Reliance on self-reports and estimated food composition data in nutrition research introduces significant bias that can only be addressed with biomarkers

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Abstract The chemical composition of foods is complex, variable, and dependent on many factors. This has a major impact on nutrition research as it foundationally affects our ability to adequately assess actual intake of nutrients and other compounds. Despite of this, accurate data on nutrient intake are key for investigating associations and causal relationships between intake, health, and disease risk at the service of developing evidence-based dietary guidance that enables improvements in population health. Here, we exemplify the importance of this challenge by investigating the impact of food content variability on nutrition research using three bioactives as model: flavan-3-ols, (–)-epicatechin, and nitrate. Our results show that common approaches aimed at addressing the high compositional variability of even the same foods impede the accurate assessment of nutrient intake, generally. This suggests that the results of many nutrition studies using food composition data are potentially unreliable and carry greater limitations than commonly appreciated, consequently resulting in dietary recommendations with significant limitations and unreliable impact on public health. Thus, current challenges related to nutrient intake assessments need to be addressed and mitigated by the development of improved dietary assessment methods involving the use of nutritional biomarkers.

Introduction

Nutrition is a crucial factor for public health ***Global Burden of Disease 2017 Diet Collaborators*** (***2019***); ***The National Academies of Sciences and Engineering and Medicine Health*** (***2017***). However, despite considerable methodological progress, nutrition research still relies mostly on self-reported dietary information and limited food composition data to investigate links between health and nutrition. Indeed, food composition data is the bedrock on which nutrition research rests today: it allows to estimate the intake of specific nutrients and other dietary compounds, and thus enables investigations into associations between nutrient intake and health outcomes. Such data inform policy makers in the development of dietary recommendations and risk assessments, and support the development of guidance for the general public and the food industry. However, this approach is not without significant challenges and limitations. One key challenge is the construction and maintenance of food composition data that underpin intake assessments for specific nutrients, as foods are highly complex and widely variable in their chemical makeup. Multiple factors

affect the ultimate nutrient content of foods, including cultivar or breed, climate, growing- and harvest conditions, storage, processing, and methods of culinary preparation ***Greenfield and Southgate*** (***2003***). Even apples harvested at the same time from the self-same tree show more than a two-fold difference in the amount of many micronutrients ***Wilkinson and Perring*** (***1961***). Moreover, processed foods are usually not standardised for composition but taste, texture and consumer preferences and thus vary in their chemical composition. Significant efforts have been made to generate extensive and detailed food composition tables, and complex sampling paradigms are used to obtain representative samples. Despite all these efforts, food composition data are generally used by relying on single point estimates, the mean food composition, de facto assuming that foods have a consistent composition. This approach introduces a considerable degree of error, bias, and uncertainty – and these are exacerbated by the limitations of self-reported dietary data which are known to carry substantial bias ***Subar et al.*** (***2015***).

Moreover, current approaches also assume that intake directly correlates with the systemic presence of a given nutrient, as it is through their systemic presence that many nutrients mediate much of their health-related biological effects. This introduces even more complexity when assessing true nutrient intake, as inter- and intra-individual aspects of absorption, metabolism, distribution and excretion, processes also impacted by the gut microbiome and other potentially highly variable and individual modulators of nutrient levels in the human body, should ideally be taken into account.

While all of this is well known in the nutrition expert community ***Gibney et al.*** (***2020***), the impact on both the interpretation of research findings and the development of dietary guidance and advice has been largely neglected, and there are only limited data exploring the impact on research outcomes ***Kipnis et al.*** (***2002***). It seems to be tenable that these limitations are a key contributor to the inconsistent and often contradictory outcomes of nutrition research and dietary guidance, which have received a high level of public attention and significant criticism in recent years ***Ioannidis*** (***2018***).

The EPIC Norfolk study (n=25,618, data available for 18,684 ***Day et al.*** (***1999***)) is ideally suited to investigate the impact of the variability in bioactive content on nutritional research because it has detailed dietary data based on the combination of self-reporting and food-composition data, nutritional biomarkers, as well as health endpoints, collected at the same time. Bioactives are food constituents that are not considered essential to human life but can affect health and are therefore extensively investigated ***Ottaviani et al.*** (***2022***). We used three dietary bioactives as model compounds, including flavan-3-ols, (–)-epicatechin and nitrate (Table 1) as: i) there are widely-used food composition data tables used to estimate their dietary intake (Figure 1); ii) there exist suitable nutritional biomarkers, which can provide accurate information on actual intake ***Kaaks et al.*** (***1997***) and iii) there are data from dietary intervention studies that support associations between intake and health outcomes ***Larsen et al.*** (***2006***); ***Ottaviani et al.*** (***2018b***) (Table 1). For the purposes of this investigation, we determined bioactive intakes in a single cohort using data and samples collected at the same time. We used two different methods: the commonly deployed approached based on combing self-reported dietary intake with data from food composition tables (DD-FCT) as well as a method based on measuring nutritional biomarkers in urine samples (biomarker method). In the context of the first approach, we also considered taking into consideration nutrient content variability data provided by current food content tables. This was achieved by not only using single point estimates (mean values) as is common practice, but also by considering reported content ranges ***Blekkenhorst et al.*** (***2017***); ***Rothwell et al.*** (***2013***), using a probabilistic-type modelling approach. While our study focuses on bioactives, it is likely that the results will also apply to nutrients and other food constituents with high variability such as minerals, where more than two fold-variabilities were previously observed ***Wilkinson and Perring*** (***1961***), and other nutrients including macronutrients such as fatty acids ***Reig et al.*** (***2013***); ***Schwendel et al.*** (***2015***). The findings of our study aim to test whether current approaches most often relying on the standardised, single point food content estimates obtained from food composition data can provide useful estimates

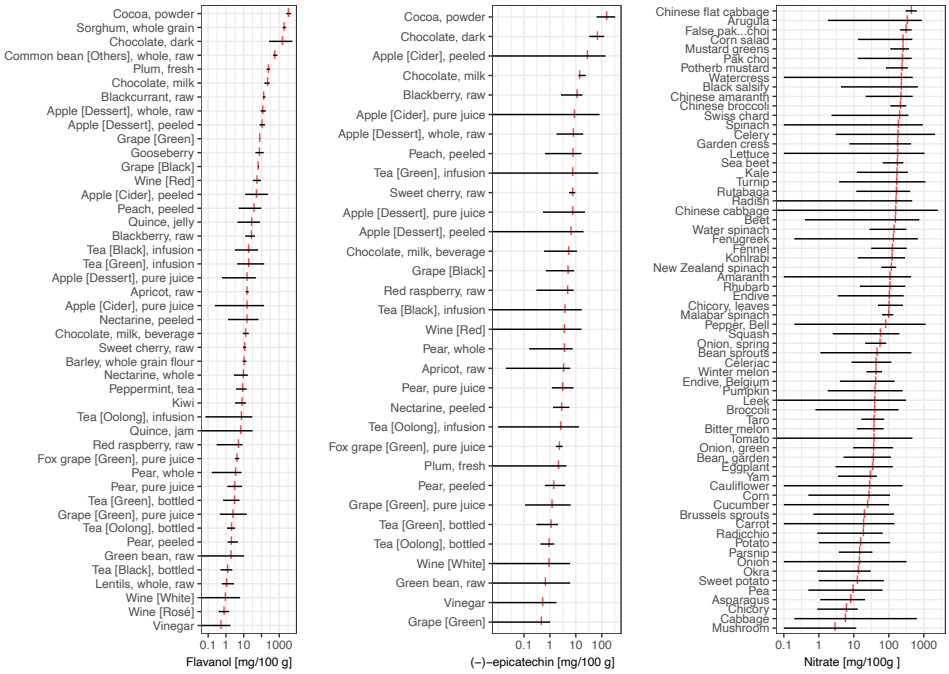

Figure 1. Variability in flavan-3-ol, (–)-epicatechin ***Rothwell et al.*** (***2013***) and nitrate ***Blekkenhorst et al.*** (***2017***) content of foods commonly eaten. Data show range of food content (black) and mean (red).

of actual dietary intake and allow the investigation and meaningful interpretation of associations with health.

Table 1. Characteristics of dietary bioactives used as model system of dietary compounds to investigate the limitation of using single-point estimates of to assess intake and investigate health outcomes in nutrition research.

Dietary compound	Dietary distribution	Factors for variability	Biomarker of intake	Potential health effect
Flavan-3-ols	tea, apple and cocoa-derived products	cultivar, agricultural conditions, storage and processing	Urinary concentrations of gut microbiome-derived flavan-3-ol metabolites (phenyl-γ-valerolactone metabolites) ***Ottaviani et al.*** (***2018a***)	Reduce cardiovascular events and deaths ***Sesso et al.*** (***2022***). Reduce blood pressure ***Ottaviani et al.*** (***2018b***). Improve cognitive performance ***Sloan et al.*** (***2021***)
(–)-epicatechin	tea, apple and cocoa-derived products	cultivar, agricultural conditions, storage and processing (including epimerization)	Urinary concentrations of structural related metabolites derived from phase II conjugation ***Ottaviani et al.*** (***2019***)	Improve vascular function ***Schroeter et al.*** (***2006***) ***Dicks et al.*** (***2022***) and reduce blood pressure ***Ottaviani et al.*** (***2018b***)
Nitrate	vegetables, drinking water	depends on a wide range of environmental factors such as fertilisation, light exposure and water supply	Urinary nitrate status can be used as a surrogate marker of intake ***Green et al.*** (***1981***); ***Pannala et al.*** (***2003***); ***Smallwood et al.*** (***2017***)	Dietary nitrate can reduce blood pressure ***Larsen et al.*** (***2006***)

Table 2. Study population and baseline-characteristics of 18,684 participants of EPIC Norfolk, for whom all data were available. Data shown are mean (SD) or absolute number and proportion. Data for urinary nitrate was available for 1027 samples.

	Women	Men
n	10167	8517
Age [years]	59 (9)	59 (9)
BMI [kg/m^2]	26.1 (4.2)	26.4 (3)
Systolic BP [mmHg]	134 (19)	138 (17.6)
Physical activity		
Inactive	2997 (30%)	2577 (30%)
Moderately Inactive	3258 (32%)	2096 (25%)
Moderately Active	2309 (23%)	1990 (23%)
Active	1603 (16%)	1854 (22%)
Smoking status		
Current	1121 (11%)	998 (12%)
Former	3250 (32%)	4647 (55%)
Never	5796 (57%)	2872 (34%)

Table 3. Intake of different bioactive compounds in EPIC Norfolk (median and IQR) when determined using different food composition data. Results are shown for estimates calculated using minimum, mean and maximum food content. and self-reported dietary data based on 24h diet recall (24HDR).

	Bioactive intake [mg/d]		
	Minimum food content	Mean food content	Maximum food content
Flavan-3-ols	48 (28 — 82)	120 (70 – 190)	329 (172 – 451)
(–)-epicatechin	1.5 (1.0 — 2.5)	19 (9 – 25)	33 (65 – 100)
Nitrate	5.5 (4.6 — 57)	100 (80 – 124)	204 (151 – 305)

Results

Impact of bioactive content variability when assessing dietary intake

The intake of an individual nutrient or bioactive is usually calculated by using self-reported dietary data and the mean food content as single point estimate. While the high variability in food composition is well known and recognised as a source of bias ***National Research Council; Coordinating Committee on Evaluation of Food Consumption Surveys; Subcommittee on Criteria for Dietary, Evaluation and ProQuest*** (***1986***), this is rarely acknowledged in such estimates and often assumed to have only little impact due to a regression to the mean. However, there is a paucity of data investigating the actual impact of this variability on estimated intakes. We have estimated the potential impact of the variability in flavan-3-ols, (–)-epicatechin, and nitrate food content on estimated intakes of the respective compounds and compound classes in 18,684 participants of EPIC-Norfolk for whom all relevant data were available (Table 2). Table 3 shows a comparison of estimated intakes when calculated using the DD-FCT approach with mean food content, as is current practice, as well as minimum and maximum reported food content. These results demonstrate a large uncertainty for estimating actual intake when taking the large variability in bioactive content into consideration. In comparison to the uncertainty introduced by the variability in food composition, the uncertainty associated with the use of self-reported methods of 2% to 25% ***Stubbs et al.*** (***2014***) appears to be small. There is an overlap in the possible range of bioactive intake between study

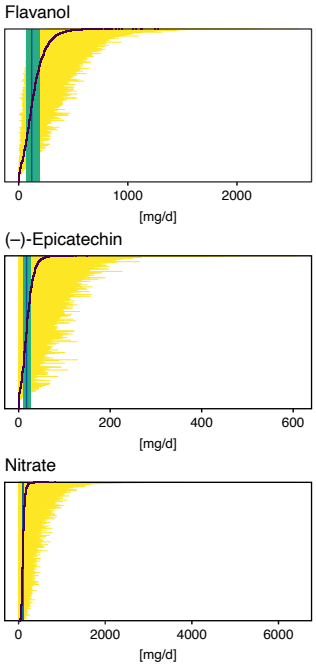

Figure 2. Possible intake ranges of flavan-3-ols, (–)-epicatechin and nitrate in each individual study participant displayed from low to high possible bioactive intake level. Range of bioactive intake was calculated using an approach similar to probabilistic modelling by sampling randomly from the distribution of possible food composition (n=10,000 iterations). Intake based on mean bioactive content, as is common practice, is indicated by a black line. Green line shows median intake of the entire cohort and the green box the inter-quartile range.

participants (Figure 2), making it difficult to identify low and high consumers or to rank participants by intake (see also below). These results show that bioactive content variability significantly contributes to the uncertainty in the estimation of dietary intake, even more than the error incurred by self-report methods that have attracted a lot of attention and discussion in nutritional research ***Subar et al.*** (***2015***).

Impact of food composition variability when assessing relative intake

In many studies relative instead of absolute intakes, for example quintiles, are used ***Altman and Bland*** (***1994***). It is assumed that relative intake is less affected by measurement error than absolute intake, and thus can mitigate some of the limitations of estimating dietary intake ***Streppel et al.*** (***2013***). We have therefore investigated how the ranking of participants is affected by the variability in bioactive content and compared the relative intake of participants with low (p25 – based on mean bioactive content), medium (p50) and high (p75) intake. Bioactive content variability was introduced in the analysis using an approach similar to probabilistic modelling by sampling randomly from the distribution of possible food composition for each food consumed by each participant. Figure 3 shows the result of 10,000 of such simulations. They suggest that the high variability in bioactive content makes estimates of relative intakes unreliable. Indeed, depending on the actual food consumed, the self-same diet could put the self-same study participant in the bottom or top quintile of intake. This suggest that it is difficult to obtain reliable relative intakes from dietary data alone, and that ranking by those data is unreliable.

In order to confirm the findings of our simulations, we have compared relative intakes estimated using data from DD-FCT and biomarker method. The biomarkers used in this study ***Green et al.*** (***1981***); ***Ottaviani et al.*** (***2018a***, 2019); ***Pannala et al.*** (***2003***); ***Smallwood et al.*** (***2017***) have been validated and characterised previously (Table 1) and are suitable to estimate relative intake

Keogh et al. (***2013***). Like the 24h dietary recall data used here, biomarkers reflect acute intake. Intake estimated from DD-FCT method was calculated using the common approach based on mean bioactive content in databases. The association between this self-reported intake and biomarker is weak with a maximum Kendall's τ of 0.16 for (–)-epicatechin and lower for flavan-3-ols (0.06) and nitrate (-0.05). Figure 4 illustrates this by comparing respective quantiles of intake, as these are commonly used to categorise relative intake. The data show very modest agreement between the two measurement methods (only 20% to 30% of participants assigned to the same quantile) and confirm that ranking is not suitable to address the measurement error and uncertainty introduced by the high variability in bioactive content. Overall, this shows that relying on a single value of bioactive content in food for all participants introduces bias when assessing relative intake of dietary compounds.

Impact of bioactive content variability on estimated association between intake and health endpoints

We have shown above that the high variability in food composition has an impact on estimates of intake using the DD-FCT method. However, it is not known whether this affects estimated associations between intake and health endpoints. Here, we use simulations to explore how the variability in food compositions affects such estimates in a "vibration of effects" type approach ***Patel et al.*** (***2015***), and compare these with results derived from biomarker estimated intakes. We use the cross-sectional association with blood pressure as example, as all three compounds have a well-established acute effect on vascular function ***Larsen et al.*** (***2006***); ***Ottaviani et al.*** (***2018b***); ***Schroeter et al.*** (***2006***).

Figure 5 shows the high variability in estimated associations for all three bioactives under investigation. Each estimate shown is based on identical dietary data and thus represents a possible true association between bioactive intake and blood pressure, depending on actual bioactive content. It is noticeable that we observe a Janus-effect with DD-FCT method estimated associations being in opposing positions. This is very noticeable for nitrate, where estimated differences in blood pressure range from -1.0 (95% CI -1.6; -0.4) mmHg between bottom and top decile of intake, suggesting a potentially beneficial, to 0.8 (0.2; 1.4) mmHg, suggesting a potentially detrimental effect on health. As the actual food composition is unknown, it is not possible to obtain a reliable estimate of this association, or even to identify the likely direction of such an association. Using the mean bioactive content, as is common practice, does not resolve this challenge. Biomarker-derived data, while not deprived of limitations but certainly not affected by the factors that modulate variability in food content in DD-FCT method, show a strong and significant inverse association between intake and blood pressure, and this association would have been missed when relying exclusively on dietary data.

These results show that the variability in bioactive content can impact estimated associations between DD-FCT method intake assessments and health endpoints. It demonstrates that even when using the self-same food intake data, differences in bioactive content can result in diametrically opposite results. Considering that most studies investigating associations between the intake of bioactives and health do not take variability in food composition into account, it is likely that many reported associations are unreliable.

Discussion

In this study, we have investigated how the variability in food composition affects nutritional research. Our results, based on three bioactives, show that the variability in food composition represents a significant factor that needs to be taken into consideration. The use of single point estimates of food composition data represents a significant oversimplification that yields unreliable data as the actual intake can be considerably different from the estimated intake. This is often exacerbated by errors that arise from imputing data into food composition tables from analyses conducted in different countries or by changes in the formulation of foods from food manufacturers.

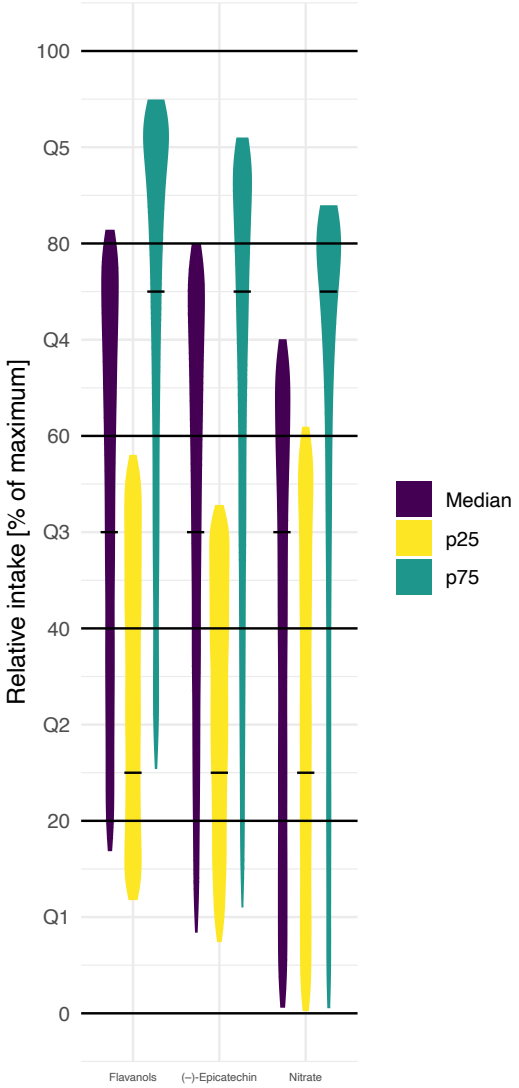

Figure 3. Simulation of the effect of variability in food composition on relative intakes of flavanols, (–)-epicatechin and nitrate of EPIC Norfolk participants with low (25th centile, p25), medium (median) and high (75th centile, p75) estimated intake of bioactive (based on 24HDR and mean food content – indicated by black line). Data shown are relative intake (100% is maximum intake) of 10,000 simulations.

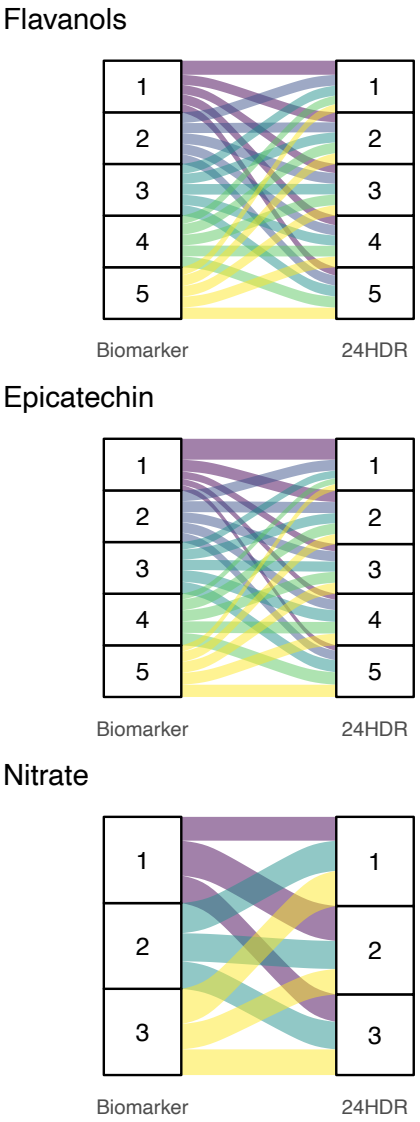

Figure 4. Alluvial plots comparing quantiles of bioactive intake estimated with biomarkers (left) and 24h dietary recall with food-composition data (24HDR – DD-FCT method, right). Bands of the same colour show participants in the same biomarker-estimated quantile of intake and their respective quantile of intake based on the DD-FCT method. 24h dietary recall estimated quantiles of intake were determined using the common approach of using mean content of flavan-3-ols, (–)-epicatechin and nitrate in each food item as reported in databases.

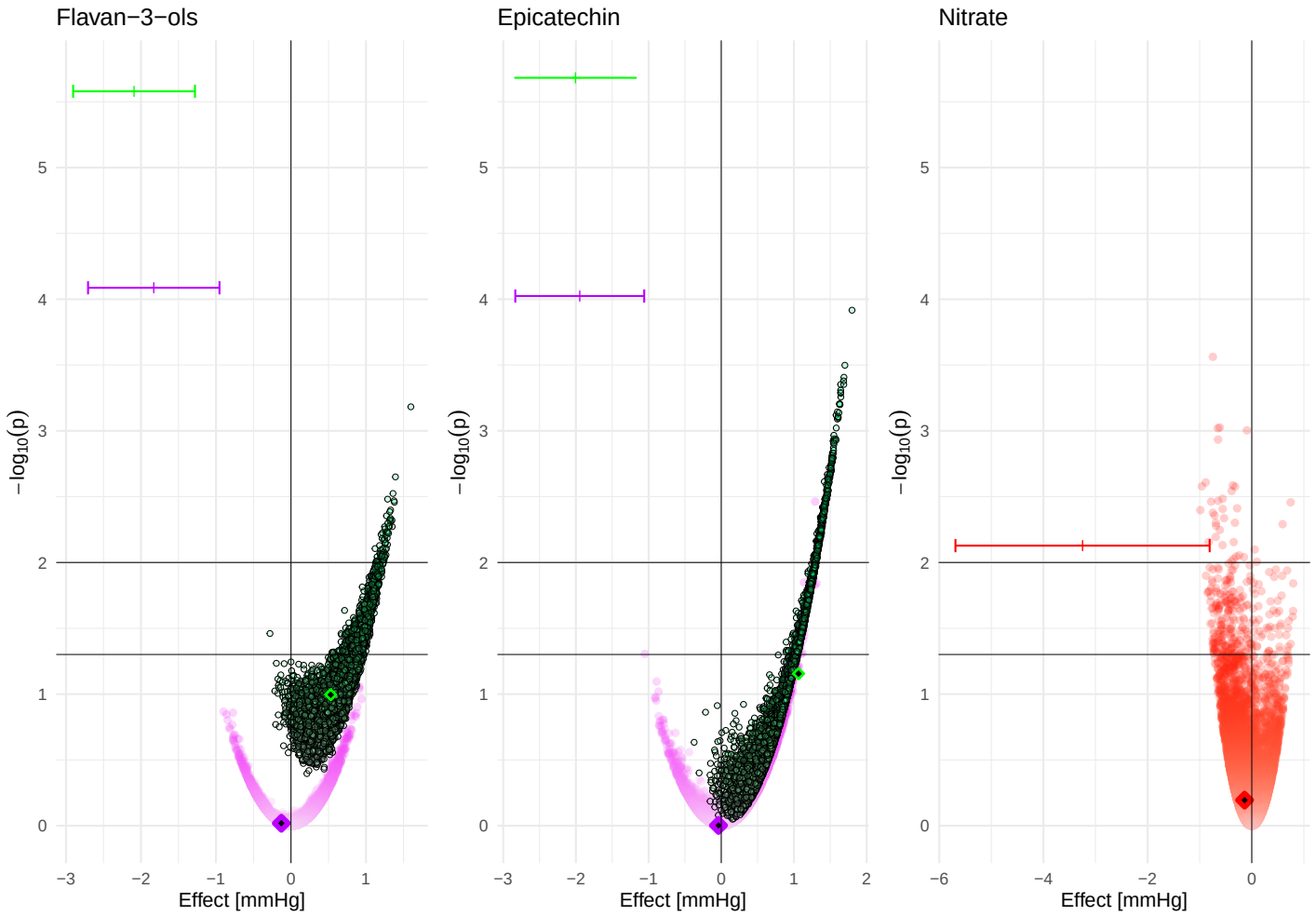

Figure 5. Association between estimated bioactive intake (flavan-3-ols, (–)-epicatechin and nitrate, based on 24h dietary recall and food composition data [DD-FCT method]) and systolic blood pressure at baseline (estimated difference between low [p10] and high [p90] intake and p-value for Wald-test (as -log10(p)) in men (purple), women (green) and all participants (red). Data are based on 10,000 simulations and adjusted for age, BMI, plasma vitamin C, smoking status, physical activity and self-reported health at baseline; additionally for menopausal status for women and sex for nitrate. Results based on intake estimated by simulating food content within minimum and maximum food content reported in databases (circle), intake based on mean food content as reported in databases (diamond) and intake based on biomarker data (|). 95% CI is shown for biomarker only.

These findings are not only important for observational studies, but also for dietary intervention studies, where such methods are often used to estimate background dietary intake and the trial designs of a given intervention. Our results show that the variability in food composition makes reliable estimates of both, absolute and relative intake of bioactives challenging and potentially highly unreliable when solely relying on a combination of self-reported dietary data and food composition databases. This significant limitation is further applied by the bias introduced through limitations of dietary assessment itself (e.g. reporting bias). Thus, any associations between intake and health outcomes derived from using many current approaches are unreliable. Indeed, for the three food constituents under investigation, we found a Janus-like effect with negative and positive associations using the self-same food consumption data and food content within the reported range. This might help explain why nutritional advise given to the general public can feel inconstant, and even contradictory, at times, especially when observing the evolution of advisory statements on the same foods, food groups, or nutrients over time.

Validated nutritional biomarkers, especially recovery biomarkers ***Kaaks et al.*** (***1997***), can provide a reliable estimate of nutrient and bioactive intake as they are based on their systemic presence and do not rely on assumptions about food composition data. In contrast to the duplicate diet method, which relies on the full analysis of all foods consumed, biomarkers provide better information to investigate associations between intake and health biomarkers as they reflect not just consumption but also nutrient-nutrient interactions and bioavailability, which can affect the systemic presence of many bioactives ***Ottaviani et al.*** (***2023***).

The application of biomarkers to assess nutrient intakes is not without limitations, however, these limitations can be addressed today. Doing so is often of greater technical feasibility and tenably delivers greater overall improvements than to address limitations of current non-biomarker based approaches, including self-report bias and the imprecisions and other limitations of today's food composition databases. This is due to the fact that even successfully mitigating limitations related to reporting bias and food composition analyses, does not address inherent shortfalls of non-biomarker based methods. These include the unknown impact of pre- and post-prandial nutrient-nutrient interactions, inter-subject variations in absorption and metabolism, and the often unknown effects of food processing, preparation, and storage on nutrient composition of foods, which can be addressed through use of biomarkers.

An important challenge when developing biomarker-based methods for assessing intake is related to the inter-subject variance in the absorption and metabolism of a specific nutrient or bioactive. It is therefore important to establish a physiological link as well as a strong statistical association between intake and biomarker, such as has been done for the biomarkers used in this study ***Ottaviani et al.*** (***2018a***, 2019); ***Pannala et al.*** (***2003***). Biomarkers need to be evaluated using data of actual bioactive intake and should not rely on published food composition data due to the limitations described above. Except for recovery biomarkers in 24h urine, most biomarkers are used to provide relative intake data in order to rank participants according to intake. Our results show however that biomarker-based ranking of intake is much more reliable than rankings based on methods relying on self-reported data and food composition databases.

High variability in food composition has been described for a range of compounds, e.g. for the fatty acid composition of dairy ***Moate et al.*** (***2007***); ***Stergiadis et al.*** (***2019***) or vitamins ***Phillips et al.*** (***2018***). There is also a longitudinal variation in food composition, in particular due to changes to cultivars, production practices, distribution and processing methods ***Davis et al.*** (***2004***), and climate change is likely to exaggerate this ***Macdiarmid and Whybrow*** (***2019***). Thus, bioactive and nutrient content variability must be taken into consideration when choosing the tools to investigate not only dietary bioactives but also micro- and macro-nutrients.

Methods commonly used to address measurement error in nutritional research, such as regression calibration ***Spiegelman et al.*** (***1997***), are not suitable to address the limitations introduced by the high variabilty. These methods rely on a known relationship between reported and actual intake in a calibration study to predict actual intake in a larger cohort. However, the composition

of the food actually consumed by participants is impossible to predict as it depends on a range of factors, many of which are unknown to consumers and researchers as outlined in the introduction.

There are of course also other sources of bias and variability that affect dietary assessment. We have excluded those from our study as much as possible by using the self-same dietary data for all analyses, using only acute intake data (24h dietary recall and spot urine samples) and an endpoint that is affected directly by intake. This allows us to attribute our findings mainly to the variability in food composition.

In our study, we have used the identical dietary data to investigate the impact of the variability in food content. This has allowed us to exclude other sources of variability in dietary assessment, in particular misreporting of dietary intake. We have also used measures of acute intake (24h dietary recalls and spot urine samples) and used a health endpoint that is directly affected by intake.

Prospective studies

In our study, we have focused on cross-sectional associations between bioactives and blood pressure as the acute effect of these compounds is well established. It is expected that the variability in food composition affects prospective analyses more than cross-sectional analyses: in addition to the variability in food content, the composition of foods changes over time ***Davis et al.*** (***2004***); ***White and Broadley*** (***2005***).

Biomarker-predicted dietary patterns

The high variability in the content of dietary compounds in food has also implications for the development of biomarkers for individual foods or dietary patterns. A number of biomarkers have been proposed to estimate intake of individual foods, for example proline-betaine as biomarker of citrus fruit intake ***Gibbons et al.*** (***2017***), but content in citrus fruits is highly variable (14.3 — 110 mg/100 mL in various citrus fruit juice ***Lang et al.*** (***2017***)) and it is thus not possible to estimate actual food intake without using foods in which the content of the dietary compound to use as a biomarker is standardised.

The same applies to metabolomics-based biomarkers of dietary patterns. They are usually developed under highly standardised conditions and reflect the composition of the foods consumed during these studies. Changes in the composition of these foods affect the concentration of metabolites and thereby reduces the reliability of metabolite-based biomarkers of individual foods or dietary pattern. This diminishes the suitability of such markers for longitudinal or multi-centre studies where a high variability in food composition is likely. These limitations do not apply for the development of biomarkers of specific bioactives or other nutrients as the variability of bioactive and nutrient content are reflected in the variation of biomarker levels.

Effect on dietary recommendations and risk assessment

The findings presented in this work have a considerable impact on dietary recommendations and guidelines. Our data clearly show that results based on DD-FCT method are likely to be biased and unreliable. Dietary recommendation based on such data emanated from that approach are therefore also likely to be unreliable and misleading. However, the high variability in food composition also has an impact on the translation of health-based guidance values into food-based dietary recommendations. For example, the amount of flavan-3-ols required to achieve a vasculoprotective effect according to the EFSA health claim is 200 mg/d ***EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA)*** (***2014***). When using mean food composition data ***Rothwell et al.*** (***2013***), this could be achieved by 5 cups of tea. However, when using the lowest reported food content, at least 22 cups of tea would have to be consumed to meet recommended intake. Similarly, 5–6 apples would be sufficient to consume the 50 mg/d (–)-epicatechin assumed to be sufficient to improve vascular function ***Ellinger et al.*** (***2012***); ***Hooper et al.*** (***2012***) when using mean food content, but it could be up to 27 when assuming a low content in food. In this manner, it would not be possible to determine whether or not a population is already meeting dietary recommendation for

flavan-3-ols without the development of biomarker-based methods ***Crowe-White et al.*** (***2022***).

These findings also have an impact on the risk assessment of food components, in particular those that are naturally present in foods and used as additives such as nitrates ***EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al.*** (***2017***) or phosphates ***EFSA Panel on Food Additives and Flavourings (FAF) et al.*** (***2019***). Results from observational studies and intervention studies relying on food content data will be affected by inaccurate assessment of intake as described above. More importantly however, the exposure assessment will be affected by the variability of data, with consequences for consumers and food producers as an overestimation of exposure could result in unnecessary restrictions in use, whereas an underestimation could put consumers at risk. For example in EPIC Norfolk, none or only very few study participants exceed the ADI of 3.7 mg/kg BW/d ***EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al.*** (***2017***) for nitrate when estimating intake with minimum and mean food content respectively. However, when using the maximum food content, one third of study participants exceed the ADI for nitrate, and almost 10% exceed it by 2-fold. Each of these scenarios would result in very different actions by risk managers due to the different impact on population health and in the latter case more stringent restrictions were necessary.

Conclusions

Our data suggest that the results of many interventional and observational nutrition studies using dietary surveys in combination with food composition data are potentially unreliable and carry greater limitations than commonly appreciated. As these studies are used to derive evidence-based dietary recommendations and disease risk assessments, their limitations could have a considerable impact on public health. We demonstrated that results relying solely on food composition data not only failed to identify beneficial associations between three bioactives and blood pressure, but even suggested possible adverse associations. It is highly likely that findings of this nature are not limited to the model compounds that served as examples in our investigation here but broadly apply to other dietary components as well. Given the importance of diet in the maintenance of health and disease risk reduction it is crucial to address this limitation: both by revisiting previous studies and by taking these limitations into consideration in future studies. We think it essential to develop and use nutritional biomarkers to determine actual nutrient intakes that ensure more reliable and actionable insights. This means that the development of more and better biomarkers for accurate dietary assessment remains crucial ***Prentice*** (***2018***). The challenges associated with developing biomarker-based approaches are not insignificant, but the technical capabilities required are broadly available today, and the advantages of deploying improved approaches to establishing links between diet and health are so significant, timely, and needed, that it should become a standard tool in nutrition research.

Methods

Study population

Between 1993 and 1997, 30,447 women and men aged between 40 and 79 years were recruited for the Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, and 25,639 attended a health examination ***Day et al.*** (***1999***). Health and lifestyle characteristics, including data on smoking, social class and family medical history, were assessed by questionnaire. Height and weight measurements were collected following a standardised protocol by trained research nurses. Physical activity, representing occupational and leisure activity, was assessed using a validated questionnaire ***Wareham et al.*** (***2002***). Blood pressure was measured by using a non-invasive oscillometric blood pressure monitor (Acutorr; Datascope Medical, Huntingdon, UK; validated against sphygmomanometers every 6 months) after the participant had been seated in a comfortable environment for 5 minutes. The arm was horizontal and supported at the level of the mid-sternum; the mean of two readings was used for analysis. Non-fasting blood

samples were taken by venepuncture and stored in serum tubes in liquid nitrogen. Serum levels of total cholesterol were measured on fresh samples with the RA 1000 autoanalyzer (Bayer Diagnostics, Basingstoke, UK). Plasma vitamin C was measured using a fluorometric assay as described previously ***Khaw et al.*** (***2001***). Spot urine samples were collected during the health examination and stored at -20°C until analysis. The study was approved by the Norwich Local Research Ethics Committee and all participants gave written, informed consent and all methods were carried out in accordance with relevant guidelines and regulations.

Diet was assessed by 7-day diary (7DD), whereby the first day of the diary was completed as a 24-h recall (24HDR) with a trained interviewer and the remainder completed during subsequent days. Diary data were entered, checked and calculated using the in-house dietary assessment software DINER (Data into Nutrients for Epidemiological Research) and DINERMO ***Welch et al.*** (***2001***). Flavan-3-ol intake (the sum of epicatechin, catechin, epicatechin-3-O-gallate, catechin-3-O-gallate and proanthocyanidins) was estimated as described previously ***Vogiatzoglou et al.*** (***2015***); minimum and maximum estimated flavan-3-ol intake was estimated using the minimum and maximum food content data provided by Phenol Explorer und USDA databases ***Rothwell et al.*** (***2013***). Nitrite and nitrate intake, based on minimum, maximum and mean food content, were estimated using a database published previously ***Blekkenhorst et al.*** (***2017***).

Nutritional biomarker

Flavan-3-ols and (–)-epicatechin

We have used two different biomarkers to estimate flavan-3-ol and (–)-epicatechin intake: $gVLM_B$ that includes the metabolites 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-O-glucuronide (gVL3G) and 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-sulphate (gVL3S), and $SREM_B$ that includes the metabolites (–)-epicatechin-3′-glucuronide (E3G), (–)-epicatechin-3′-sulfate (E3S) and 3′-methoxy(–)-epicatechin-5-sulfate (3Me5S). $gVLM_B$ are specific for estimating the intake of flavan-3-ols in general, including (±)-epicatechin, (±)-catechin, (±)-epicatechin-3-O-gallate, (±)- catechin-3-O-gallate and procyanidins and excluding the flavan-3-ols gallocatechin, epigallocatechin, gallocatechin-3-O-gallate, epigallocatechin-3-O-gallate, theaflavins and thearubigins ***Ottaviani et al.*** (***2018a***). $SREM_B$ are specific for (–)-epicatechin intake ***Ottaviani et al.*** (***2019***). Spot urine samples were collected during the baseline health examination and stored in glass bottles at -20°C until analysis. Stability analyses confirmed that biomarkers are stable under these conditions ***Ottaviani et al.*** (***2019***). Samples were analysed in random order using the method described previously ***Ottaviani et al.*** (***2018a***, 2019), with automated sample preparation (Hamilton Star robot; Hamilton, Bonaduz, Switzerland). Concentrations below the lower limit of quantification (LLOQ, 0.1 μM) were used for the analysis to avoid the bias of substituting a range of values by a single value. Concentrations were adjusted by specific gravity for dilution as the endpoint of the analysis, systolic blood pressure, was strongly correlated with urinary creatinine. We have used specific gravity to adjust for dilution previously when there was a strong association between creatinine and study endpoint ***Bingham et al.*** (***2007***).

Flavan-3-ol and (–)-epicatechin biomarker data, as well as data for all other variables were available for 18,864 participants. Data for nitrate biomarker were available for 1,027 participants.

Nitrate

Urinary nitrate concentration, adjusted for dilution by specific gravity, was used as biomarker of nitrate intake, as between 50% and 80% of dietary nitrate are recovered in urine, whereas endogenous production is relatively stable at 0.57 (95% CI 0.27 – 0.86) mmol/d ***Green et al.*** (***1981***); ***Packer et al.*** (***1989***). A random subset of 1,027 samples were analysed by ion chromatography with colorimetric detection (NOx Analyser ENO-30, EICOM, San Diego, CA).

Simulation of variability

We have conducted 10,000 simulations to explore the impact of the variability in bioactive content. For each simulation, we assigned each participant a possible intake of total flavan-3-ol, (–

)-epicatechin and nitrate based on their self-reported dietary intake and the minimum and maximum reported content of each compound in the foods consumed. The data available do not suggest that food composition follows a normal distribution, and we have therefore assumed a uniform distribution.

Data analysis

Data analyses were carried out using R 3.6 ***R Core Team*** (***2023***), using the packages rms ***Harrell Jr*** (***2023***) for regression analyses, ggplot2 ***Wickham*** (***2016***) and gridExtra ***Auguie*** (***2017***) for the generation of graphics. Regression analyses were conducted using `ols` as regression function. We have used the Wald statistics calculated by the *rms* `anova` function to investigate the relationship between dependent and independent variables, and to test for linearity. The *tableone* package ***Yoshida and Bartel*** (***2022***) was used to prepare tables. Unless indicated otherwise, results are shown with 95% confidence intervals.

Descriptive statistics

Descriptive characteristics of the study population were summarised using mean (standard deviation) for continuous variables and frequency (percentage) for categorical variables.

Data transformation

Biomarker data were positively skewed (log-normal distribution) and therefore log2-transformed data were used for all analyses. Restricted cubic splines (3 knots, outer quantiles 0.1 and 0.9; using the rcs function ***Harrell Jr*** (***2023***)) were used for all continuous variables unless indicated otherwise.

Cross-sectional analyses

In cross-sectional analyses, stratified by sex, we investigated associations between biomarker and 24h recall estimated flavan-3-ol, (–)-epicatechin and nitrate intake (biomarkers adjusted by specific gravity adjusted, dietary data by energy, log2-transformed), as independent variable and systolic and diastolic blood pressure [mmHg] using multiple regression analyses. Analyses were adjusted by age (continuous; years), BMI (continuous, kg/m^2), plasma vitamin C, smoking status (categorical; never, ever, former), physical activity (categorical; inactive, moderately inactive, moderately active, active) and health at baseline (self-reported diabetes mellitus, myocardial infarction, cerebrovascular accident). Analyses with flavan-3-ol and (–)-epicatechin as independent variable were stratified by sex, and analyses for women additionally adjusted by menopausal status; analyses with nitrate as independent variable were adjusted by sex and menopausal status.

Availability of data and code

Data from the EPIC-Norfolk study must be requested directly from their data request team by completing a data request form. Code will be available on request from the authors.

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Declaration of competing interest

HS and JIO are employed by Mars, Inc. a company engaged in flavanol research and flavanol-related commercial activities. GGCK has received unrestricted research grants from Mars, Inc.

References

Altman DG, Bland JM. Quartiles, quintiles, centiles, and other quantiles. BMJ. 1994; 309(6960):996. doi: 10.1136/bmj.309.6960.996.

Auguie B. gridExtra: Miscellaneous Functions for "Grid" Graphics; 2017.

Bingham SA, Luben R, Welch AA, Tasevska N, Wareham NJ, Khaw KT. Epidemiologic assessment of sugars consumption using biomarkers: comparisons of obese and nonobese individuals in the European prospective investigation of cancer Norfolk. Cancer Epidemiology, Biomarkers & Prevention. 2007; 16(8):1651–1654. doi: 10.1158/1055-9965.EPI-06-1050.

Blekkenhorst LC, Prince RL, Ward NC, Croft KD, Lewis JR, Devine A, Shinde S, Woodman RJ, Hodgson JM, Bondonno CP. Development of a reference database for assessing dietary nitrate in vegetables. Molecular Nutrition and Food Research. 2017; 61(8):1600982. doi: 10.1002/mnfr.201600982.

Crowe-White KM, Evans LW, Kuhnle GGC, Milenkovic D, Stote K, Wallace T, Handu D, Senkus KE. Flavan-3-ols and Cardiometabolic Health: a Guideline Recommendation by the Academy of Nutrition and Dietetics. Advances in Nutrition. 2022; 13(6):2070–2083. doi: 10.1093/advances/nmac105.

Davis DR, Epp MD, Riordan HD. Changes in USDA food composition data for 43 garden crops, 1950 to 1999. Journal of the American College of Nutrition. 2004; 23(6):669–682. doi: 10.1080/07315724.2004.10719409.

Day N, Oakes S, Luben R, Khaw KT, Bingham SA, Welch AA, Wareham NJ. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. British Journal of Cancer. 1999; 80 Suppl 1(6):95–103. doi: 10.2337/diacare.23.6.726.

Dicks L, Haddad Z, Deisling S, Ellinger S. Effect of an (-)-Epicatechin Intake on Cardiometabolic Parameters-A Systematic Review of Randomized Controlled Trials. Nutrients. 2022; 14(21). doi: 10.3390/nu14214500.

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). Scientific Opinion on the modification of the authorisation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006 following a request in accordance with Article 19 of Regulation (EC) No 1924/2006. EFSA Journal. 2014; 10:2809. doi: 10.2903/j.efsa.2014.3654.

EFSA Panel on Food Additives and Flavourings (FAF), Younes M, Aquilina G, Castle L, Engel KH, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Mennes W, Moldeus P, Oskarsson A, Shah R, Waalkens Berendsen I, Wölfle D, Aggett P, Cupisti A, Fortes C, Kuhnle GG, et al. Re-evaluation of phosphoric acid–phosphates – di-, tri- and polyphosphates (E 338–341, E 343, E 450–452) as food additives and the safety of proposed extension of use. EFSA Journal. 2019; 17(6):920. doi: 10.2903/j.efsa.2019.5674.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambré C, Leblanc JC, Lindtner O, Moldeus P, Mosesso P, Oskarsson A, Parent Massin D, Stankovic I, Waalkens Berendsen I, Woutersen RA, et al. Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. EFSA Journal. 2017; 15(6):689. doi: 10.2903/j.efsa.2017.4787.

Ellinger S, Reusch A, Stehle P, Helfrich HP. Epicatechin ingested via cocoa products reduces blood pressure in humans: a nonlinear regression model with a Bayesian approach. American Journal of Clinical Nutrition. 2012; 95(6):1365–1377. doi: 10.3945/ajcn.111.029330.

Gibbons H, Michielsen CJR, Rundle M, Frost G, McNulty BA, Nugent AP, Walton J, Flynn A, Gibney MJ, Brennan L. Demonstration of the utility of biomarkers for dietary intake assessment; proline betaine as an example. Molecular Nutrition and Food Research. 2017; 61(10). doi: 10.1002/mnfr.201700037.

Gibney M, Allison D, Bier D, Dwyer J. Uncertainty in human nutrition research. Nature Food. 2020; 1:247–249. doi: 10.1038/s43016-020-0073-2.

Global Burden of Disease 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2019; 393(10184):1958–1972. doi: 10.1016/S0140-6736(19)30041-8.

Green LC, Ruiz de Luzuriaga K, Wagner DA, Rand W, Istfan N, Young VR, Tannenbaum SR. Nitrate biosynthesis in man. Proceedings of the National Academy of Sciences of the United States of America. 1981; 78(12):7764–7768.

Greenfield H, Southgate DAT. Food Composition Data. Food & Agriculture Organisation; 2003.

Harrell Jr FE. rms: Regression Modeling Strategies; 2023.

Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, Cassidy A. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. American Journal of Clinical Nutrition. 2012; 95(3):740–751. doi: 10.3945/ajcn.111.023457.

Ioannidis JPA. The Challenge of Reforming Nutritional Epidemiologic Research. JAMA: The Journal of the American Medical Association. 2018; 320(10):969–970. doi: 10.1001/jama.2018.11025.

Kaaks R, Riboli E, Sinha R. Biochemical markers of dietary intake. IARC Scientific Publication. 1997; (142):103–126.

Keogh RH, White IR, Bingham SA. Using surrogate biomarkers to improve measurement error models in nutritional epidemiology. Statistics in medicine. 2013; 32(22):3838–3861. doi: 10.1002/sim.5803.

Khaw KT, Bingham SA, Welch AA, Luben R, Wareham NJ, Oakes S, Day N. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. Lancet. 2001; 357(9257):657–663. doi: 10.1016/s0140-6736(00)04128-3.

Kipnis V, Midthune D, Freedman L, Bingham SA, Day NE, Riboli E, Ferrari P, Carroll RJ. Bias in dietary-report instruments and its implications for nutritional epidemiology. Public Health Nutrition. 2002; 5(6A):915–923. doi: 10.1079/PHN2002383.

Lang R, Lang T, Bader M, Beusch A, Schlagbauer V, Hofmann T. High-Throughput Quantitation of Proline Betaine in Foods and Suitability as a Valid Biomarker for Citrus Consumption. Journal of Agricultural and Food Chemistry. 2017; 65(8):1613–1619. doi: 10.1021/acs.jafc.6b05824, lang, Roman Lang, Tatjana Bader, Matthias Beusch, Anja Schlagbauer, Verena Hofmann, Thomas eng J Agric Food Chem. 2017 Mar 1;65(8):1613-1619. doi: 10.1021/acs.jafc.6b05824. Epub 2017 Feb 16.

Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. New England Journal of Medicine. 2006; 355(26):2792–2793. doi: 10.1056/NEJMc062800.

Macdiarmid JI, Whybrow S. Nutrition from a climate change perspective. Proceedings of the Nutrition Society. 2019; 78(3):380–387. doi: 10.1017/S0029665118002896.

Moate PJ, Chalupa W, Boston RC, Lean IJ. Milk fatty acids. I. Variation in the concentration of individual fatty acids in bovine milk. Journal of Dairy Science. 2007; 90(10):4730–4739. doi: 10.3168/jds.2007-0225.

National Research Council; Coordinating Committee on Evaluation of Food Consumption Surveys; Subcommittee on Criteria for Dietary, Evaluation and ProQuest. In: Modeling of Sources of Variability and Biases Washington, D.C: National Academy Press; 1986. .

Ottaviani JI, Ensunsa JL, Fong RY, Kimball J, Medici V, Kuhnle GGC, Crozier A, Schroeter H, Kwik-Uribe C. Impact of polyphenol oxidase on the bioavailability of flavan-3-ols in fruit smoothies: a controlled, single blinded, cross-over study. Food and Function. 2023; 14:8217–8228. doi: 10.1039/D3FO01599H.

Ottaviani JI, Fong R, Kimball J, Ensunsa JL, Britten A, Lucarelli D, Luben R, Grace PB, Mawson DH, Tym A, Wierzbicki A, Khaw KT, Schroeter H, Kuhnle GG. Evaluation at scale of microbiome-derived metabolites as biomarker of flavan-3-ol intake in epidemiological studies. Scientific Reports. 2018; 8(1):9859. doi: 10.1038/s41598-018-28333-w.

Ottaviani JI, Fong R, Kimball J, Ensunsa JL, Gray N, Vogiatzoglou A, Britten A, Lucarelli D, Luben R, Grace PB, Mawson DH, Tym A, Wierzbicki A, Smith AD, Wareham NJ, Forouhi NG, Khaw KT, Schroeter H, Kuhnle GGC. Evaluation of (–)-epicatechin metabolites as recovery biomarker of dietary flavan-3-ol intake. Scientific Reports. 2019; 9(1):13108–13110. doi: 10.1038/s41598-019-49702-z.

Ottaviani JI, Heiss C, Spencer JPE, Kelm M, Schroeter H. Recommending flavanols and procyanidins for cardiovascular health: Revisited. Molecular Aspects of Medicine. 2018; 61:63–75. doi: 10.1016/j.mam.2018.02.001.

Ottaviani JI, Schroeter H, Kuhnle GG. Measuring the intake of dietary bioactives: Pitfalls and how to avoid them. Molecular Aspects of Medicine. 2022; p. 101139. doi: 10.1016/j.mam.2022.101139.

Packer PJ, Leach SA, Duncan SN, Thompson MH, Hill MJ. The effect of different sources of nitrate exposure on urinary nitrate recovery in humans and its relevance to the methods of estimating nitrate exposure in epidemiological studies. Carcinogenesis. 1989; 10(11):1989–1996.

Pannala AS, Mani AR, Spencer JP, Skinner V, Bruckdorfer KR, Moore KP, Rice-Evans CA. The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. Free Radical Biology and Medicine. 2003; 34(5):576–84. doi: 10.1016/s0891-5849(02)01353-9.

Patel CJ, Burford B, Ioannidis JPA. Assessment of vibration of effects due to model specification can demonstrate the instability of observational associations. Journal of Clinica Epidemiology. 2015; 68(9):1046–1058. doi: 10.1016/j.jclinepi.2015.05.029.

Phillips KM, Tarrago-Trani MT, McGinty RC, Rasor AS, Haytowitz DB, Pehrsson PR. Seasonal variability of the vitamin C content of fresh fruits and vegetables in a local retail market. Journal of the Science of Food and Agriculture. 2018; 98(11):4191–4204. doi: 10.1002/jsfa.8941.

Prentice RL. Intake biomarkers and the chronic disease nutritional epidemiology research agenda. American Journal of Clinical Nutrition. 2018; 108(3):433–434. doi: 10.1093/ajcn/nqy206.

R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria; 2023.

Reig M, Aristoy MC, Toldra F. Variability in the contents of pork meat nutrients and how it may affect food composition databases. Food Chemistry. 2013; 140(3):478–82. doi: 10.1016/j.foodchem.2012.11.085, reig, Milagro Aristoy, M-Concepcion Toldra, Fidel eng Research Support, Non-U.S. Gov't England 2013/04/23 Food Chem. 2013 Oct 1;140(3):478-82. doi: 10.1016/j.foodchem.2012.11.085. Epub 2012 Nov 29.

Rothwell JA, Pérez-Jiménez J, Neveu V, Medina-Remón A, M'Hiri N, García-Lobato P, Manach C, Knox C, Eisner R, Wishart DS, Scalbert A. Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. Database. 2013; 2013:bat070–bat070. doi: 10.1093/database/bat070.

Schroeter H, Heiss C, Balzer J, Balzer J, Kleinbongard P, Kleinbongard P, Keen CL, Keen C, Hollenberg N, Hollenberg NK, Sies H, Sies H, Kwik-Uribe C, Kwik-Uribe C, Schmitz HH, Schmitz HH, Kelm M, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(4):1024–1029. doi: 10.1073/pnas.0510168103.

Schwendel BH, Wester TJ, Morel PC, Tavendale MH, Deadman C, Shadbolt NM, Otter DE. Invited review: organic and conventionally produced milk-an evaluation of factors influencing milk composition. J Dairy Sci. 2015; 98(2):721–46. doi: 10.3168/jds.2014-8389, schwendel, B H Wester, T J Morel, P C H Tavendale, M H Deadman, C Shadbolt, N M Otter, D E eng Research Support, Non-U.S. Gov't Review J Dairy Sci. 2015 Feb;98(2):721-46. doi: 10.3168/jds.2014-8389. Epub 2014 Dec 12.

Sesso HD, Manson JE, Aragaki AK, Rist PM, Johnson LG, Friedenberg G, Copeland T, Clar A, Mora S, Moorthy MV, Sarkissian A, Carrick WR, Anderson GL, Group CR. Effect of cocoa flavanol supplementation for the prevention of cardiovascular disease events: the COcoa Supplement and Multivitamin Outcomes Study (COSMOS) randomized clinical trial. American Journal of Clinical Nutrition. 2022; 115(6):1490–1500. doi: 10.1093/ajcn/nqac055.

Sloan RP, Wall M, Yeung LK, Feng T, Feng X, Provenzano F, Schroeter H, Lauriola V, Brickman AM, Small SA. Insights into the role of diet and dietary flavanols in cognitive aging: results of a randomized controlled trial. Scientific Reports. 2021; 11(1):3837. doi: 10.1038/s41598-021-83370-2.

Smallwood MJ, Ble A, Melzer D, Winyard PG, Benjamin N, Shore AC, Gilchrist M. Relationship Between Urinary Nitrate Excretion and Blood Pressure in the InChianti Cohort. American Journal of Hypertension. 2017; 30(7):707–712. doi: 10.1093/ajh/hpx035.

Spiegelman D, McDermott A, Rosner B. Regression calibration method for correcting measurement-error bias in nutritional epidemiology. American Journal of Epidemiology. 1997; 65(4 Suppl):1179S–1186S. doi: 10.1093/oxfordjournals.aje.a009089.

Stergiadis S, Berlitz CB, Hunt B, Garg S, Ian Givens D, Kliem KE. An update to the fatty acid profiles of bovine retail milk in the United Kingdom: Implications for nutrition in different age and gender groups. Food chemistry. 2019; 276:218–230. doi: 10.1016/j.foodchem.2018.09.165.

Streppel MT, de Vries JH, Meijboom S, Beekman M, de Craen AJ, Slagboom PE, Feskens EJ. Relative validity of the food frequency questionnaire used to assess dietary intake in the Leiden Longevity Study. Nutrition Journal. 2013; 12:75. doi: 10.1186/1475-2891-12-75.

Stubbs RJ, O'Reilly LM, Whybrow S, Fuller Z, Johnstone AM, Livingstone MBE, Ritz P, Horgan GW. Measuring the difference between actual and reported food intakes in the context of energy balance under laboratory conditions. British Journal of Nutrition. 2014; 111(11):2032–2043. doi: 10.1017/S0007114514000154.

Subar AF, Freedman LS, Tooze JA, Kirkpatrick SI, Boushey C, Neuhouser ML, Thompson FE, Potischman N, Guenther PM, Tarasuk V, Reedy J, Krebs-Smith SM. Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. Journal of Nutrition. 2015; 145(12):2639–2645. doi: 10.3945/jn.115.219634.

The National Academies of Sciences and Engineering and Medicine Health. Guiding Principles for Developing Dietary Reference Intakes Based on Chronic Disease Guiding Principles for Developing Dietary Reference Intakes Based on Chronic Disease; 2017. doi: 10.17226/24828.

Vogiatzoglou A, Mulligan AA, Bhaniani A, Lentjes M, McTaggart A, Luben RN, Heiss C, Kelm M, Merx MW, Spencer JPE, Schroeter H, Khaw KT, Kuhnle GG. Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). Free Radical Biology and Medicine. 2015; 84:1–10. doi: 10.1016/j.freeradbiomed.2015.03.005.

Wareham NJ, Jakes RW, Rennie KL, Mitchell J, Hennings S, Day NE. Validity and repeatability of the EPIC-Norfolk Physical Activity Questionnaire. International Journal of Epidemiology. 2002; 31(1):168–174. doi: 10.1093/ije/31.1.168.

Welch AA, McTaggart A, Mulligan AA, Luben R, Walker N, Khaw KT, Day NE, Bingham SA. DINER (Data Into Nutrients for Epidemiological Research) - a new data-entry program for nutritional analysis in the EPIC-Norfolk cohort and the 7-day diary method. Public Health Nutrition. 2001; 4(6):1253–1265. doi: 10.1079/phn2001196.

White PJ, Broadley MR. Historical variation in the mineral composition of edible horticultural products. Journal of Horticultural Science and Biotechnology. 2005; 80(6):660–667. doi: 10.1080/14620316.2005.11511995.

Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York; 2016.

Wilkinson BG, Perring MA. Variation in mineral composition of Cox's Orange Pippin apples. Journal of the Science of Food and Agriculture. 1961; 12(1):74–80.

Yoshida K, Bartel A. tableone: Create 'Table 1' to Describe Baseline Characteristics with or without Propensity Score Weights; 2022.