Commentary

Novel strategies for improving dietary exposure assessment: Multiple-data fusion is a more accurate measure than the traditional single-biomarker approach

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Abstract

Background: Accurate measurement of food intake is the cornerstone of understanding the links between diet and optimal health status or risk of disease. The utilization of metabolomics approaches is revolutionizing the field of dietary assessment by associating metabolic profiles with intake of specific foods or dietary patterns and/or investigating human health status in nutritional trials. Combining dietary biomarkers with conventional dietary assessment methods is considered a potential strategy for tackling the complexity of dietary exposure fingerprinting.

Scope and approach: We discuss existing approaches among dietary assessment methods and dietary biomarkers. A combined approach taking into consideration data from dietary questionnaires with measurements of dietary biomarkers is emphasized.

Key findings and conclusions: Trends in novel strategies for improving dietary exposure assessment will be influenced by the discovery and validation of dietary exposure biomarkers. Among different strategies, multi-metabolite biomarker panels enable more reliable estimation of dietary exposure than does the traditional single-biomarker approach. Therefore, a combined approach using data from dietary questionnaires along with measurements of dietary biomarkers is considered an excellent strategy for improving dietary exposure assessment.

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1. Introduction

Diet and nutrition are major determinants of human health. Accurate measurement of food intake is the cornerstone of understanding the links between diet and optimal health status or risk of disease. Dietary assessment has been traditionally performed using conventional methodologies of food surveys such as food frequency questionnaires (FFQs), 24-h dietary recalls or food records. However, the accuracy of the dietary intake and nutritional status is frequently challenged due to the subjective nature of these dietary instruments. This limitation can be improved by the application of metabolomics to characterize dietary exposure.

Metabolomics approaches are revolutionizing the field of dietary assessment by associating metabolic profiles with intake of specific foods or dietary patterns, and/or investigating human health status in nutritional trials. Recently, exploring the food metabolome has been defined as a data-driven strategy for identifying novel biomarkers and improving the accuracy of measurement of dietary exposures by traditional dietary assessment instruments (Scalbert et al., 2014). In this way, the use of metabolomics has enabled the identification of novel and robust biomarkers of food or nutrient intake, which provide an objective measure of exposure that is devoid of many of the biases and errors associated with self-reported methods (Scalbert et al., 2014).

However, there are some factors not present in the traditional dietary assessment instruments that could misrepresent biomarker measures of dietary intake. These factors include genetic variability (e.g. biological variation in nutrient absorption and metabolism,
epigenetic variation or gene-gene interactions), lifestyle/physiological factors (e.g. smoking, alcohol consumption, physical exercise or influence of microbiota), dietary factors (e.g. nutrient bioavailability or nutrient-nutrient interactions), biological samples and analytical methodology (Scalbert et al., 2014). Further research will address this issue and identify the best emerging dietary biomarkers. Therefore, as biomarkers cannot replace conventional dietary assessment methods, the use of conventional dietary instruments together with dietary biomarkers is considered the best strategy for tackling the complexity of dietary exposure fingerprinting. Fig. 1 represents a schematic diagram for combining dietary questionnaires with biomarkers.

The main aim of this paper is to highlight existing approaches aimed at improving dietary exposure assessment and the novel combined measure considering data from dietary questionnaires with measurements of dietary biomarkers.

2. Dietary assessment methods

2.1. Dietary questionnaires

In nutritional studies, the traditional method of collecting data on foods and beverages consumed over a prescribed period of time relies on information gathered using dietary assessment questionnaires. As previously mentioned, the most commonly used dietary assessment methods are open-ended questionnaires such as food records or 24-h dietary recalls, or closed-ended questionnaires including FFQs (Tucker, 2007). Generally, these instruments require a systematic estimation of the frequency and usual serving size of foods, as well as detailed information about the ingredients of a meal recipe, combinations of foods consumed together, and sometimes cooking processes, which may influence the estimation of exposure to a particular dietary constituent. Moreover, the estimation of nutrient/compound intakes depends largely on the existence of appropriate, complete, reliable and up-to-date food composition tables or databases. Many users are often not conscious of the high composition variability, particularly in terms of micronutrients (e.g. vitamins) and phytochemicals, between similar foods, and even in the same type of food (e.g. raw, cooked or processed foods). For this reason, a great dependency on professionals’ knowledge is needed to generate, compile, update and use the food composition data adequately. Indeed, the Food and Agriculture Organization of the United Nations (FAO) and the International Network of Food Data Systems (INFOODS) have recently published three comprehensive guidelines on conversions, data evaluation and food matching in order to improve and harmonize the compilation of food composition data, which could also lead to more accurate nutrient intake estimations (Charondiere et al., 2016).

The consumption data obtained through these methods are used to compute the intake of whole diets/dietary patterns, food groups, foods, energy, nutrients, bioactive compounds and other food components. Recently, dietary pattern analysis has emerged as a useful approach for investigating diet-disease associations. Thus, eating patterns may be more predictive of disease risk than isolated analysis on foods or nutrients (Hu, 2002). The 2015 Dietary Guidelines Advisory Committee recognized the advantages that dietary patterns offer as an approach for informing public health recommendations (U.S. Department of Health and Human Services & U.S. Department of Agriculture, 2015, p. 2). Different approaches for developing dietary patterns exist (Lassale et al., 2016). The most prominent methods are, a priori, numerical indexes, scores that measure adherence to disease-specific dietary and lifestyle guidelines (e.g. Dietary Approaches to Stop Hypertension (DASH)), scores that measure adherence to a regional diet (e.g. Mediterranean Diet Score) and scores based on nutritional guidelines (e.g. Diet Quality Index International (DQI-I)). Other approaches have been proposed to derive patterns by using all food groups available, such as principal component analysis (PCA), reduced rank regression, partial least-squares regression (PLS), confirmatory factor analysis (CFA) and treelet transform (Hu, 2002; Imamura & Jacques, 2011; Varraso et al., 2012).

The strengths of these methods are the lower relative cost, the ease with which the questionnaires can be completed with the help of a trained interviewer (dietician) or by participants themselves (self-reported questionnaires), and the chance to gather a large amount of dietary data. However, the use of questionnaires is also subject to some limitations (Tucker, 2007). The main one is that they are mostly self-reported, wherein the estimation of food portion size is an important source of errors (perception, conceptualization and memory), which could be inappropriate for some populations (children, obese people, and elderly people with cognitive impairment, among others). Such systematic errors inherent in self-reported data plus random errors (e.g. the accuracy of the food composition tables) can bias the estimation of dietary intake. However, the FFQ is the most common dietary assessment method used to estimate habitual dietary intake (e.g. the previous 12-months) of specific individual dietary exposures related to a certain disease or various dietary components in large-population studies, due to its self- or interviewer-administered and economical machine-readable features. In this context, the use of a previously validated FFQ is an essential requirement for improving the measurement of errors previously mentioned. FFQs should be developed specifically for the research objective because diet may be influenced by participant’s characteristics such as ethnicity, culture, dietary habits and lifestyle, among others. Nevertheless, more precise measurements are obtained when using multiple 24-h dietary recalls or dietary records, but only short-term intake (actual intake information over the previous 24 hours) is estimated. In this context, long-term intake can also be estimated if repeated during the year. Recently, it has been suggested that a combination of two different dietary assessment instruments, such as four to six 24-h dietary recalls with a FFQ, could improve estimates of dietary intakes with regard to the methods separately. In this study, the association between diet and disease was statistically significant with food records but not with a FFQ (Carroll et al., 2012). Therefore, if new nutritional studies are designed to include FFQs plus repeat 24-h evaluations, further improvements to minimize their measurement errors might be seen by combining data from the two methods. Our group has recently observed that the highest tertile of total dietary polyphenols, which were estimated using a validated FFQ and an ad hoc database on polyphenol content in foods, was not associated with the risk of cognitive and physical decline, frailty and total mortality, in comparison with the lowest tertile (Rabassa, Cherubini, et al., 2015; Rabassa, Zamora-Ros, et al., 2015, 2016; Zamora-Ros et al., 2013). However, an association with total urinary polyphenols was observed. Moreover these studies have demonstrated the importance of assessing dietary polyphenol exposure whenever possible, using dietary biomarkers and not only through dietary questionnaires.

Despite these strengths, more refined and improved techniques of dietary assessment intake are essential to reduce the limitations of traditional dietary questionnaires and also to reduce the cost associated with the collection and processing of dietary data (Illner et al., 2012; Stumbo, 2013). This is being met with intense methodological research and innovative technologies. Many applications of information and communication technologies are currently under development and validation, and great strides have been made. An example of interactive computer-based techniques is a menu-driven standardized 24-h dietary recall program (called EPIC-
SOFT) developed by the European Prospective Investigation into Cancer and Nutrition study (Slimani et al., 2011). The National Cancer Institute in the US has also developed an interactive computer-based approach but with an Internet-based technology, called the Automated Self-Administered 24-h Dietary Recall, which is based on the Automated Multiple-Pass Method approach (Schatzkin et al., 2009). In addition, mobile phone applications have been released such as Nutricam, which allows users to capture images of foods and verbally describe their items before intake, and then to upload both the image and voice file onto a website for analysis (Rollo, Ash, Lyons-Wall, & Russell, 2011). Another example is a wearable electronic device that resembles a necklace and includes a microphone, camera and other sensors. In this case, dietary intakes are collected from the video recording and are calculated automatically (Sun et al., 2010). However, these methods have not yet been widely implemented in large-population studies due to their related technical issues (data transfer, storage, battery life and others) and methodological difficulties such as self-reporting and higher costs. In addition, certain users are not familiar with innovative technologies or new devices. Despite these limitations, automated versions promise to overcome the labour-intensive and costly coding of the 24-h dietary recalls (Shim, Oh, & Kim, 2014).

2.2. Dietary biomarkers

Thus far, individual biomarkers of dietary intake have been used to assess exposure to specific foods or food groups. However, this strategy has important limitations and only in some cases it has been successful. The more relevant limitations can be grouped into a wide distribution of food components (low specificity of biomarkers), high interindividual variation and the microbiota metabolism, among others. A common approach when the research community looks for a biomarker of intake is first to study the food composition, then try to identify the possible modifications caused by the host metabolism and finally look for metabolites (e.g. biomarkers) in the biofluid (mostly blood and/or urine). Usually, the food composition is very complex (from the quantitative and qualitative point of view) and many of the compounds are widely distributed in different foods. For instance, most polyphenols are present in a wide range of plant foods, such as chlorogenic acids (e.g. coffee and apple) and flavan-3-ols (e.g. cocoa and tea) (Clifford, 2000; Monagas et al., 2010). Another example of a compound widely distributed in many plant foods is vitamin C. This compound has been used as a biomarker of consumption of fruits and vegetables, although differences in concentration between different foods reduces their ability to be a good biomarker (Scalbert et al., 2014). Therefore using the single-biomarker strategy compromises its usefulness because there are a number of factors that limit the prediction of dietary exposure.

It is worth noting that in some cases, similar compounds from different food sources could produce the same biomarker. One of the most relevant cases is ellagitannins. This class of polyphenols (with some differences) is present in foods such as pomegranate, strawberries and walnuts (Espín, Larrosa, García-Conesa, & Tomás-Barberán, 2013). They are poorly absorbed and when they reach the gut they are largely metabolized by the microbiota, producing urolithin derivatives (Espín et al., 2013). Selma, Beltrán, García-Villalba, Espín, & Tomás-Barberán (2014) showed the ability of the bacteria Cordinobacter to produce urolithins from ellagitannins. This means that urolithins are biomarkers of ellagitannin intake instead of particular foods. This relevance of the microbial effect is crucial because a significant number of food components are degraded by the colonic microbiota and after absorption and distribution are excreted in urine. Therefore, the real biomarker of intake is provided by the microbiota instead of the host metabolism.

Procyanidins are a good example of this behaviour. This class of
polyphenols is present in many dietary sources, such as cocoa, tea, wine and apples (Monagas et al., 2010). These polyphenols show low bioavailability. However, gut microbiota have the capacity to degrade these metabolites and produce other compounds called hydroxyphenylvalerolactones and hydroxypheynylvaleanic acids (Monagas et al., 2010). In fact, these metabolites have been used as biomarkers of procyanidin-rich foods. Both urolithins and hydroxyphenylvalerolactones have been used as biomarkers of intake of single foods (e.g. walnuts and tea, respectively). However, taking into account the variety of dietary sources that could provide these parent compounds (e.g. ellagitannins and procyanidins), these compounds are not suitable for use as single accurate biomarkers.

In relation to the individual variation, there are some interesting examples. According to Lars O. Dragsted (2010), creatinine can be considered a potential marker of meat intake (even cooked). However, endogenous levels connected with creatine turnover showed important variations between subjects (Dragsted, 2010). Another example is a recent study about polyphenols and their urinary quantification (Achaïtre et al., 2016). In this paper the authors showed that in urine from 475 EPIC participants a total of 34 polyphenols were evaluated and in general these compounds showed large interindividual variations (Achaïtre et al., 2016).

The way that metabolites are formed through microbial metabolism of polyphenols (5-(3′,4′-dihydroxyphenyl)-valerolactone glucuronide). Both metabolites have been proposed as biomarkers of cocoa intake in studies with different designs (i.e., acute interventions, long-term intervention trials and observational studies) and provided the model with complementary information about habitual cocoa intake (Garcia-Aloy et al., 2015). Finally, an additional MBP was highly predictive for wholegrain bread intake [AUC = 93.1% (88.7–97.4%) and 93.7% (89.4–98.1%) for data from positive and negative ionization mode, respectively], while the MBP designed to evaluate white bread consumption had a reasonably good predictive ability [AUC = 80.6% (72.1–89.0%) and 77.8% (69.1–86.4%) for data from positive and negative ionization mode, respectively] (Garcia-Aloy et al., 2015). Previously, Campbell et al. (1994) published one of the first studies suggesting a combination of biomarkers of intake. They used a stepwise logistic regression analysis to assess fruit and vegetable consumption. The resultant prediction model included compounds measured in biological samples (three carotenoids determined in plasma) and data from dietary questionnaires (energy intake) (Gross et al., 1994). Later, Nielsen, Freese, Kleemola, and Mutanen (2002) proposed measuring the sum of different flavonoids determined in urine for examining the intake of fruits and vegetables (Nielsen et al., 2002), an approach also suggested in other studies (Brevik, Rasmussen, Drevon, & Andersen, 2004; Krogholm, Haraldsdóttir, Knuthsen, & Rasmussen, 2004). In the same vein, summing individual resveratrol or alkylresorcinol metabolites has also been attempted for assessing wine (Queipo-Ortuno et al., 2012; Rotches-Ribalta et al., 2012; Zamora-Ros et al., 2009) and wholegrain wheat and rye consumptions (Kristensen et al., 2012; Landberg et al., 2009; Linko-Pavinen, Landberg, Tikkanen, Adlercreutz, & Penaalvo, 2007), respectively. In parallel, the ratio between alkylresorcinols, C17:0/C21:0, has shown the ability to discern between wholegrain wheat and rye intakes (Landberg et al., 2009; Linko-Pavinen et al., 2007). However, although these later examples exhibited a good predictive capacity, these statistical approaches could not give the real weight of each metabolite within the biomarker panel, and therefore more sophisticated approaches could be required for the assessment of dietary exposures for more complex foods, food groups or dietary patterns. At the same time, it is important to bear in mind that using metabolites from the same class could not deal with the problem of specificity previously highlighted.

As mentioned above, recent work from our laboratory applied a
<table>
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<th>Food item</th>
<th>Statistical test</th>
<th>Metabolites in the panel</th>
<th>Study design</th>
<th>TS &amp; VS Panel-diet associations</th>
<th>Was the panel better than single biomarkers?</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Walnuts                   | Stepwise logistic regression | 3-Indolecarboxylic acid glucuronide
10-Hydroxy-decane-4,6-dienoic acid sulphate
Urolithin A glucuronide
Tridecadienoic/tridecynoic acid glucuronide
Urolithin A sulphate | Observational (cross-sectional) | Yes  
AUC (95% CI):
- TS = 93.4% (90.1–96.8%)
- VS = 90.2% (85.9–94.6%) | Yes | Garcia-Aloy et al., 2014 |
| Cocoa                     | Stepwise logistic regression | 7-Methylxanthine
5-(3',4'-dihydroxyphenyl)-valerolactone GlcA | Observational (cross-sectional) | Yes  
AUC (95% CI):
- TS = 95.7%
  (89.8–100%)
- VS = 92.6%
  (81.9–100%) | Yes | Garcia-Aloy, Llorach, Urpi-Sarda, Jäuregui, et al., 2015 |
| Bread                     | Stepwise logistic regression | White Bread: HPAA GlcA
HMBOA
Riboflavin
Wholegrain Bread: HHPAA
HPAA
HMBOA
Enterolactone GlcA
Pyralline
3-Indolecarboxylic acid GlcA
Riboflavin | Observational (cross-sectional) | No  
AUC (95% CI): from 77.8% (69.1–86.4%)
to 93.7% (89.4–98.1%) | Yes | Garcia-Aloy, Llorach, Urpi-Sarda, Tulipani, et al., 2015 |
| Wine                      | Stepwise logistic regression | Tartrate
Ethyl glucuronide | Sustained intervention [TS]
Observational (cross-sectional) [VS] | Yes  
AUC (95% CI):
- TS = 90.7% (84.5–96.4%)
- VS = 92.4% (84.1–100%) | Yes | Vázquez-Fresno et al., 2015 |
| Orange juice              | Random forest | Stachydrine
Methyl glucopyranoside (α+β)
Dihydroferulic acid
Galactonate | Sustained intervention | Yes  
AUC (95% CI): 99.6%
(96–100%)
Accuracy:
- Entire data set = 93%
- Hold-out data set = 87.5%
- Permutation test: Entire data set: p-value = 0.006
- Hold-out dataset: p-value = 0.004 | Yes for some metabolites | Rangel-Huerta et al., 2017 |
| Coffee                    | Support vector machines | Cyclo(isoleucylprolyl) 1-Methylxanthine
Trigonelline | Observational (cross-sectional) | Yes  
AUC (95% CI): 98% (93–100%) | Yes | Rothwell et al., 2014 |
| Sugar-sweetened beverages | PLS regression | Formate
Citrulline
Taurine
Isocitrinate | Observational [TS]
Acute intervention [VS] | Yes  
AUC [TS]: 80%
Specificity [TS]: 80%
Sensitivity [TS]: 70% | Yes | Gibbons et al., 2015 |
| Nordic diet               | PLS-DA | (2-Oxo-2,3-dihydro-1H-indol-3-yl)acetacid
6-Amino-5-(N-methylformamidino)-1- methyluracil
Hydroquinone GlcA
3,4,5,6-Tetrahydrohippurate
3-Indoleacetic acid GlcA
Limonele-1,2-diol GlcA
Limonele-8,9-diol-GlcA
p-Menth-1-1ne-6,8,9-triol
Trimethylamine N-oxide | Sustained intervention | Yes*  
Misclassified samples:
- TS: 35%
- VS: 19% | NR | Andersen et al., 2014 |
multivariate statistical approach to link dietary data with both targeted and untargeted metabolomics data to identify a series of MBPs (Garcia-Aloy et al., 2014, 2015, 2015; Urpi-Sarda et al., 2015; Vázquez-Fresno et al., 2015). In this approach, stepwise logistic regression analysis was used to include more than one metabolite in biomarker panels and regressed against dietary data to identify MBPs. Other research groups also applied regression analysis for addressing this issue (Jin et al., 2014; McGrath et al., 2016; Neuhouser et al., 2003), whereas, more recently, other multivariate statistical strategies such as PCA, PLS, random forest and support vector machine algorithms have also been used to build MBPs for assessing the consumption of different foods or dietary patterns (Andersen et al., 2014; Gibbons et al., 2015; Marklund et al., 2014; O’Gorman et al., 2014; Rangel-Huerta et al., 2017; Rothwell et al., 2014). These multivariate methods may be of interest in terms of the further discovery of dietary exposure biomarkers. For example, Marklund et al. (2014) created a novel dietary biomarker score based on a combination of several individual biomarker concentrations, using rank scores or principal component analysis to assess compliance in a dietary randomized controlled trial, Systems Biology in Controlled Dietary Interventions and Cohort Studies (SYSDIET), and to investigate how a healthy Nordic diet influences cardiometabolic risk factors (Marklund et al., 2014). Their results suggest that this dietary biomarker score provides a better reflection of the dietary intake and thereby increases the strength of detecting potential cardiometabolic health effects. In addition, they proposed that future studies should evaluate the combined use of dietary biomarkers and reported dietary intake methods for assessing compliance.

Nevertheless, since until now MBPs have been proposed for a limited number of foods, food groups and dietary patterns, further work in this line is required. This will enable a broad coverage of dietary exposure, including the assessment of exposure to bioactive compounds, foods, food groups or complex dietary patterns reflective of habitual dietary intake. The challenge that lies ahead will be finding the simplest combination of metabolites that is able to properly evaluate dietary exposure. In the same vein, the capacity of metabolomics to measure simultaneously a high number of metabolites offers a great opportunity to propose new MBPs clustering in the same model metabolites of different classes, providing the panels with complementary information about dietary intake.

However, before translating MBPs into nutritional epidemiology, appropriate validation steps in the post-discovery phase are essential in order to assess their robustness. Firstly, it is necessary to develop analytical methods for accurate and specific quantification of the metabolites included in each MBP, as recently suggested for simultaneous quantification of multiple biomarkers associated with alcohol intake (Monosik & Dragsted, 2016). Additionally, the specificity, sensitivity, kinetics and dose-response relationships of proposed MBPs should be investigated in separate studies with different designs and populations, including people with different genetic and dietary backgrounds.

### 2.3. Methods of combining dietary questionnaires and biomarkers

Taking into account all of the above, and with a view to evaluating the diet-disease relationship, the use of validated dietary biomarkers helps to improve precision in the measure of dietary intake of different foods, nutrients and other components, and to then strengthen the ability to detect dietary effects. In addition, a combined approach using data from dietary questionnaires with measurements of dietary biomarkers has emerged as a good strategy, especially when the extent to which the biomarker mediates the dietary effect is unknown. Freedman, Kipnis, et al.
<table>
<thead>
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<th>Reference</th>
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<tbody>
<tr>
<td>Wholegrain wheat and rye</td>
<td>Sum Ratio</td>
<td>Sum of AR homologues (AR C17:0-C25:0) AR C17:0/C21:0 ratio</td>
<td>Sustained intervention</td>
<td>Increase: p-value &lt; 0.05</td>
<td>NR</td>
<td>Linko-Parvinen et al., 2007</td>
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<tr>
<td>Rye wholegrain/bran</td>
<td>Sum Ratio</td>
<td>Sum of AR homologues (C17:0, C19:0, C21:0, C23:0, C25:0) AR C17:0/C21:0 ratio</td>
<td>Sustained intervention</td>
<td>Increase: p-value &lt; 0.0001</td>
<td>NR</td>
<td>Landberg et al., 2009</td>
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<tr>
<td>Wholegrain wheat</td>
<td>Sum</td>
<td>Sum of AR homologues (C17:0, C19:0, C21:0, C23:0, C25:0)</td>
<td>Sustained intervention</td>
<td>Increase: p-value &lt; 0.001</td>
<td>NR</td>
<td>Kristensen M, Toubro S, Jensen MG, Ross AB, Riboldi G, Petronio M, Bügel S, &amp; Tetens I, 2012</td>
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<td>Wine</td>
<td>Sum</td>
<td>cis-Resveratrol-3-O-GlcA trans-Resveratrol-3-O-GlcA</td>
<td>Observational (cross-sectional)</td>
<td>Correlation: r = 0.895 (p-value &lt; 0.001)</td>
<td>AUC (95% CI) = 98.3% (97.3–99.0%)</td>
<td>Sensitivity (95% CI) = 93.3% (91.5–94.7%)</td>
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<td>Sum</td>
<td>Resveratrol Metabolites:</td>
<td>Sustained intervention</td>
<td>Increase: p-value &lt; 0.05</td>
<td>NR</td>
<td>Queipo-Ortuño et al., 2012</td>
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<td>Sum</td>
<td>Resveratrol Phase II Metabolites</td>
<td>Sustained intervention</td>
<td>Increase: p-value &lt; 0.05</td>
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<td>Rotches-Ribalta et al., 2012</td>
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<td>Piceid sulphate</td>
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<td>Urine - hydrolysed samples:</td>
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<td></td>
<td></td>
<td>2,4-Dihydroxybenzoic acid Gallic acid Ethylgallate</td>
<td></td>
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<td>Urpi-Sarda et al., 2015</td>
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### Stepwise Logistic Regression

**Urine - non-hydrolysed samples:**
- Methylgallic acid sulphate
- Ethylgallate sulphate

**Plasma - hydrolysed samples:**
- 3-Hydroxyphenylacetic acid
- Gallic acid
- p-Coumaric acid

<table>
<thead>
<tr>
<th>Fruit &amp; vegetables</th>
<th>Sum Biomarkers</th>
<th>Sustained intervention</th>
<th>Increase: p-value</th>
<th>NR</th>
<th>Brevik et al., 2004</th>
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<td>Phloretin</td>
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<td></td>
<td>Vitamin C</td>
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<td></td>
<td>b-Carotene</td>
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<td>Lutein</td>
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### Acute Intervention

- 24 h urine: $r = 0.86$ (p-value < 0.0000001)
- Morning urine: $r = 0.43$ (p-value < 0.01)

Yes for 24 h urine  
No for morning urine

**Krogholm et al., 2004**

### Observational (cross-sectional)

- Correlation: $r = 0.35$ (p-value < 0.0001)

Yes for most single biomarkers

**Nielsen et al., 2002**

### Stepwise Logistic Regression

**Regression model**
- Vitamin C
- Carotenoids (cholesterol-adjusted)
- Ferric-reducing antioxidant power

### Logistic Regression

**Diet Quality Index Score**
- Stepwise linear regressions
- Vitamin C
- α-Tocopherol
- α-Carotene
- β-Cryptoxanthin
- Oleic acid
- Stearic acid

**Observational (cross-sectional)**
- Correct allocation: 45–86%  
Yes for most studies

**McGrath et al., 2016**

### Mediterranean diet
- NR
- Carotenones
- β-Vitamin E
- EPA
- DHA

**Observational (cross-sectional)**
- Correlation: $r = -0.52$ (p-value = 0.03)

**Gerber, 2006**

### Nordic diet
- Rank scores
- PCA
- α-Linolenic acid
- β-Carotene
- Alkylresorcinols
- EPA
- DHA

**Observational (cross-sectional)**
- Correlation: $r = -0.36$ (p-value < 0.05)

**Marklund et al., 2014**

AR, alkylresorcinol; AUC, area under the curve; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GlcA, glucuronide; MBP, multi-metabolite biomarker panel; NR, not reported; PCA, principal component analysis; TS, training set; VS, validation set.

* Samples of the training and validation sets were from the same subjects.
and Freedman, Tasevska, et al. (2010) proposed two main direct approaches, Howe’s method with ranks or PCA, for combining questionnaires and biomarkers related to whole diet, food group, food, nutrient or other food components (Freedman, Kipnis, et al., 2010; Freedman, Tasevska, et al., 2010). Comparing the results obtained from the two approaches, Freedman, Kipnis, et al. (2010) and Freedman, Tasevska, et al. (2010) found that Howe’s method gives results close to those from the PCA approach (Freedman, Kipnis, et al., 2010; Freedman, Tasevska, et al., 2010). It can be said that Howe’s method is more appropriate due to its simplicity than the approach based on PCA. Later, these authors proposed a more complex modelling-based approach, namely the regression calibration method, for combining dietary biomarkers and reports that recovers lost power and gives unbiased relative risk estimates (Freedman et al., 2011). However, this method is not applicable to food patterns or foods that have no known specific biomarkers. Unlike this, PCA and Howe’s method do not require knowledge of the quantitative relationship between biomarker level and true usual intake. Freedman, Kipnis, et al. (2010) and Freedman, Tasevska, et al. (2010) applied Howe’s method in an analysis of a diet-disease association in a real example from the Carotenoids and Age-Related Eye Disease Study (CAREDS) (Freedman, Kipnis, et al., 2010; Freedman, Tasevska, et al., 2010). In their study, the estimated odds ratios [OR (95%CI)] for the primary nuclear cataract outcome using the reported dietary intake (FFQ-lutein plus zeaxanthin), the biomarker level (serum lutein plus zeaxanthin) and the combined FFQ-biomarker were 0.77 (0.57–1.02), 0.69 (0.51–0.94) and 0.66 (0.48–0.91), respectively. As can be seen from the statistical significance, the combination of exposure was higher than that for the FFQ and biomarker alone. In addition, an increase in statistical power was also observed in detecting a diet-disease association. Recently, Rabassa, Cherubini, et al. (2015) and Rabassa, Zamora-Ros, et al. (2015) applied this approach to study the association between habitual dietary resveratrol exposure and the development of frailty syndrome in older adults from the InCHIANTI study (Rabassa et al., 2015). Inverse associations between resveratrol exposure and frailty syndrome risk were observed for FFQ-total resveratrol [OR for comparison of extreme tertiles = 0.17 (0.05–0.63)] biomarker-total urinary resveratrol [0.32 (0.09–1.11)] and FFQxbiomarker-total resveratrol [0.11 (0.03–0.45)]. The most successful results from the combined exposure measure will emerge when the strength of the associations for each separate exposure is similar, as occurs in the examples described in this section.

3. Future trends

Future research trends should focus on exploring more novel approaches for the discovery and validation of dietary exposure biomarkers. While considerable progress has been made in demonstrating how dietary biomarkers can be used as dietary assessment tools, a number of challenges have still to be overcome before they can achieve their complete validation, including both analytical and biological perspectives. Recent studies have suggested different strategies for identifying panels of biomarkers. They have also demonstrated that MBPs offer a more reliable estimation of dietary exposure than the traditional single-biomarker approach used until now. Therefore, future studies will face up to the complexity of evaluating the use of MBPs in combination with conventional dietary questionnaires to assess dietary exposure fingerprinting. These advances will enable more detailed information to be obtained about the associations between diet and health, providing better evidence of the development of health claims and dietary advice for both public health institutions and the food industry.

Conflict of interest

The authors have declared no conflict of interest.

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