or tissue samples[54–56]. Combining these novel and sensitive techniques with non-professional biological sampling (e.g. blood collection by finger-pricking or filters/sticks) offers the opportunity to collect biological data without the employment of health professionals. Recently, an extensive literature review has been performed summarizing the present evidence with respect to food intake biomarkers from different body fluids, e.g. blood, leucocytes, plasma, serum, urine, stool, saliva, sweat, hair, nails, skin, adipose tissue and skeletal muscle[57]. It is feasible to collect and submit dried blood spot samples via regular mail in large epidemiological settings with more than 70 % response rates similar to saliva collection[58]. Documentation of dried blood spot application on biomarkers is available for fatty acids[59], 25-hydroxyvitamin D[60] and 8-epi-Prostaglandin F2α [61]. Presently, the FoodBAll consortium continues the work on alternative food intake biomarker sampling techniques by conducting validation studies on the use of dried blood spot and Mitra sticks for the analyses of lipid biomarkers as well as different nutrients, including sodium, magnesium, potassium, calcium, iron, cupper, zinc, selenium and iodine.

## Bridging dietary biomarkers to health pathways

In order to strengthen evidence for the application of intake/health efficacy biomarkers, future research should aim to relate food intake biomarkers to diet-related disease risk by (1) taking a pathway- and network-based bioinformatics approach and (2) building on existing transcriptomic and metabolomics data[62]. O'Gorman *et al.* recently applied this approach in the Metabolic Challenge Study and identified twenty-two lipid biomarkers that were associated with total dietary fat intake[63]. Five of these twenty-two lipid biomarkers were significantly elevated in participants with increased risk of the metabolic syndrome[63]. Wittenbecher *et al.* also applied a metabolomics approach and identified six out of twenty-one potential biomarkers for red meat consumption to be associated with type 2 diabetes[64]. A Spanish group observed urolithin A glucuronide as the most discriminant marker of nuts consumption, which in turn showed a significant inverse correlation with abdominal overweight and glycaemia[65]. The FoodBAll consortium is presently conducting additional studies on the relevance and value of transcriptomics and metabolomics data patterns relating dietary intake with health.

## Online (food) metabolomics databases

Although many food compounds can be found in human bio specimens and detected in metabolomic profiles, the

interest in food metabolomics is relatively new, and annotation of the food metabolome is still very limited as compared to annotation of the endogenous metabolome. Thus, identification and validation of potentially new food intake biomarkers using metabolomics techniques requires knowledge of presently available electronic resources, such as food composition, compound and biomarker databases, libraries of spectra, software tools and various online tools for food metabolome profiles annotation and interpretation. A new public web portal developed by the FoodBAll consortium has been launched in 2017 that provides descriptions, links and tutorials for more than fifty selected databases, spectral libraries, software programmes, and online tools useful for food metabolome profiles annotation and food intake biomarker discovery[45]. One of the major online resources is the human metabolome database, which contains detailed data about small molecule metabolites identified in the human tissues[66]. The most comprehensive metabolome database specifically focusing on the food compounds is FooDB (http://foodb.ca/), covering more than 25 000 native food compounds, food additives and man-made compounds also formed during food processing with their dietary sources (>900 foods)[67]. However, online databases are still incomplete, especially regarding the human metabolites of all non-nutrients present in foods. Initiatives are ongoing to manually collect information available in the literature and to add *in silico* predicted metabolites of all food components. PhytoHub is a compound database focused on food phytochemicals and their human metabolites manually extracted from the literature, initially built by Institut national de la recherche agronomique for nutrimetabolomics studies[68]. Besides databases useful for annotation in metabolomics, International Agency for Research on Cancer (France) and University of Alberta developed the Exposome-Explorer database on biomarkers of environmental exposure (http://exposome-explorer.iarc.fr/) in order to compare the performance between biomarkers and their fields of application[46]. Exposome-Explorer contains data on many attributes related to 142 dietary biomarkers extracted, such as type of exposure, biomarker concentrations in various human body fluids or tissue and associated analytical techniques, biomarker reproducibility over time, population characteristics, study design and with references to publications. FooDB, PhytoHub and Exposome-Explorer are being further developed within the FoodBall project to better meet all needs for the discovery and validation of new nutritional biomarkers. The FoodComEx 'Food Compound Exchange' may be another valuable online source for metabolomics researchers. FoodComEx is a chemical library recently developed by FoodBAll partners that aims to facilitate the sharing of standards of food compounds, their metabolites and other reference materials[69]. These standards are essential for the validation or identification of candidate biomarkers in metabolomics studies. An interface has been developed that facilitates the exchange between researchers interested in using a specific compound and researchers owning that specific compound. Presently, about forty rare food-derived metabolites and 1005 commercial compounds are included in this database. The database will be further extended by attracting more contributors and as such more compounds. Furthermore, additional compounds will be produced by using *in vitro* (microbial fermentation, liver microsomes and isolated enzymes) and *in vivo* (rodents) systems to 'metabolise' food chemicals.

## Conclusion

Food intake biomarkers provide a valuable alternative/addition to traditional self-reported dietary intake assessment methods. However, although many biomarkers have been described, only relatively few are sufficiently validated and accepted as food intake biomarkers. In the identification of new potential biomarkers of food intake, many factors need to be considered, such as metabolism and lifestyle. Additionally, the specificity of the metabolite for the food under study needs to be evaluated, where also the increasing use of fortified foods and supplements should be considered. By joining international multidisciplinary expertise, the FoodBAll consortium intends to contribute to the discovery of new food intake biomarkers, using novel metabolomics techniques. Ultimately this effort aims to facilitate three main applications, such as (1) validation/adjustment of self-reported dietary assessment tools, (2) serve as markers of compliance in intervention studies and (3) improve the value of many observational studies investigating nutrition and health associations by providing less biased intake estimates.

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## Conflict of interest

C. A. D. is a cofounder, board member, stock-owner and consultant for Vitas AS (http://vitas.no) performing the dried blood spot analyses.

## Authorship

E. J. M. F., L. B., C. A. D., L. O. D., C. M., H. M. R. and H. van K. are the work-package leaders of the FoodBAll, and responsible for the design and conduct of the project. C. A.-L., S. J. L. B., J. B., L. B., F. C., S. D. S., L. O. D., C. A. D., T. E. G., H. van K., M. K., R. L., J. L., C. M., F. M., R. P. M., H. M. R., A. S., S. K., C. S., T. S., I. T., G. V., D. W. and E. J. M. F. are principal investigators at the partaking institutes. E. M. B.-B. and E. J. M. F. drafted the manuscript. All authors provide feedback on the draft manuscript and approved the final manuscript.

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