The balance between food and dietary supplements in the general population

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Abstract

In the past, vitamins and minerals were used to cure deficiency diseases. Supplements nowadays are used with the aim of reducing the risk of chronic diseases of which the origins are complex. Dietary supplement use has increased in the UK over recent decades, contributing to the nutrient intake in the population, but not necessarily the proportion of the population that is sub optimally nourished; therefore, not reducing the proportion below the estimated average requirement and potentially increasing the number at risk of an intake above the safety limits. The supplement nutrient intake may be objectively monitored using circulation biomarkers. The influence of the researcher in how the supplements are grouped and how the nutrient intakes are quantified may however result in different conclusions regarding their nutrient contribution, the associations with biomarkers in general, and dose-response associations specifically. The diet might be sufficient in micronutrients, but lacking in a balanced food intake. Since public health nutrition guidelines are expressed in terms of foods, there is potentially a discrepancy between the nutrient-orientated supplement and the quality of the dietary pattern. To promote health, current public health messages only advocate supplements in specific circumstances, but not in optimally nourished populations.

Introduction

The micronutrients that we have come to know as 'vitamins', had their road of discovery pathed by a multitude of deficiency diseases. A clear intervention, then still in the form of foods, relieved symptoms and cured diseases such as limes & scurvy, unpolished rice & beri beri and cod liver oil & rickets. Diseases nowadays are not marked by deficiency, rather overconsumption of foods tends to be the major cause of chronic diseases such as cardiovascular disease, diabetes and cancer [1–3]. These lifestyle diseases are multifactorial, where diet/nutrients play a role in disease development; however, more than a narrow focus on micronutrients is necessary to treat or prevent them.

Yet, dietary supplements remain popular in the general population where supplement users have been labelled as the 'worried well'. Positive beliefs about supplements, such as "Help me to be healthy", "Stop me getting ill", "Not do me any harm" and "Be the best I can do for myself" have been observed among supplement users in the UK [4]. A Dutch survey found that 61% thought that supplements were 'sufficiently proven' and 48% believed that supplements were 'an easy way to stay healthy' [5]. Also in NHANES (US), reasons for supplement use relate to disease prevention/treatment and supplementing the diet [6]. These opinions are in contrast with public health guidelines in these countries, where there is -in general- no role for supplement use for adults, apart from illness/special conditions, and more recently, for vitamin D supplementation in at risk groups in the UK [7,8].

So, is there a role for dietary supplements? Should we have to make up a balance of food *vs.* supplements even if health guidelines are not encouraging the use of dietary supplements? The fact that supplements continue to be used, means that the general population derives nutrients from both foods and supplements and the supplement contribution may be substantial. Supplement use is therefore an exposure that cannot be ignored in relation to (i) nutrient deficiency, sufficiency and toxicity, (ii) biomarker associations and sometimes (iii) disease, in case of suboptimal nutrient status or food intake (*e.g.* fish *vs.* fish oil and the association with cardiovascular disease). Alternatively, in observational research it is not always about establishing whether there is a benefit from supplement use itself, but also, how can we control for this health-seeking behaviour when we are interested in this (or another) exposure and health [9]. 'The typical supplement user' does not exist, there is heterogeneity in the characteristics of supplement users, depending on the type of supplement consumed [10–13]. Therefore, adjusting the supplement-disease analyses for 'yes/no supplement use' might not take away the suspected confounding, but could potentially create (more) noise/attenuation in the associations.

This paper aims to describe dietary supplement assessment methodology in the context of observational research and characterise the heterogeneity amongst supplement users. A secondary aim is to focus on the role of supplements in the nutrient distribution, circulating biomarkers and disease, using a variety of examples illustrating their (in)effectiveness in public health.

Dietary supplement assessment: definition, instruments and prevalence of use

Within Europe since 2002, dietary supplements have been regulated by the directive 2002/46/EC which defines supplements as [14]: "*Food* stuffs the purpose of which is to supplement the normal diet and which are *concentrated* sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured *small unit quantities*." Definitions of what are considered to be 'dietary supplements', or indeed specific types of supplements, have been reported to vary across American surveys [15]. Also in UK studies, definitions are lacking although the answer categories or the examples given to participants in the questionnaires give an indication of what was studied [10,16,17]. Depending on the aim of the study, prescribed medication (as sources of folate, calcium and iron) can be included in order to calculate what is known as 'total nutrient intake' (TNI), *i.e.* the sum of nutrient intake from foods and supplements [18]. Moreover, separating medication-derived nutrients from dietary supplements (or indeed food intake from dietary supplement intake) might provide additional information regarding reverse causality or confounding by indication, which might obscure the association with biomarkers or illness, *e.g.* the use of prescribed ferrous sulphate for anaemia, which itself might be caused by an underlying illness/treatment, will be differently associated with health than ferrous sulphate part of a multivitamin/multimineral (MVMM) supplement consumed out of choice.

The following issues arise when wanting to assess the nutrient contribution from supplements: (i) the potential for short-term use by participants, (ii) constant change in the supplement supply and (iii) constant change in supplement composition. The choice of the dietary supplement assessment instrument will have consequences for how well these issues can be dealt with. Dietary supplement use is assessed in similar ways to diet. There is self-reported data, using a variety of questionnaires, as well as objective measures, in the form of biochemical markers each with advantages and disadvantages (Table 1). The gold standard in supplement assessment is considered to be a face-to-face *supplement inventory*, which enables label transcription and/or collection of supplement bottles

to retrieve nutrient composition as well as tablet count and hence provides very detailed information. This method has been applied in sub-cohorts or pilot studies, mainly to validate questionnaires [19,20]. Label transcription has also been applied in the UK National Diet and Nutrition Surveys (NDNS) and the North/South Ireland Food Consumption Survey. General *questionnaires* can include question(s) regarding supplement use. Answer categories will enable categorisation into non-supplement users (NSU) and supplement users (SU) and might ask more detailed (possibly in free text) information on the type of supplement used, such as frequency or dose. The recall time and words such as 'regular', 'usual' or 'seasonal' will reflect the prevalence of supplement use obtained [21,22]. In a *Supplement Frequency Questionnaire (SFQ)*, supplements are grouped, for example 'fish oils', 'vitamin C', 'one a day multivitamins' and frequency and/or amount of use are asked for each supplement group, sometimes specifying a minimal frequency of use required [23]. The nutrient intake is calculated by assuming a nutrient formulation for each of these supplement groups. The recall period varies between studies and can be up to 10 years [23]. A *recall* covers a period of 24h, whereby supplement nutrient intake can be calculated using default nutrient profiles or manufacturers' data matched to the exact supplement used, multiplied by the frequency of consumption. The number of days collected will influence the findings regarding prevalence of supplement use [24]. In *records*, supplements can be recorded as they are consumed, which could minimise omissions due to forgetfulness (and thereby the potential for recall bias) and capture full label content. Participants are asked to fully describe the supplement, the dose (or enclose the label), the quantity and potentially also the clock time. The number of days collected will influence the results regarding prevalence of supplement use. *Biomarkers*, such as blood or urine samples, tend to be used to measure concentrations of the compound of interest or its metabolite. Biomarkers cannot differentiate between sources of the nutrient (*i.e.* whether the vitamin C was derived from foods or supplements), they vary in reference time (they may reflect recent or long-term exposure) and some nutrients are homeostatic or may be affected by illness. Laboratory measures are independent of errors made during self-report, but sample collection can be burdensome for the participant as well as expensive.

In summary, all these instruments have limitations and the quality of the data obtained will influence how the obtained data may be used in analysis. Supplement-disease analysis may be fraught with confounding when simply comparing SU against NSU; supplement nutrient intake may require researchers to maintain time-consuming, detailed supplement composition data; while biomarkers will leave the researcher with a sample concentration, but without an idea of what was actually consumed. Indeed, a combination of instruments might be a better way forward [18,25].

The choice of instrument is reflected in the prevalence of dietary supplement use observed. By using a similar instrument, secular trends can be monitored. Using a one year recall, the NDNS in 2012/13-2013/14 estimated the use of any type of dietary supplement in the UK among adults aged 19-64 years to be 15% in men and 24% in women and for those >=65 years, 30% and 41% respectively [26]. In years 5 and 6 of the rolling programme, the percentage using dietary supplements has not changed greatly for the oldest age category (38% and 41% respectively); for the younger age groups, up to a threefold increase was observed. Compared to earlier adult survey data collections in 1986/87, the change has been substantial since it was estimated to be approx. 9% and 17% respectively [27]. Secular trends have also been observed in the US, where the use of any type of supplement might have stabilised, but, for example, vitamin D supplementation increased between 1999 and 2012 from 5% to 19% and omega-3 containing supplements increased 7-fold up to 13% [28]. A trend analysis of supplement use in the Health Professionals Follow-Up Study and the Nurses' Health Study indicated continued increase of supplement use up to 2006, but a marked decrease of beta-carotene after 1994, partly because trials suggested potential harm [29]. The changes in trends may be a consequence of health policies (*e.g.* Healthy Start) and/or media coverage of trials. Supplement use varies greatly across Europe [30], both in prevalence and in the type of supplement consumed. Comparisons across countries are hampered by the variety in recall time and choice of instrument. In EPIC-Europe, the choice of a single 24h recall between 1995-2000 might have underestimated the 'usual' supplement exposure; however, a clear North-South gradient was observed (Figure 1), as well as positive trends with age [31]. The stark differences in the prevalence of supplement use between countries and continents needs to be considered when comparing results regarding supplement-sourced nutrient intake between studies.

Supplement nutrient intake - extremes of the distribution

All of the above listed assessment instrument -except the biomarkers- require the researcher to make assumptions regarding the supplement nutrient composition. The pre-structured questionnaires will assume a default nutrient composition. Open-ended questionnaires, such as used in the NDNS [32,33] and in the Norfolk arm of the European Prospective Investigation into Cancer (EPIC-Norfolk) study [34], can be more specific, but will equally rely on the labels printed on dietary supplement packaging, and therefore the potential for label-transcription errors [35]. The packaging may contain errors, the supplement may have been kept in poor storage conditions or the supplement may contain 'overages', the latter mainly for vitamins, and taking into account safety limits, in the range of 5-100% of the label value [36,37]. All these factors make what is 'on the label' not an accurate reflection of what is 'in the dietary supplement' and therefore a less accurate -or possibly even biased- measure of supplement nutrient intake (at least attenuating any association

between nutrient intake and the biomarker or disease). A long-term process of developing a composition table based on analytical data has for these reasons been proposed and developed [38,39].

Once the nutrient intake from supplements is assessed, it can be added to the food-sourced intake, to obtain TNI. This widens the range of the studied nutrient, and therefore enables risk assessment at either side of the nutrient intake distribution (Figure 2). The 'at risk' population is situated in the tails of the nutrient intake distribution (either because the intake remains low or becomes too high after inclusion of supplement sources), the intakes of which are less accurately measured. For this reason, researchers may take the upper/lower 5th centile of the nutrient intake distribution as a more stable assessment rather than the proportion in the distribution above or below the exact cutoff set by the *Dietary Reference Values* (DRV) [40,41]. When a limited number of dietary intake days are collected, researchers prefer application of statistical techniques such as 'Shrink & add' or 'Add & shrink' (see the measurement error webinar series for information about these methods [42]). The TNI distributions are used to establish the contribution that supplements make in meeting or exceeding DRVs. The *Estimated Average Requirement* (EAR) is used for comparing populations against a standard. It is the average nutrient requirement in a healthy group of people meant to maintain sufficient concentrations of a particular biomarker (blood/tissue concentration; enzyme saturation) in order to prevent nutrient deficiencies. The exact requirement is often unknown and assumed to be symmetrical [40], but reasonable estimates of the proportion at risk can be obtained using the EAR cut-point method [43], which assumes that the proportion below the average nutrient intake is -under certain conditions- approximately the same as the proportion of people with an intake below their average nutrient requirement. The *Lower Reference Nutrient Intake* (LRNI) is the EAR value *minus* two standard deviations and is likely to cover the need of only 2% of the population. The *Reference Nutrient Intake* (RNI) is the EAR value *plus* two standard deviations, and covers the need of 98% of individuals in a population [40,43]. The RNI might provide a good estimate for comparison against an individual's requirement; however, at the population level, this measure is (too) cautious [43]. The *Safe Upper Level* (SUL) is defined by the Expert Group on Vitamins and Minerals (EVM) to "represent an intake that can be consumed daily over a lifetime without significant risk to health on the basis of available evidence" [36] and refers to the supplement-sourced intake only. The *Guidance Level* (GL) is defined by the EVM as "an approximate indication of levels that would not be expected to cause adverse effect, but have been derived from limited data and are less secure than SULs" [36].

Considering the variation in supplement use across Europe [30,31], supplements vary in the contribution that they make to food-sourced intake and the proportion of the populations at risk of not meeting the sufficiency DRVs. There are however various complications when wanting to

assess this across countries, not in the least because of different dietary assessment methodologies applied in surveys, but also what is considered 'sufficient' across countries varies due to [44,45]: different expert panels, the currency of the evidence assessed, use of different DRVs, different cut-off points for age groups, criteria for adequacy (*i.e.* the condition that the nutrient needs to prevent) and the extrapolation of data. Mensink *et al.* [46] streamlined participant-level data with regard to DRVs and age cutoffs from dietary surveys in eight countries in the European Union, with data collections between 1997 and 2010. Using vitamin C from this publication as an example, mean food-sourced intake in adults aged 18-60 years varied from 81 (PO) - 152 (G) mg/d in women and from 81 (F, NL) -152 (D) mg/d in men. After the contribution of supplements, TNI ranged from 96 (F) -175 (D) mg/d in women and from 87 (F) -173 (D) mg/d in men. There was a very small decrease (0-1% women; 0-0.7% men) in the percentage of the populations meeting the EAR after inclusion of supplements; only among the 65+ age group were reductions of 0-4% obtained. Particularly for the vitamins A, D and E, and the minerals iron (among women) and selenium, a lower prevalence of intakes below the EAR (up to 34% decrease for vitamin D) were observed after inclusion of supplement sources of these nutrients in adults. When it comes to exceeding upper limits due to supplements, Flynn *et al.* [30] studied dietary survey data of seven vitamin and eight mineral nutrient distributions gathered in a selection of European countries between 1994 and 2006. Food-sourced intake (with fortified foods making a small contribution) was responsible for the majority of the populations' intakes. The nutrient intake associated with the 95th centile of retinol, zinc, iodine, copper and magnesium increased considerably after inclusion of supplement sources; however, it only exceeded the upper limits in a small percentage of the studied populations.

When supplement use is compared between countries or continents, its use and contribution do not only vary because of participant-associated variation (*i.e.* the choice of supplement), but also due to the choices in data handling and analysis by researchers. When comparing publications, large differences between studies may be explained due to SUs all being grouped together *vs.* nutrient-by-nutrient distinction among SUs. This is the case when interpreting publications using NHANES data for example [47–49]. Here, far greater effects on meeting the EAR and exceeding the TUL are obtained because of different supplement nutrient groupings of participants (on top of different DRV cut-offs and the majority of the supplements being MVMM-type supplements). Applying this nutrient-by-nutrient grouping strategy and UK DRVs to the vitamin C intake as assessed in the NDNS data of years 1-4 of the rolling programme [32], then SUPP-Table 2 is obtained. When the food-sourced vitamin C intake of *all* the men or *all* the women within the same age group are compared against the TNI, the median intake increased with 3-9 mg/d and the percentage of participants in this population *not* meeting the EAR was maximally 0.1-1.1% lower once

supplements were included, as was observed EU-wide [46]. When we additionally ask the question *"Who is at risk?"* and stratify the strata further by supplement status, we can allocate the supplement exposure to those who were truly exposed and not dilute the exposure with non-vitamin C containing supplements. When the vitamin C supplement users (SU+C) are identified, the contribution of the supplement was approximately twofold that of the food-sourced intake (SUPP-Table 2). The SU+C group had a lower risk of not meeting the sufficiency DRVs (not just because of the supplement, but also because of higher food-sourced vitamin C intake among the SU-C and SU+C); moreover, only the SU+C group, and only when studying TNI, were exceeding quantities >1000 mg/d, intakes which have been associated with GI-problems [36]. A visual representation of this TNI distribution and DRVs is provided in Figure 3.

Conclusion - intake

Supplement intakes shift the nutrient exposure distribution to the right; however, nutrient sufficiency -in most cases- may be obtained from food sources only. The (small) reduction in the proportion at risk after including supplements depends on the nutrient, but also on the grouping of the supplements. There is a modest higher risk of exceeding the upper limits when supplement intake is included (among those using that nutrient in supplement form).

Association between supplement intake and biomarkers

Objectively measured nutrient biomarkers may serve to validate the self-reported nutrient intake, by providing an indication of the 'internal dose', the absorption. Biomarkers may be influenced by a variety of factors described in detail elsewhere [50,51]; however, with regard to dietary supplements as a source of nutrient intake, a few points stand out. First, the range of nutrient intake is made wider and different dose-response associations may be detected with TNI vs. food-sourced intake alone. Secondly, the statistical parameters chosen in observational research are mostly there to establish correlations and quantify reclassification of participants, but a dose-response association is different and some of these results may be counterintuitive with regards to the 'internal dose'. Thirdly, just as foods contain multiple nutrients which may interact (*e.g.* fat-soluble vitamins as antioxidants in high fat foods), colinearity in supplement nutrient ingestion exists (*e.g.* use of MVMM-type supplements). Therefore, biomarkers other than the nutrients studied may be affected (*e.g.* vitamin C supplement use and tocopherol concentrations). These points are illustrated below.

In (large) cohort studies, circulating biomarkers are commonly used as an indicator of absorption/bio-availability. The nutrient exposure may be classified into *N*-tiles (e.g. tertiles, quintiles) and the means of both intakes and biomarkers may be presented for each *N*-tile, this to establish any type of dose-response association. Researchers may be interested in the (improvement

of the) agreement in classification between the objectively and subjectively collected data, *i.e.* establish whether participants ranked and placed into a specific *N*-tile according to the biomarker are the same participants as those placed in this *N*-tile according to the questionnaire (comparing this agreement using the intake without and with supplements). Alternatively, researchers may wish to summarise the association between intake and biomarker in a single number, using either (i) a correlation or (ii) a beta-coefficient. A correlation is a standardised measure (disregarding the unit) indicating the strength between two variables. If the correlation is high, then a standardised higher intake is associated with a standardised higher or lower biomarker concentration; however, it does not reflect a dose-response association (even when the value approaches 1 or -1), since the standardisation process has removed this aspect from the results. Using linear regression, which obtains the (adjusted) beta-coefficient, the unit in which the variables are measured remains (though the input variables might be 'transformed'), and the results may be interpreted as a 'dose-response' since the intake of *x* amount of mg/d can be associated with a higher/lower *y* amount of the biomarker. For example, correlations between TNI or supplement-sourced vitamin E intake and α-tocopherol concentration biomarkers have been reported to range from 0.3-0.7 using a variety of parameters on transformed or non-transformed data [52–55]. In the VITamin And Lifestyle (VITAL) cohort [52], adjusted correlations between supplement intake and biomarker were 0.69 with a significant linear trend across *N*-tiles ($P<0.0001$); however, when plotting the means of the supplement intake groups (NSU: 0; quartiles: 18, 180, 194, 360 mg/d) against the blood biomarker (NSU: 28, quartiles: 34, 44, 50, 60 μmol/L), three issues become apparent. (i) Supplement-sourced intake exceeds food-sourced intake 30-40 fold; (ii) due to the non-normal distribution of supplement-sourced intake, a wide range of supplement-sourced intake is grouped together, creating then small, then large differences between the *N*-tile means of intake; and consequently (iii) the dose-response of supplement intake is not the same at every amount of supplement-sourced vitamin E intake. Such observations were also observed by Zhao et al. in the Irish National Adult Nutrition Survey (NANS) data [56]. α-Tocopherol concentrations are positively associated with vitamin E intake, γ-tocopherol is negatively associated with vitamin E intake due to preference of hepatic α-tocopherol transfer proteinase; furthermore, potential differences in the associations of plasma tocopherol and natural *vs.* synthetic forms of vitamin E may exist [57].

When assessing the association between nutrient intake (from both food and supplement sources) and a biomarker, Block *et al.* draw an analogy with smoking [58]. When the association between smoking and a nicotine biomarker is assessed, we could analyse the amount smoked at home separately from the amount smoked at work, or analyse the amount smoked at work adjusted for the amount smoked at home, however the total amount smoked is the exposure of interest in aetiology

[58]. Moreover, when applied to nutrient-biomarker associations, the biomarker has no ability to detect a difference between food or supplement sources. One more analogy may be added to the ones listed by Block *et al.* and that is that we would not average the number of cigarettes smoked whilst including the non-smokers. However, this is what happens by grouping all SUs into a single group, the supplement contribution of a nutrient is diluted by SUs who consume different types of supplements. A nutrient-by-nutrient supplement group distinction can provide insights not only in potentially differential food-sourced intakes (as described above in the intake distribution section), but also in potentially differential dose-response associations. Particularly so, since supplement-sourced intake could surpass food-sourced intake and therefore approach intakes associated with biomarker saturation. In the EPIC-Norfolk study, dose-response associations have been observed to vary across subgroups of SUs. A sex-adjusted analysis of published results [59], obtains the following associations between food-sourced vitamin E intake (per 10 mg/d) and back-transformed log-biomarkers of α-tocopherol concentrations (and therefore representing a percentage change [95%CI]) among NSU, SU-E and SU+E respectively of: 10% (9,12%), 9% (6,12%) and 5% (2, 9%). When replacing food-sourced intake with TNI, the associations in the SU+E group weakened to 1% (1,2%); although the adjusted correlation strengthened from 0.09 (food only) to 0.43 (TNI) among the SU+E (since supplement-sourced vitamin E intake may be over 10-fold higher than food-sourced intake in the UK). This linear model indicates saturation, which has been reported with intakes varying between 9-17 mg/d [54,60]; and indeed, when only participants with TNI <17 mg/d were included, the coefficient among the SU+E was 9%, although with wide confidence intervals (1-16%). The urinary excretion products of vitamin E have for this reason been studied as a substitute to indicate sufficiency, or very high ingested doses [54]. Saturation thresholds also exist for vitamin C since kidneys excrete vitamin C at intakes higher than 120 mg/d [40]; whereas retinol concentrations are largely homeostatic, even after a state of toxicity has been reached [61] and therefore dose-response associations are not observed in replete individuals.

The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are mostly obtained from oily fish, for which the most recent dietary guideline recommendations (1 portion of oily fish per week, approx. 0.45 g/day or 3.15 g/week of EPA+DHA) [62] have not been met in the UK population [32,33]. A source of EPA and DHA may also be obtained from cod liver oil and fish oil type supplements (referred to as 'EPA/DHA-containing supplements'), which could approximately double the exposure among those using EPA/DHA-containing supplements (SU+EPA/DHA). In EPIC-Norfolk, a general population-based cohort, aged between 39 and 79 years, the median TNI was 0.39 g/d in men and 0.29 g/d in women among SU+EPA/DHA between 1993-1998 [59]. For EPA or DHA supplements, when these nutrients are ingested separately or

combined, in doses up to 7 g/d (*i.e.* over 15 times the SACN recommendation), dose-response associations in trials have resulted in increased plasma concentrations with the most efficient dose-response when the respective fatty acids is supplemented [63]. Dose-response associations between the sum of EPA and DHA intake (3:2 ratio) and plasma EPA and DHA, have been found to be linear up to 3 g/d in a trial of healthy young men who consumed fish <1 times/week at baseline [64]. A trial among healthy men and women aged 20-80 years, who did not consume fish or supplements thereof, showed linear dose-response associations up to 4 portions of oily fish per week (where six capsules totalling 3.27 g of EPA+DHA reflected a single portion) [65]. However in a cohort study where SU+EPA/DHA were excluded and fish consumption was 0.5-1 serving per week, a linear association was observed up to 0.5 g/d of EPA+DHA intake [66,67]. The differences in dose-response between cohorts and trials may be explained by differences in bio-availability of food-sourced and supplement-sourced EPA+DHA due to varying fat content of meals and biochemical form of the supplemented fatty acids [68,69] or the frequency of EPA+DHA consumption. Supplements in trials are advised to be taken daily, whereas fish is an episodically consumed food. Browning *et al.* observed that similar weekly doses of EPA and DHA (6.54 g/wk, *i.e.* 2 times the SACN recommendation), but taken either daily or dispersed over only 2 days per week, resulted in faster and sustained incorporation into plasma, platelets and red blood cells when supplements were taken daily, although after 12 months no difference was observed in plasma concentration when comparing the weekly *vs.* the daily regime [70].

Not just pharmaceutical supplement doses, but also supplement doses not exceeding the RNI are associated with circulating biomarker concentrations. A recent publication from the Lung Cohort Cancer Consortium (LC3) combined cohorts across four continents and analysed biomarkers in a single laboratory [71]. It illustrated a wide range in vitamin status across the continents, with higher concentration among MVMM-type SUs. In the 1994/95 NDNS 65+ sample, vitamin but not mineral intake from supplements, was associated with higher status indices, regardless of the supplement assessment tool used [18]. In the UK, vitamin D is mostly contained in cod liver/fish oil supplements as well as multivitamin and MVMM supplements. Here, the doses do not tend to exceed 5 mcg/d and still 10 nmol/L higher 25(OH)D concentrations were observed among participants in the 1958 Birth Cohort who took such supplements [72], lowering their risk of a 25(OH)D concentration being <40 nmol/L by 64% (95%CI: 56-70%).

Conclusion - biomarker

The supplemented nutrients are capable of raising plasma concentrations of the respective nutrients, particularly vitamins and fatty acids. Supplements at pharmaceutical doses might obtain high correlations between intakes and biomarker; however, the dose-response associations indicate

saturation. A biomarker may be influenced by many other factors (see for example Proc Nut Soc McMillan); moreover, it does not automatically mean that higher circulating concentrations indicate better health or functionality, since circulating biomarkers might not reflect storage or the effectiveness of the nutrient in an organ.

Health outcomes

In this last section, the balance between food and supplements is discussed in light of positive and negative health outcomes. Evidence for causality of a putative beneficial nutrient is generally taken from (double-blinded, placebo-controlled) trials; however, evidence with regards to side effects, contamination or toxicity are mostly gathered from extensive risk assessment using animal models, observational studies and case reports or sensitivity analysis from trial data. I will first contrast these study designs, followed by a summary of systematic reviews evaluating the role of dietary supplements and emphasizing the differences between foods *vs*. supplements.

Trials and observational studies have advantages and disadvantages when studying associations between supplement use and health/disease (Table 3). Trials are limited in the number of exposures that can be tested in a single experiment [23,73,74]. The conclusion of dietary supplement efficacy in relation to the outcome is hence limited to the number of compounds tested, the dose tested (potentially higher than a commonly available dose) and the outcome tested. Moreover, particularly when the outcome is cancer, the follow-up in trials tends to be too short since the disease might take 10-20 years to develop [75–77]. Trial findings can be obscured by the use of supplements beside the trial dose, particularly when these are unrecorded. Similarly, past use of supplements by trial participants (treatment or control) could obscure findings as well as pre-cancerous stages which may modify the risk to the intervention arm [13,77,78]. Regarding observational studies and supplements, such studies can be more inclusive in their eligibility criteria and the follow-up time tends to be longer than in trials. They can assess a wide range of commonly used dietary supplements and doses [23]. Depending on the frequency of assessment, cohorts can take into account the variability of supplement use over time, since a single measure cannot be considered to reflect habitual supplement use [79,80]. On the other hand, observational studies suffer from confounding and, if retrospective measures are used, potentially recall bias [75,81]. The distribution of socio-demographic characteristics, behavioural factors, and prevalent illnesses are not uniformly distributed between SU and NSU [23,73,82]. Additionally, the role of specific nutrients is difficult to assess due to colinearity, *i.e.* nutrients are commonly consumed as part of a MVMM-type supplement for which factorial trial designs are better equipped [23,73,77].

Since supplements contain (isolated) nutrients in concentrated forms, TNI may lead to chronic intakes exceeding safe upper levels [83] (Figure 2). In the Iowa Women's Health Study, supplement use has -potentially for this reason, but also due to confounding by indication- observed harmful associations between supplemental iron and mortality [84]. High retinol TNI (~2500 µg/d) in combination with low vitamin D TNI (< 11 µg/d) has been associated with fractures in post-menopausal women [85]. For Vitamin C the difference between the RNI and (reversible) harm in the form of GI problems ranges between 40 mg/d and 1000 mg/d; whereas for retinol this is 600 µg/d *vs.* 1500 µg/d (the difference being just over a common vitamin A dose in a supplement). The European Food Safety Authority [86] and the Expert group on Vitamins and Minerals in the UK have extensively reviewed trials and safety reports for a wide range of nutrients [36]. A selection of the SULs set by the EVM are provided in Table 4. When compared against the 95th centile of supplement-sourced intake among the adult population in the NDNS, it is observed that the intake of Zinc and vitamin B6 could exceed the SUL. Although such intakes would need to be sustained over a long period of time to affect health and the collection of a single 4-day diary might not be sufficient to reflect a person's usual intake or capture the varying behaviour of supplement use.

Systematic reviews with meta-analyses of trials randomising participants to placebo or single/combinations of anti-oxidant supplements (Vitamin A, C, E, β-carotene, selenium), observed significant associations with harm in unbiased trials (RR 1.04; 95%CI: 1.01, 1.07), but significant beneficial associations (RR 0.91; 95%CI: 0.85, 0.98) for biased trials [87]. Significantly higher all-cause mortality risks were observed for β-carotene (RR 1.05; 95%CI: 1.01, 1.09), and potentially for vitamins A and E, but not for vitamin C or selenium. Also the U.S. Preventive services Task Force recommendation statement concluded that overall no benefit could be observed for primary prevention of cancer or cardiovascular disease when using single nutrient supplements [88,89]. A meta-analysis of MVMM-type supplement trials concluded no benefit with regards to total, cardiovascular or cancer mortality [90].

The Linxian Nutrition Intervention Trials in the general population, studied the effects of the use of any of the four supplement combinations: retinol & zinc, riboflavin & niacin, vitamin C & molybdenum, or ß-carotene, vitamin E & selenium in the prevention of all-cause mortality, cancer mortality and cancer incidence [91]. It observed significant reductions in mortality (9%), cancer mortality (13%), but particularly for stomach cancer (21%) when ß-carotene, vitamin E & selenium were supplemented. Potential explanations for the observed effects were marginal micronutrient intake at baseline due to low consumption of fruits and vegetables. Indeed, plasma vitamin C concentrations were low at the start of the trial and a daily supplement doses of 120 mg/d raised these concentrations comparable to or just below the UK mean. Suboptimal circulating vitamin

concentrations have also been proposed as an explanation for the decrease in cancer incidence in the supplementation *vs.* placebo arm in men of the SUpplementation en VItamines et Mineraux AntioXydants (SU.VI.MAX) trial, since the baseline antioxidant concentrations were lower in men. In post-hoc analysis, an interaction (P=0.04) between baseline concentrations and trial arm could only be observed for vitamin C and only among men [92].

Since nutrients may be derived from a variety of (potentially fortified) foods, and not necessarily from foods which are recommended for public health, one can argue that food intake might be a better marker of optimal intake rather than nutrient intake. For example, median vitamin C TNI expressed as a percentage of the RNI was 185% and 197% in men aged 19-64 y and 65+ y respectively, and 192% and 209% in women [32]. Contrasting this to fruit and vegetable consumption, the UK diet meets 30% and 40% of the 5-a-day guidelines in both men and women aged 19-64 y and 65+ y respectively [32]. The role of multivitamins in the past was partly seen as a means to compensate poor dietary choices [73]; or, where after various considerations, the likely benefits outweighed harm of supplement use [93]. However, as observed in above described meta-analyses, such use has not been successful in the prevention of disease or early death in populations. Potentially, since foods contain more than vitamins and minerals alone and dietary patterns as a whole play an important role in health [3].

An example of a sub optimally consumed food group in the UK is fish, of which the recommendation is to consume 2 portions/week (~280 g/week). In men, intake reached 161 g/week and 252 g/week for the age groups 19-64 y and 65+ y respectively, in women 154 g/week and 189 g/week [32]. Data on the contribution of EPA+DHA from the most commonly consumed supplement, cod liver oils & fish oils, are lacking in the national surveys. These results are available from the baseline EPIC-Norfolk cohort (SUPP-Table 5). The low dose EPA+DHA from mainly cod liver oil resulted in 15-20% more participants meeting the EAR of 0.45 g/d.

Higher fish consumption has been associated with lower CHD/CVD mortality in cohort studies, despite differences across the globe due to differences in dietary assessment methods, absolute amounts of fish consumed, fish preparation and water contamination [94,95]. Various biological mechanisms relating to long chain omega-3 fatty acids and CHD have recently been reviewed in these Proceedings, including the prevention of arrhythmia and anti-inflammatory properties [96,97]. Fish may also exert its benefit as a source of protein, vitamin D, iodine, calcium (bones), or due to the substitution effect when consumed as part of a meal [98,99]. Although, trials using EPA+DHA supplements in secondary/tertiary prevention groups showed promising results initially, later trials observed no benefit [100]. A recent review by the Omega-3 Treatment Trialists' Collaboration confirmed no benefit in relation to fatal CHD or nonfatal myocardial infarction among those with

existing CHD [101]. Supplementation with omega-3 fatty acids for primary prevention of CVD has not been advised due to lack of trial results in primary prevention [102,103] (the results from the first primary prevention trial on Vitamin D and EPA+DHA, the VITamin D and OmegA-3 TriaL [VITAL], are not yet available [104]), only the consumption of oily fish and seafood is currently advocated. Since cod liver oil is a low dose source of EPA+DHA and a commonly consumed supplement in the EPIC-Norfolk study (SUPP-Table 5), it was possible to assess the role of this supplement in *primary* prevention of CHD mortality. A low dose of 250 mg/d of EPA/DHA is considered sufficient for prevention of arrhythmia [105]. Due to supplement use, an additional 19-24% of the participants met this threshold. The confounding associated with SU+EPA/DHA and SU-EPA/DHA as well as the changes over time in supplement use were modelled using time-varying covariates analysis. It was observed that CHD mortality was 26% lower (95%CI: 16-34%) among SU+EPA/DHA compared to NSU, but no significant association was observed when comparing SU-EPA/DHA vs. NSU [106]. Due to the observational nature of the study, residual confounding and collinearity of nutrients could have occurred.

Conclusion – health

Whenever supplement use and health are being associated, the heterogeneity among SUs cannot be ignored. 'The typical supplement user' does not exist. The obvious distinction between SUs lies in the variety of the supplements consumed, but also in the many other disease risk factors which might confound or bias the supplement-health association in observational research. Supplements may be considered 'natural'; however, the concentrated form puts the user at risk of harm when overdosed. Meta-analyses of trials studying MVMM supplements thus far have indicated that if populations are optimally nourished, there is no role for supplement use - "Enough is enough" [107].

Closing remarks

How does the balance tip between foods and supplements? Supplements continue to be used by an increasing proportion of the population, so their contribution to diet, health and disease needs to be monitored. Traditionally, essential nutrients have been studied in relation to health, and although micronutrient deficiencies are still prevalent in the UK population, the relatively high nutrient intake may not be a marker of healthy food choices, as reflected in the low fruit, vegetable and fish consumption from national surveys. Resolving unhealthy dietary patterns with micronutrient supplements is a too narrow-minded solution. Nowadays, public health nutrition guidelines take the role of the nutrient, its food source and its place in the diet into account to optimise diet. The current role of supplements herein seems restricted to certain age groups, life circumstances or diseases with impaired nutrient absorption [7,108]. The challenge in observational research

methodology is to assess and describe nutrient intake, as well as diet as a whole, in the general population and to clarify the role -if any- of nutrient supplements in primary disease prevention.

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Conflicts of interest

None.

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Figures

Figure 1: Prevalence of any type of dietary supplement in EPIC-Europe as assessed by 24-hour recall [31]. Data collection of the calibration study between 1995-2000.

Figure 2: Schematic of the various DRVs. Adapted and combined from [40,83,109].

DRV, dietary reference value; LRNI, lower reference nutrient intake; EAR, estimated average requirement; RNI, reference nutrient intake; SUL, safe upper level.

Figure 3: Vitamin C TNI distribution by vitamin C supplement user group status among men and women >18 years. Data from NDNS from years 1-4 of the rolling programme [26].

TNI, total nutrient intake (food + supplements); NSU, non-supplement users; SU, supplement users; SU+C, supplement user consumes a vitamin C containing supplement; SU-C, supplement user consumes a supplement without vitamin C; NDNS; national diet and nutrition survey; LRNI, lower reference nutrient intake (10 mg/d); EAR, estimated average requirement (25 mg/d); RNI, reference nutrient intake (40 mg/d); 1000 mg/d being the intake at which GI-problems have been reported.

Tables

Table 1: Overview of dietary supplement assessment instruments and characteristics of collected data. A summary based on Dwyer *et al.* [110]

	Retrospective/ Memory	Time/burden participant	Supplement composition database	Short term	Open ended
Supplement inventory		✓	✓	✓	✓
Diet record (diary)		✓	✓	✓	✓
Supplement Frequency Questionnaire	✓		✓		
24-hour Diet Recall*	✓		✓	✓	✓
Screeners/brief questionnaires	✓				
Biomarker		✓		(✓)	

* When repeated measures are taken, the time/burden approaches that of the diet record method.
Bracketed ticks (✓) indicate that the measure is not uniform in its characteristic/use, see examples in text.

SUPP-Table 2: Vitamin C intake from food and supplement sources by supplement user subgroups and the prevalence of meeting/exceeding of dietary reference values using UK-weighted NDNS data from the rolling programme years 1-4 [26].

Sex	Age (y)	Supplement status	N base	Food Vitamin C (mg/d) Median (IQR)	< EAR 25 mg (%)	>1000 mg/d (%)*	TNI Vitamin C (mg/d) Median (IQR)	< EAR 25 mg (%)	>1000 mg/d (%)*
Men	19-64	ALL	1126	71 (41, 109)	10.6	0	74 (44, 116)	9.5	0.4
		NSU	925	69 (41, 105)	10.7	0	69 (41, 105)	10.7	0
		SU	201	82 (42, 133)	9.9	0	123 (75, 194)	3.9	2.5
		SU-C	91	83 (42, 148)	8.4	0	83 (42, 148)	8.4	0
		SU+C	110	77 (42, 117)	11.7	0	173 (105, 278)	0	4.6
	65+	ALL	317	75 (43, 114)	9.3	0	79 (44, 120)	9.3	0.1
		NSU	211	65 (39, 104)	12.8	0	65 (39, 104)	12.8	0
		SU	106	88 (55, 119)	2.9	0	115 (69, 157)	2.3	0.6
		SU-C	73	87 (54, 116)	3.4	0	87 (54, 116)	3.4	0
		SU+C	33	107 (55, 130)	0	0	174 (130, 263)	0	1.8
Women	19-64	ALL	1571	68 (42, 104)	8.3	0	77 (44, 120)	7.6	1.1
		NSU	1148	62 (40, 99)	9.5	0	62 (40, 99)	9.5	0
		SU	423	83 (49, 118)	5.0	0	129 (82, 206)	2.6	4.1
		SU-C	207	86 (51, 128)	5.5	0	86 (51, 128)	5.5	0
		SU+C	216	76 (46, 117)	4.6	0	181 (124, 365)	0	7.8
	65+	ALL	436	78 (47, 115)	3.9	0	84 (50, 125)	3.8	0.8
		NSU	251	69 (43, 106)	6.4	0	69 (43, 106)	6.4	0
		SU	185	82 (51, 122)	1.0	0	102 (66, 150)	0.7	1.7
		SU-C	118	81 (51, 119)	1.1	0	81 (51, 119)	1.1	0
		SU+C	67	84 (50, 125)	0.9	0	154 (110, 282)	0	4.4

TNI, total nutrient intake (food + supplement); NSU, non-supplement users; SU, supplement users; SU+C, supplement user consumes a vitamin C containing supplement; SU-C, supplement user consumes a supplement without vitamin C; EAR, estimated average requirement; NDNS, national diet and nutrition survey; IQR, interquartile range.

* No SUL or GL are set by the EVM, but intakes >1000 mg have been associated with GI-problems in certain populations [36]. This cutoff value was taken as an illustration of high intakes.

The inclusion of an additional stratification among the SU (SU-C and SU+C, rather than the combined group of SU) might have made the median, IQR and prevalence estimates unstable.

Table 3: The advantages and disadvantages of using observational or trial data to ascertain efficacy of dietary supplements in disease prevention.

	Prospective cohort	Trial
Advantages	Long follow-up time Data collection/hypothesis can be adjusted based on latest findings	Confounding minimised Clear exposure measure
Disadvantages	Residual/unmeasured confounding Colinearity of nutrients Supplement databases are laborious to maintain Repeated measures of exposures & confounders necessary	Short-medium follow-up Testing a specific supplement, component or dose Selective inclusion of participants

Table 4: Safe Upper Limits as set by EVM [36], applied to NDNS rolling programme years 1-4 where participants were 18 years or older [26].

Nutrient	EVM (SUL)	95th centile of food-sourced intake (mg/d)						Supplement intake (among SU+ only, mg/d)			
		Men			Women			Men		Women	
		NSU	SU-	SU+	NSU	SU-	SU+	Median (IQR)	95th centile	Median (IQR)	95th centile
Vitamin B6	0.17 mg/kg BW/d	4	5	6	3	3	3	2 (2,3)	11	2 (2,5)	25
Vitamin E	540 mg/d	18	17	18	14	15	15	5 (2,10)	18	10 (2,12)	62
Copper	0.16 mg/kg BW/d	2	3	3	2	2	3	1 (1,2)	3	1 (1,1)	2
Zinc	25 mg/d	15	15	17	12	12	13	15 (6,15)	28	15 (5,15)	30

EVM, expert group on vitamins and minerals; NDNS, national diet and nutrition survey: IQR, interquartile range; BW, body weight; NSU, non-supplement users; SU, supplement users; SU+, supplement user consuming the nutrient of interest in supplement form; SU-, supplement user *not* consuming the nutrient of interest in supplement form.

SUPP - Table 5: EPA/DHA intake from food and supplement sources by supplement user subgroups and the prevalence of meeting/exceeding the EAR using baseline 7dDD data (>= 3 completed days) from the EPIC-Norfolk study (1993-1998) – re-analysed data by age/sex groups as used in Lentjes *et al.* 2015 and 2017 [59,106].

Sex	Age (y)	Supplement status	N	Median (IQR) Food EPA+DHA (g/d)	DRVs using food sources		Median (IQR) TNI EPA+DHA (g/d)	DRVs using TNI		Meeting 0.25 (g/d)**	
					<EAR 0.45 g/d	*>5 g/d**		*<EAR 0.45 g/d*	*>5 g/d**	*Food*	*TNI*
					%	N		%	N	%	%
Men	39-64	ALL	6675	0.13 (0.07, 0.35)	80	0	0.16 (0.08, 0.41)	77	1	67	63
		NSU	4712	0.12 (0.06, 0.32)	82	0	0.12 (0.06, 0.32)	82	0	69	69
		SU	1963	0.16 (0.07, 0.42)	77	0	0.27 (0.14, 0.64)	66	1	63	48
		SU-EPA/DHA	683	0.16 (0.07, 0.41)	78	0	0.16 (0.07, 0.41)	78	0	62	62
		SU+EPA/DHA	1280	0.15 (0.07, 0.43)	77	0	0.31 (0.18, 0.81)	59	1	63	40
	65+	ALL	3545	0.16 (0.07, 0.40)	78	0	0.21 (0.09, 0.50)	73	0	62	56
		NSU	2260	0.15 (0.07, 0.38)	80	0	0.15 (0.07, 0.38)	80	0	65	65
		SU	1285	0.18 (0.08, 0.45)	75	0	0.32 (0.16, 0.77)	60	0	58	41
		SU-EPA/DHA	352	0.20 (0.07, 0.46)	75	0	0.20 (0.07, 0.46)	75	0	57	57
		SU+EPA/DHA	933	0.18 (0.08, 0.45)	75	0	0.38 (0.19, 0.92)	55	0	59	35
Women	39-64	ALL	8776	0.11 (0.05, 0.30)	84	0	0.15 (0.07, 0.36)	80	0	71	66
		NSU	4822	0.10 (0.05, 0.27)	86	0	0.10 (0.05, 0.27)	86	0	73	73
		SU	3954	0.12 (0.06, 0.35)	82	0	0.20 (0.10, 0.48)	73	0	68	57
		SU-EPA/DHA	1767	0.11 (0.05, 0.32)	83	0	0.11 (0.05, 0.32)	83	0	70	70
		SU+EPA/DHA	2187	0.12 (0.06, 0.36)	81	0	0.27 (0.16, 0.62)	66	0	66	47
	65+	ALL	3960	0.14 (0.06, 0.36)	82	0	0.19 (0.08, 0.42)	77	1	65	59
		NSU	2192	0.13 (0.06, 0.34)	83	0	0.13 (0.06, 0.34)	83	0	67	67
		SU	1768	0.15 (0.07, 0.37)	81	0	0.25 (0.14, 0.56)	69	1	64	50
		SU-EPA/DHA	575	0.16 (0.06, 0.37)	81	0	0.16 (0.06, 0.37)	81	0	62	62
		SU+EPA/DHA	1193	0.15 (0.07, 0.37)	81	0	0.31 (0.17, 0.71)	63	1	64	44

TNI, total nutrient intake (food + supplement); EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; NSU, non-supplement users; SU, supplement users; SU+EPA/DHA, supplement user consumes a EPA/DHA containing supplement (mostly cod liver oil and fish oil supplements); SU-EPA/DHA, supplement user consumes a supplement without EPA/DHA; DRV, daily reference value; EAR, estimated average requirement; IQR, interquartile range.

* Amounts > 5 g/d have been associated with adverse events, but EFSA has not set a TUL for EPA+DHA [111].
** Amounts of >0.25 g/d have been associated with anti-arrhythmic effects [105].