Correlations between fruit, vegetables, fish, vitamins and fatty acids estimated by web-based non-consecutive dietary records and respective biomarkers of nutritional status

List of abbreviations:

DR, dietary record

BMI, body mass index

CI, confidence interval

DHA, docosahexaenoic acid (C22:6 n-3)

EPA, eicosapentaenoic acid (20:5 n-3)

F&V, fruit and vegetables

FFQ, food frequency questionnaire

FPQ, food propensity questionnaire

PUFA, poly-unsaturated fatty acid

r, Spearman's correlation coefficient

Abstract

Background: It is of major importance to measure the validity of self-reported dietary intake using web-based instruments before applying them in large-scale studies.

Objective: This study aimed to validate self-reported intake of fish, fruit and vegetables and selected micronutrient intakes assessed by a web-based self-administered dietary record (DR) tool used in the NutriNet-Santé prospective cohort study, against concentration biomarkers: plasma β-carotene, vitamin C and n-3 polyunsaturated fatty acids.

Participants/setting: One hundred ninety eight adult volunteers (103 men and 95 women, mean age=50.5y) were included in the protocol: they completed 3 non-consecutive-day DRs and two blood samples were drawn, 3 weeks apart. The study was conducted in the area of Paris, France, between October 2012 and May 2013.

Main outcome measures: Reported fish, fruit and vegetables, selected micronutrient intakes and plasma β-carotene, vitamin C and n-3 polyunsaturated fatty acids.

Statistical analyses: Simple and adjusted Spearman's rank correlation coefficients were estimated, after deattenuation for intra-individual variation.

Results: Regarding food groups, in men, adjusted correlations ranged from 0.20 for vegetables and plasma vitamin C to 0.49 for fruits and plasma vitamin C, and from 0.40 for fish and plasma c20:5 n-3 (EPA) to 0.55 for fish and plasma c22:6 n-3 (DHA). In women correlations ranged from 0.13 (non-significant) for vegetables and plasma vitamin C to 0.41 for fruits & vegetables and plasma β-carotene, and from 0.27 for fatty fish and EPA to 0.54 for fish and EPA+DHA. Regarding micronutrients, adjusted correlations ranged from 0.36 (EPA) to 0.58 (Vitamin C) in men and from 0.32 (vitamin C) to 0.38 (EPA) in women.

Conclusion: The findings suggest that three non-consecutive web-based DRs provide reasonable estimates of true intake of fruits, vegetables, fish, β-carotene, vitamin C and n-3 fatty acids. In addition to other validation studies, our study shows acceptable validity of

using such diet assessment methods in large epidemiologic surveys and broadens new perspectives for epidemiology.

Introduction

Consumption of fruit and vegetables (F&V) and fish may play a critical role in the prevention of some cancers and cardiovascular disease [1;2], which together represent the heaviest global disease burden. These food groups are of particular interest as the consumption of non-starchy vegetables and fruits is one of the recommendations issued by the World Cancer Research Fund [1] and according to the World Health Organization, low intake of F&V and fish are linked to cardiovascular disease risk [2]. In large-scale epidemiological studies, from which an important part of the evidence is based, dietary information is reported through self-administered instruments such as multiple 24h recalls, diet records, or Food Frequency Questionnaires (FFQ). Inherently to the self-reporting administration mode, none of these instruments provide unbiased estimates of the true intakes [3], and this measurement error can bias or attenuate the observed relationships between F&V or fish and health outcomes. For instance it is known that F&V consumption is overestimated by FFQs [4]. To assess individual usual intake as accurately as possible, the data collection tool that performs optimally is suggested to be several non-consecutive days of diet records or recalls [5-7], where within-individual error can be taken into account. In turn, it is of major importance to measure the validity of such instruments, i.e. their ability to properly assess food group consumption or nutrient intake, before applying them in large-scale studies.

Only a handful of biomarkers can adequately reflect true dietary intake and can be used to validate specific dietary assessment instruments. They are qualified as 'recovery biomarkers' [8] and are specifically: energy (doubly labeled water), nitrogen, potassium and sodium (24 hour urinary excretion). Even if they do not relate directly to intakes of F&V or fish due to complex metabolic regulations and influence of individual characteristics [9], plasma levels of β-carotene, vitamin C [10-13] and polyunsaturated fatty acids [14;15] have proven to be reliable 'concentration biomarkers' of intake. This means that they can be used to capture the validity of reported intake of F&V and fish, respectively.

Most epidemiological studies on large populations to date have used FFQs because traditional diet records or 24h recalls by a dietitian require substantial logistic resources. The Internet, among other new technologies, may help overcome logistical and cost issues by the implementation of web-based self-administered instruments. However, very few studies have evaluated the validity of Internet-based dietary data collection tools in regards to F&V intake [16;17] and to our knowledge no study focused on validating fish intake with such a tool. NutriNet-Santé is a French web-based prospective cohort study that aims to investigate the relationship between nutrition and health [18]. Diet is assessed by three non-consecutive records at baseline and at each year of follow-up. Dietary records are self-administered through a specific web-based tool, which has shown high agreement (median intra-class correlation and Pearson's correlation 0.7-0.8) with an interview with a dietitian [19].

In a companion paper [20], it was shown that the web-based repeated non-consecutive-day DR tool used in the NutriNet-Santé cohort study performs well in estimating protein, potassium and sodium intake, with correlations of 0.61, 0.78 and 0.47 for men and 0.64, 0.42 and 0.37 for women, respectively. In the present study the aim was to investigate the validity of intake of F&V and fish and of a range of micronutrients reported through three web-based self-administered dietary records (DRs) against corresponding concentration biomarkers.

METHODS

Study population and ethics statement

Participants were a sample of volunteers from the NutriNet-Santé study, an on-going web-based cohort study launched in France in May 2009, whose aims and methods have been described elsewhere [18;21]. Using a dedicated website, adult volunteers (aged >18 years) are followed for at least 10 years (recruitment still on-going). Informed consent is obtained electronically from all participants. All procedures were approved by the International Research Board of the French Institute for Health and Medical Research (IRB Inserm No.

0000388FWA00005831) and the French National Information and Citizen Freedom Committee "CNIL" (No. 908450 and 909216). Briefly, at the beginning of the study, participants complete a set of questionnaires assessing demographic, socioeconomic and lifestyle factors, dietary intake (three DRs), physical activity (PA), anthropometry and health status. Dietary intake is evaluated again every year and questionnaires on health status are sent on a regular basis.

Among participants of the NutriNet-Santé study living in Paris and greater area (chosen for logistical reasons), a total of 1400 randomly selected participants stratified by sex, age (<45y, >45y) and educational level (primary and secondary up to some college, university graduate) were invited by e-mail to take part in the dietary validation study. The objective was to include 200 stable-weight participants, free from chronic disease in the NutriNet-Santé Dietary Validation Study. For enrolment in the NutriNet-Santé study, they had to have at least basic computer knowledge and no difficulty in understanding or reading French language. The ancillary protocol of the NutriNet-Santé Dietary Validation Study was approved by the Consultation committee for the Protection of Participants in Biomedical Research of Paris Saint-Louis (No. 2011/22) and the "CNIL" (DR-2012-467). Participants provided written informed consent at their first visit.

Study design

Study schematic of the NutriNet-Santé Dietary Validation Study is presented in **Figure 1**. Recruitment was carried out between October 2012 and April 2013. The study consisted of two visits at the clinical center (Hôtel Dieu hospital, Paris) in a fasting state (at least 6 hours). At the first visit, a blood sample was drawn and clinical measurements were taken. Two questionnaires were given to complete at home (paper, self-administered) before the second visit. The first was a physical activity questionnaire (PAQ) on occupational, transport and leisure time physical activity during the last 4 weeks. The second was a food propensity questionnaire (FPQ) on usual consumption (frequency, no quantity) of 11 major

food groups over the last year. The DR days were scheduled in advance (1 weekend day, 2 week days) over the following 2 weeks. To complete the three DRs, a specific login and password were given to the participants. The second visit was scheduled approximately 3 weeks after the first visit, where participants provided a second fasting blood specimen. Between the two visits, three DRs were self-administered on the specific web-based tool, with a time-lag of approximately 2 weeks between first and third DR. These procedures correspond to the design in the NutriNet-Santé study. Participants received a €100 (US$110) incentive after the second visit.

Dietary data collection

The web-based tool is designed for self-administration and based on a secured user-friendly interface, designed by Medical Expert Systems MXS © (Paris, France). Participants report all foods and beverages (type and quantity) consumed at each meal (breakfast, lunch, dinner) or any other eating occasion. The system allows logging in on the day to fill the questionnaire straight away and access to the questionnaire is maintained open for two weeks. Participants first fill out a list of every food item consumed at an eating occasion that they can find through two ways: a food browser (foods are grouped by category) or a search engine that accepts spelling errors. Then portion sizes are estimated with the help of photographs, derived from a previously validated picture booklet [22]. It represents more than 250 generic foods, corresponding to more than 2000 specific food items, presented in three different portion sizes (A, B, C) and allows to choose also from two intermediate (e.g. between A and B) and two extreme portions (smaller than A, greater than C), hence there are seven choices of amounts. Participants could also enter the specific quantity consumed in grams or by volume, or use purchased units or standard household units (e.g. teaspoon, tablespoon). To avoid omissions, prompting is integrated, similar to the additional questions asked by trained dietitians when performing an interview for a 24h dietary recall to identify missing foods and food details. For each participant, daily nutrient intakes were calculated using the ad-hoc

NutriNet-Santé composition table [23] that links each item reported in the DR to its nutrient content. This includes energy, macronutrients, specific fatty acids and cholesterol, dietary fiber and 26 vitamins and minerals and consists primarily of available public data on French food composition [24]. An intake below 500kcal/day for women, or 800kcal/day for men was considered implausible and excluded [25] and the final analyses included only participants with at least two valid DRs.

Food items were grouped into broad categories as described [23]: the food groups used for the present validation study were fruits, vegetables, total fish and fatty fish. Fruits included whole fruits as well as the fruit part of mixed dishes containing fruit, e.g. the apples in an apple tart. Vegetables did not include potatoes, pulses, or other starchy vegetables [26] and the same rule was applied to take into account the part of vegetables from soup and other mixed dishes. Fatty fish included anchovies, haddock, herring, mackerel, sardine, salmon, tuna and trout.

The FPQ gives information on frequency of consumption of the following food groups over the last 12 months: bread and cereals (4 items); rice, pasta, potatoes (6 items); vegetables (1 "overall consumption" then 9 more detailed items, some of which take into account the season of consumption); meat, poultry and meat products (9 items); fish and other seafood (1 overall, then 6 subcategories including a fatty fish one); eggs and egg products (3 items); dairy products (8 with 1 on ice creams divided in two according to the season); fruits (1 overall and 7 subcategories divided in two according to the season); sweets and cakes (7 items); non-alcoholic beverages (4 items); alcoholic beverages (4 items). For each of the 82 items, participants indicated their frequency of consumption out of 8 possible choices ranging from never to every day.

Biomarker assessment

Participants were instructed to be fasting for at least 6 hours if their visit was in the morning, 4 hours if it was in late morning or afternoon and to limit their fat and sugar intake at their last meal. Blood samples were drawn in two 9mL vacutainers. One tube was immediately centrifuged (to obtain plasma), while the other was allowed to clot for 30 minutes at room temperature before centrifugation (serum). For vitamin C assessment, plasma was diluted (1:10) with a 5% metaphosphoric acid solution. Plasma and serum aliquots were then stored at -80°C. All frozen samples were shipped to Grenoble Hospital in May 2013 where assays were conducted.

Lipids were extracted from aliquots of plasma with hexane/isopropanol (3:2, v:v), saponified with NaOH in dry methanol at 100°C, and the fatty acids were methylated with boron trifluoride (14%) in methanol. The fatty acid methyl esters were quantified by gas chromatography using a capillary column (AT-WAX polar 30 m length, 0.25 mm i.d., film thickness 0.25 µm), and hydrogen as carrier gas. Peak identification was made by comparison of their elution times with that of a mixture of commercial standards. Fatty acid composition was expressed as absolute values and as percentages of the total area of all fatty acid peaks. The coefficients of variation were <12.8% for C20:5 n-3 (EPA), <6.7% for C22:5 n-3 (DPA) and <10.0% for C22:6 n-3 (DHA). Plasma vitamin C was assessed using fluorometric determination by high-performance liquid chromatography (HPLC). β-carotene was measured with a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific Courtaboeuf France).

Covariate assessment

Recent dietary supplements' use, frequency, brand name, active components and doses were determined by written questionnaire and participants were asked to bring packaging of consumed supplements to the visit in order to assess their composition precisely. We identified 5 types of dietary supplements commonly used: multivitamin, containing vitamin C, containing beta-carotene, fish oil/omega 3 and vitamin D/calcium.

Height was measured for participants without wearing shoes by a trained technician with a wall-mounted stadiometer to the nearest 0.5 cm [27]. Weight (to the nearest 0.1 kg) of participants wearing underwear only was measured with a calibrated impedance body composition analyzer (BC-418MA, TANITA ©, Tokyo, Japan). Body mass index (BMI) was calculated as the weight (kg) divided by the squared height (m^2).

Statistical analysis

Study participants' characteristics (mean ± SD or n, %) were compared by sex with t-tests or chi² tests, as appropriate.

All intake and biomarker values were natural-log transformed to improve normality. To correct for inflated within-person variance, we calculated usual intakes of fruit, vegetables, fish and fatty fish (3 DRs), using the method proposed by the National Cancer Institute: the SAS macros %MIXTRAN followed by %INDIVINT[5;28;29]. The percentages of non-consumers for fish ranged from 61% to 68% for each DR, 77% to 81% for fatty fish, 17% to 26% for fruit and 4% to 8% for vegetables. They can therefore be considered 'episodically-consumed' food groups, and a two-part model was fit. The first part considers the probability of consumption, including the frequency variable for the corresponding group in the FPQ [30], calculated to reflect the frequency of consumption of portions per day (which could be <1). The following individual characteristics likely to influence usual intake [31] were used as covariates: age, sex, BMI and educational level. The second part of the model considers the consumption/day amount and allows for the previously listed covariates. Usual intake of vitamin C, β-carotene and fatty acids (3 DRs), as well as 'usual status' in these nutrients (2 blood samples), were also estimated using the second part of the model consisting of the consumption/day only [30]. The effect of whether the DR was performed on a weekend and participant's perception that the DR day represents usual intake or not was also explored by incorporating these covariates in the models.

Comparisons between men and women's usual intakes, adjusted for age, BMI, educational level, were obtained from the amount part of the mixed model performed by the %MIXTRAN macro. Comparisons of biomarker levels were performed using ANCOVA, further adjusted for smoking status and dietary supplement use.

To assess the validity of the dietary record tool, we calculated Spearman's rank correlation coefficients, crude and adjusted (partial correlations) for age (continuous), BMI category (normal weight <25, overweight 25-29.9, obese≥30 kg/m^2), tobacco smoking (never, former, current smoker), educational level (up to high school, some college, university graduate), energy intake (by the residual method), alcohol consumption on the 3 days of DR (yes/no) and specific use of dietary supplements (yes/no). Total serum cholesterol (continuous) was further accounted for in analyses on β-carotene [32]. To interpret the correlation coefficients, conventional values 0.20 to 0.40 were deemed weak, 0.40 to 0.60 moderate and ≥0.60 high [33]. We expected the correlations to be weak to moderate because the strength of the correlation with concentration biomarkers is most often <0.60 [34].

Spearman's correlation coefficients between dietary intake of F&V and plasma vitamin C and β-carotene were calculated. Regarding fish and fatty fish consumption, the correlations with plasma fatty-acid composition of total and specific n-3 polyunsatured fatty acids (n-3 PUFAs), namely c20:5 n-3 (eicosapentaenoic acid, EPA) and C22:6 n-3 (docosahexaenoic acid, DHA), were calculated. All results are presented separately for men and women. Finally, to investigate the role of individual factors, we further stratified analyses by age category (<50y, ≥50y), BMI category (<25, ≥25), educational level (up to some college, university graduate), smoking status (current vs other) and supplement user.

All analyses were performed using SAS version 9.3 (released July 2011, SAS Institute, Inc., Cary, NC, USA).

RESULTS

Participant characteristics

Of the 1400 individuals contacted by e-mail, 237 (16.9%) responded favorably to enrolment. Of these, 7 (3%) were ineligible and 31 (13%) were not able to attend the planned visits. One man had 2 implausible DRs, and hence was excluded, leaving a sample of 198 participants for analyses. Of these, 195 (98.5%) had 3 valid DRs and 3 participants had only 2 valid DRs. All participants had available data for biomarkers at at least one time point: 3 participants had only one blood draw hence only one time point for all biomarkers, an additional 2 participants had only one assay on β-carotene and 29 on vitamin C. The median number of days between the first and third DR was 15 days. Participants' characteristics are presented in **Table 1**. The most frequent types of supplements used were multivitamins (36%) and bone health related supplements (vitamin D and calcium, 22%). Fish oil/n-3 PUFA supplements were consumed by only 7% (data not shown).

Food group and nutrient intakes

As shown in **Table 2**, men reported higher intakes than women of all food groups except fish, irrespective of age, BMI and educational level, but none of the differences were statistically significant. Vitamin C, β-carotene, total n-3 PUFA were also lower in women than men (only statistically significant for total n-3 PUFA), whereas EPA, DPA and DHA intakes were slightly higher.

Plasma biomarkers

Table 3 shows that vitamin C, total n-3 PUFA, EPA and DHA plasma concentrations were higher among women than men, with the difference in vitamin C particularly strong (1.088 vs 0.943 mg/dL [61.8 vs 53.6 μmol/L]).

Correlation

The Spearman's rank correlation coefficients between specific food group intakes and plasma biomarkers are depicted in **Figure 2**. Regarding F&V in relation with vitamin C and β-carotene (6 comparisons), the median crude correlation was 0.39 for men and 0.29 for

women, ranging from 0.14 (vegetables and vitamin C, NS p=0.16) to 0.48 (fruits and vitamin C) in men, and from 0.09 (vegetables and vitamin C, NS p=0.38) to 0.41 (fruits and β-carotene) in women. For fish and fatty fish intake in relation to plasma fatty acids (6 comparisons), the median crude correlation was 0.35 in men and 0.28 in women, ranging from 0.34 (fish and EPA) to 0.44 (fish and DHA) in men, and from 0.11 (fatty fish and EPA, NS p=0.29) to 0.40 (fish and DHA) in women. Adjustment for intra-individual variability and age, BMI, smoking status, alcohol use and dietary supplement use (and serum cholesterol for correlation with β-carotene) produced fairly similar coefficients for the F&V intakes with plasma vitamin C, but notably weaker for the correlation with plasma β-carotene in men. In contrast, for both total fish and fatty fish with plasma EPA and DHA, a great improvement in correlations was observed after correcting for intra-individual variability alone (data not shown), as well as also taking other parameters into account.

Regarding 5 nutrient intakes in relation with their respective biomarker, results are presented in **Figure 3**; median crude correlations were 0.43 for men (ranging from 0.29 for total n-3 PUFA to 0.56 for vitamin C) and 0.37 for women (ranging from 0.32 for vitamin C to 0.42 for DHA). After adjustment, these coefficients remained similar, but were weaker for β-carotene in men and for DHA in women.

Spearman's correlations were also calculated individually for each single DR with their respective biomarker: for each food group or nutrient, these coefficients varied across three DRs, and the average of these values was always lower than the correlation of the usual intake.

Individual factors influencing the correlations

All results for influencing factors are presented in the **On-line Supplemental tables**. Regarding educational level (Supplemental Table 1a), the more educated showed higher correlation coefficients for vegetables and plasma β-carotene than correlations in those in the less educated category, but the opposite was found for fruits and vitamin C in men. Overall

(for men and women combined), higher correlations between fish intake and circulating EPA and DHA were observed in those in the more educated group than in those in the less educated group.

Regarding age (Supplemental Table 1b), no clear differences were present for results in men, but there were higher correlations across all food group intakes in women over the age of 50y compared to <50y.

When stratified by BMI categories (Supplemental Table 1c), men of normal weight (BMI<25) showed either equivalent or slightly higher correlations than their overweight counterparts (BMI≥25), whereas there were higher coefficients in overweight women than in the normal weight group.

Smokers (Supplemental Table 1d) showed higher adjusted correlations than the non-smokers, for both men and women. Finally, no clear trend was observed in the crude correlations between non-consumers and consumers of dietary supplements (Supplemental Table 1e), but the adjusted correlations were overall lower for all nutrients and food groups in men and higher in women for non-consumers compared to consumers.

The effect of other variables (weekend, perception of DR being representative of usual intake) that could potentially influence the probability or amount of intake was also explored. None of these covariates were significantly associated for most food groups and nutrients and adding them in the model did not modify substantially the correlation findings.

DISCUSSION

In the present study we could assess the validity of reported usual intake of some food group intakes, namely F&V and fish, as well as micronutrients (vitamin C, β-carotene and n-3 PUFAs) based on three non-consecutive web-based self-administered dietary records (DR). Compared with associated plasma biomarkers, Spearman's coefficients showed low to moderate correlations for F&V intake, and moderate correlations for fish intake. Regarding micronutrients, correlations were moderate for vitamin C and β-carotene in men, and for EPA

and DHA in both men and women. These results are encouraging regarding the utility and precision of this web-based self-administered dietary record tool, compared to other existing tools, however they do not guarantee that this tool is giving unbiased estimates of food group and nutrient intake.

Fish and plasma polyunsaturated fatty acid profile

The correlation coefficients for fish and n-3 fatty acid profile observed in the present study are stronger than equivalents reported in the literature. A study using different dietary assessment instruments (1day 24h recall, 7day food diary, FFQ) [35] showed correlations ranging from 0.14 (1 24h recall) to 0.20 (7-d food diary) for total fish and from 0.13 (1 24h recall) to 0.23 (7d food diary) for fatty fish, with rather similar results between men and women. The European Food Consumption Validation (EFCOVAL) study is a trans-European initiative which developed and validated the EPIC-soft program in 11 European countries by administrating 2 non-consecutive 24h recalls, conducted by a dietitian. In the French center of the EFCOVAL study [31], weaker correlations were observed between fish and EPA+DHA measured in plasma phospholipids in men (0.22 crude, 0.27 fully-adjusted for the same factors, namely within-person variability, age, BMI, education, alcohol intake and smoking status), whereas results for women were similar in their study and ours (0.37 crude, 0.55 adjusted). As observed in EFCOVAL, accounting for intra-individual variability improved the correlations. Hence, our short term dietary instrument corrected for intra-individual variability proved to be reliable for reflecting medium to long-term fish intake, as demonstrated by the moderate correlations with EPA and DHA plasma concentrations.

The adjusted correlations were notably weaker for women when we considered only fatty fish (≈0.3) compared to total fish intake (≈0.5), whereas they were fairly similar between fish and fatty fish in men. Women reported higher total fish intake but lower fatty fish intake, whereas their average plasma n-3 PUFA concentration was higher than in men. This difference between men and women is unlikely to be explained by dietary supplement use (2

men and 2 women reported consuming fish oil supplements), hence the higher n-3 PUFA status and lower fatty fish intake seem to imply that women underreported their fatty fish intake. Some vegetable oils such as rapeseed oil are rich in c18:3 n-3 (ALA), which can be converted into EPA [14], and could therefore influence EPA plasma status. However, women reported lower vegetable oil intake than men (7.0 vs 14.4 g/day), but this lower amount may also be due to under-reporting. Indeed, a phenomenon of stigmatization of consumption of some fat-rich foods may exist and influence intake of these in women more than in men, due to societal pressure to be slim [36]. Finally, as shown by lower correlation coefficients, misreporting of fatty fish intake was more common among young women (<50y), in those of normal weight (BMI<25) and lower educational level.

Fruits & vegetables and β-carotene

Overall, the correlations between F&V and β-carotene in the present study are stronger than correlations observed using one 24h diet recall in EPIC [37], which ranged from 0.11 to 0.16. Here crude correlations between F&V intake and plasma β-carotene were similar between men and women, but weaker for men after adjustment, which was similar to the trend observed in male participants of the French center in EFCOVAL [31].

Fruits & vegetables and vitamin C

Because vitamin C is labile, its assessment in plasma requires stabilization by metaphosphoric acid before storage and cautious handling [38], hence for logistic purpose it is a less commonly used biomarker. However it has proven to be an interesting concentration biomarker of fruits and vegetables intake [11]. To our knowledge, no recent study in adults has shown correlation between F&V intake assessed by short-term instruments (such as 24h recall or record) and plasma vitamin C. We observed a moderate correlation for fruit intake in men (crude: 0.48) and a weak correlation in women (0.32), but correlations for vegetable intake were non-significant. These results are consistent with those of a community-based study of French adults, where diet was estimated by a FFQ administered by a dietitian, showing

correlations for fruits but not vegetables [12], as well as in a European study on adolescents [39]. Given that we excluded potatoes from our definition of vegetables, and potato consumption is an important source of vitamin C in the diet (18% in our study), this may explain why the correlations between total vitamin C intake and plasma vitamin C were stronger than coefficients for vegetable intake and plasma vitamin C.

Micronutrient intakes and respective plasma biomarkers

The coefficients we observed are slightly lower than those summarized in a recent review when usual n-3 PUFA intake was assessed by FFQ [15]: 0.42 for EPA and 0.44 for DHA, but our method still produced correlations within the moderate range (0.35 to 0.45) which suggests that 3 repeated DRs perform as well as an FFQ for the estimation of usual n-3 PUFA intake, after taking into account intra-individual variability.

Regarding vitamin C, the coefficients observed in the present study showed moderate correlations for men (0.58) and weak for women (0.32). Our method therefore recorded vitamin C intake more effectively in men, but less so in women, compared to previous validation studies where vitamin C intake measured over several diet records (up to 14 days) was correlated with plasma vitamin C (r=0.35 for men, 0.41 for women) [38].

Finally, we observed acceptable correlations of our methods for β-carotene (adjusted r=0.37 in men; 0.38 in women). A recent study aiming at validating web-based self-administered 24h recalls (four non-consecutive days) in the United States, focused on carotenoid intakes and concentration biomarkers, and reported weaker correlations than those observed in this study, ranging from 0.03 (African Americans) to 0.38 (Whites) [16].

Strengths and limitations

Our study is the first to assess validity of specific food groups and micronutrient intakes estimated by an online dietary record tool in comparison to their respective concentration biomarkers. Furthermore, it was possible to correct for within-individual error using repeated measurements, not only with DRs but also using blood biomarkers. An

important strength is the quality and precision of biomarker measurements (two time points, all analyses performed in one laboratory) and of the dietary data collected (specifically designed online tool, ad-hoc nutrient database). A further novel aspect of this study, compared to many recent validation studies, is the assessment of vitamin C status, which allowed us to observe moderate correlations between usual intakes estimated through 3 DRs and plasma vitamin C.

A major weakness is the use of concentration biomarkers, which may not reflect dietary intake directly, but also depend on individual and lifestyle parameters. Indeed, vitamin C, β-carotene and n-3 PUFA status can be influenced by age, body weight, smoking status and alcohol consumption [12;40-42]. Furthermore, blood lipids, especially cholesterol, are important determinants of plasma carotenoids [43]. To overcome this issue we calculated adjusted mean intakes and adjusted correlation coefficients, taking age, BMI, smoking status, alcohol consumption and cholesterol into account, hence the adjusted results may be more informative on the validity of the DR tool for its intended measurements. Also, nutritional status can be influenced by the use of dietary supplements, such as multivitamins, carotenoids or fish oil, but we took this parameter into account in the analyses. Finally, generalizability of our results is subject to caution as participants in this study were paid volunteers and displayed different demographic and lifestyle characteristics from the non-respondent. However, our random sampling strategy allowed obtaining a wide spectrum of age, educational level and an equal number of men and women.

Conclusion

In the present validation study of three non-consecutive DRs, self-administered through a specific online tool used in the French NutriNet-Santé study, we observed moderate correlations between self-reported fruits and vegetables intake and plasma β-carotene and vitamin C, between fish and plasma n-3 polyunsatured fatty acids, and between micronutrient intake and their plasma biomarkers. There is a need to develop new methods able to

414 objectively assess unbiased estimates of dietary intake. However, this web-based tool
415 provides substantial logistic and cost saving to collect dietary data on large populations (the
416 NutriNet-Santé study includes more than 150 000 participants). It appears to have acceptable
417 validity for assessing intake of specific food groups and micronutrients, although caution is
418 advised regarding the generalizability of these findings to other foods, nutrients and to the
419 general population.

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Table captions

Table 1. Characteristics of the Participants in the NutriNet-Santé Dietary Validation Study, France, 2012-2013

Table 2. Food and nutrient intake based on three diet records, NutriNet-Santé Dietary Validation Study, France, 2012-2013

Table 3. Plasma biomarkers based on two fasting blood draws, 3 weeks apart, NutriNet-Santé Dietary Validation Study, France, 2012-2013

Figure captions

Figure 1. Schematic of the NutriNet-Santé Dietary Validation Study, France, 2012-2013

Figure 2. Spearman's correlation coefficients between food groups reported intake (3 DRs) and plasma biomarkers, NutriNet-Santé Dietary Validation Study, France, 2012-2013.

Abbreviations*:* CI, confidence interval; DHA, docosahexaenoic acid (C22:6 n-3); EPA, eicosapentaenoic acid (20:5 n-3); r, Spearman correlation coefficient

[a] Spearman's correlation coefficient between crude mean food group intake and mean biomarker value (log-transformed values)

[b] Spearman's correlation coefficient (r) and 95% Confidence Interval (95% CI)

[c] Spearman's correlation coefficient for usual intake, i.e. de-attenuated for within-person variation, and adjusted for energy (residuals method), age, BMI, education, smoking status, alcohol consumption and specific dietary supplement use. Further adjustment was made for cholesterol for analyses with β-carotene.

[d] eicosapentaenoic acid (C20:5 n-3)

[e] docosahexaenoic acid (C22:6 n-3)

Figure 3. Spearman's correlation coefficients between nutrient reported intake (3 DRs) and corresponding plasma biomarkers, NutriNet-Santé Dietary Validation Study, France, 2012-2013

Abbreviations*:* CI, confidence interval; DHA, docosahexaenoic acid (C22:6 n-3); EPA, eicosapentaenoic acid (20:5 n-3); r, Spearman correlation coefficient

[a] Spearman's correlation coefficient on crude mean nutrient intake and mean biomarker value (log-transformed values)

[b] Spearman's correlation coefficient (r) and 95% Confidence Interval (95% CI)

[c] Spearman's correlation coefficient for usual intake, i.e. de-attenuated for within-person variation, and adjusted for energy (residuals method), age, BMI, education, smoking status, alcohol consumption and specific dietary supplement use. Further adjustment was made for cholesterol for analyses with β-carotene

[d] n-3 polyunsaturated fatty acid

[e] eicosapentaenoic acid (C20:5 n-3)

[f] docosahexaenoic acid (C22:6 n-3)

Online supplemental material

Supplemental Table 1a. Spearman's correlation coefficients between food intakes and respective biomarkers, according to educational level, NutriNet-Santé Dietary Validation Study

	Men											
	Crude [a]						Adjusted [b]					
	Up to some college n=54			University graduates n=49			Up to some college n=54			University graduates n=49		
	r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]	
F&Vs [e] and vitamin C	0.49	0.25	0.67	0.27	-0.01	0.51	0.48	0.22	0.67	0.26	-0.04	0.52
Fruits and vitamin C	0.61	0.40	0.75	0.34	0.06	0.56	0.60	0.39	0.76	0.28	-0.02	0.53
Vegetables and vitamin C	0.15	-0.12	0.41	0.17	-0.12	0.43	0.15	-0.14	0.41	0.16	-0.14	0.44
F&Vs [e] and β-carotene	0.35	0.09	0.56	0.57	0.35	0.74	0.26	-0.02	0.51	0.49	0.22	0.69
Fruits and β-carotene	0.24	-0.03	0.47	0.44	0.18	0.64	0.20	-0.09	0.46	0.40	0.12	0.63
Vegetables and β-carotene	0.32	0.06	0.54	0.52	0.28	0.70	0.26	-0.03	0.50	0.36	0.07	0.60
Fish and EPA [f]	0.30	0.04	0.53	0.40	0.13	0.61	0.41	0.14	0.62	0.46	0.19	0.67
Fish and DHA [g]	0.47	0.23	0.65	0.40	0.14	0.61	0.50	0.26	0.69	0.52	0.26	0.71
Fish and EPA+DHA [f,g]	0.36	0.11	0.58	0.44	0.18	0.64	0.47	0.22	0.66	0.56	0.32	0.74
Fatty fish and EPA [f]	0.33	0.07	0.55	0.34	0.07	0.57	0.45	0.19	0.65	0.37	0.08	0.60
Fatty fish and DHA [g]	0.43	0.18	0.63	0.27	-0.02	0.51	0.41	0.15	0.62	0.37	0.08	0.60
Fatty fish and EPA+ DHA [f,g]	0.37	0.11	0.58	0.35	0.08	0.58	0.45	0.19	0.65	0.43	0.15	0.65
	Women											
	Up to some college n=43			University graduates n=52			Up to some college n=43			University graduates n=52		
F&Vs [e] and vitamin C	0.21	-0.10	0.48	0.37	0.11	0.59	0.22	-0.11	0.51	0.38	0.10	0.60
Fruits and vitamin C	0.27	-0.03	0.53	0.36	0.10	0.58	0.30	-0.03	0.57	0.37	0.09	0.60
Vegetables and vitamin C	0.03	-0.27	0.33	0.25	-0.03	0.49	0.00	-0.32	0.32	0.17	-0.13	0.44
F&Vs [e] and β-carotene	0.47	0.19	0.67	0.29	0.02	0.52	0.47	0.18	0.69	0.04	-0.25	0.32
Fruits and β-carotene	0.57	0.33	0.74	0.23	-0.05	0.47	0.53	0.26	0.73	0.02	-0.27	0.30
Vegetables and β-carotene	0.19	-0.12	0.46	0.30	0.02	0.53	0.21	-0.12	0.50	0.12	-0.17	0.40
Fish and EPA [f]	0.19	-0.12	0.46	0.44	0.18	0.63	0.32	0.00	0.58	0.54	0.30	0.72
Fish and DHA [g]	0.18	-0.13	0.46	0.53	0.30	0.70	0.26	-0.07	0.53	0.59	0.36	0.75
Fish and EPA+DHA [f,g]	0.22	-0.09	0.49	0.48	0.24	0.67	0.32	0.00	0.58	0.58	0.35	0.74
Fatty fish and EPA [f]	0.08	-0.23	0.37	0.20	-0.08	0.45	0.42	0.12	0.65	0.40	0.12	0.61
Fatty fish and DHA [g]	0.03	-0.28	0.32	0.34	0.07	0.56	0.33	0.01	0.59	0.37	0.09	0.59
Fatty fish and EPA+ DHA [f,g]	0.09	-0.22	0.38	0.26	-0.02	0.50	0.37	0.06	0.62	0.38	0.11	0.60

[a] Spearman’s correlation coefficient on crude nutrient intake and mean biomarker value (log-transformed values)

[b] Spearman’s correlation coefficient for usual intake, i.e. de-attenuated for within-person variation, and adjusted for energy (residuals method), age, BMI, smoking status, alcohol consumption and specific dietary supplement use. Further adjustment for cholesterol for analyses with β-carotene.

[c] correlation coefficient

[d] 95% confidence interval;

[e] Fruits and vegetables

[f] eicosapentaenoic acid (C20:5 n-3)

[g] docosahexaenoic acid (C22:6 n-3)

Supplemental Table 1b. Spearman's correlation coefficients between food intakes and respective biomarkers, according to age category, NutriNet-Santé Dietary Validation Study

	Men											
	Crude [a]						Adjusted [b]					
	Age <50y n=50			Age ≥50y n=53			Age <50y n=50			Age ≥50y n=53		
	r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]	
F&Vs [e] and vitamin C	0.53	0.30	0.71	0.36	0.10	0.57	0.47	0.20	0.67	0.38	0.11	0.61
Fruits and vitamin C	0.60	0.38	0.75	0.46	0.22	0.65	0.55	0.30	0.73	0.53	0.29	0.71
Vegetables and vitamin C	0.19	-0.09	0.45	0.16	-0.12	0.41	0.21	-0.09	0.48	0.21	-0.08	0.47
F&Vs [e] and β-carotene	0.44	0.18	0.64	0.47	0.23	0.66	0.34	0.05	0.58	0.46	0.20	0.66
Fruits and β-carotene	0.34	0.06	0.56	0.32	0.06	0.54	0.24	-0.06	0.50	0.39	0.11	0.61
Vegetables and β-carotene	0.41	0.15	0.62	0.39	0.14	0.60	0.24	-0.06	0.50	0.40	0.12	0.61
Fish and EPA [f]	0.34	0.07	0.57	0.37	0.11	0.58	0.40	0.13	0.62	0.37	0.10	0.59
Fish and DHA [g]	0.44	0.18	0.64	0.46	0.22	0.65	0.59	0.36	0.75	0.47	0.21	0.66
Fish and EPA+DHA [f,g]	0.43	0.17	0.63	0.40	0.15	0.61	0.57	0.34	0.74	0.45	0.19	0.65
Fatty fish and EPA [f]	0.32	0.05	0.55	0.37	0.11	0.58	0.44	0.17	0.65	0.37	0.09	0.59
Fatty fish and DHA [g]	0.30	0.03	0.53	0.42	0.17	0.62	0.50	0.25	0.69	0.36	0.09	0.59
Fatty fish and EPA+ DHA [f,g]	0.33	0.06	0.56	0.39	0.14	0.60	0.54	0.29	0.72	0.40	0.13	0.61
	Women											
	Age <50y n=40			Age ≥50y n=55			Age <50y n=40			Age ≥50y n=55		
F&Vs [e] and vitamin C	0.32	0.01	0.58	0.34	0.08	0.55	0.15	-0.20	0.47	0.26	-0.03	0.50
Fruits and vitamin C	0.35	0.04	0.60	0.37	0.12	0.58	0.29	-0.05	0.57	0.12	-0.16	0.39
Vegetables and vitamin C	0.11	-0.21	0.41	0.13	-0.14	0.38	-0.01	-0.35	0.33	0.14	-0.15	0.40
F&Vs [e] and β-carotene	0.13	-0.18	0.43	0.31	0.05	0.53	-0.11	-0.43	0.23	0.29	0.01	0.52
Fruits and β-carotene	0.14	-0.18	0.43	0.43	0.18	0.62	-0.01	-0.34	0.33	0.31	0.03	0.54
Vegetables and β-carotene	0.19	-0.13	0.47	0.15	-0.12	0.40	-0.05	-0.38	0.29	0.23	-0.05	0.48
Fish and EPA [f]	0.08	-0.23	0.38	0.49	0.26	0.67	0.26	-0.08	0.54	0.57	0.35	0.73
Fish and DHA [g]	0.20	-0.12	0.48	0.56	0.34	0.72	0.21	-0.13	0.51	0.64	0.44	0.78
Fish and EPA+DHA [f,g]	0.14	-0.18	0.43	0.55	0.33	0.71	0.26	-0.08	0.54	0.65	0.46	0.79
Fatty fish and EPA [f]	-0.06	-0.36	0.26	0.32	0.06	0.54	-0.03	-0.36	0.30	0.54	0.31	0.71
Fatty fish and DHA [g]	0.19	-0.12	0.48	0.33	0.07	0.55	0.11	-0.23	0.43	0.55	0.32	0.72
Fatty fish and EPA+ DHA [f,g]	0.08	-0.24	0.38	0.34	0.08	0.55	0.04	-0.29	0.37	0.57	0.34	0.73

[a] Spearman’s correlation coefficient on crude nutrient intake and mean biomarker value (log-transformed values)

[b] Spearman’s correlation coefficient for usual intake, i.e. de-attenuated for within-person variation, and adjusted for energy (residuals method), age, BMI, smoking status, alcohol consumption and specific dietary supplement use. Further adjustment for cholesterol for analyses with β-carotene.

[c] correlation coefficient

[d] 95% confidence interval;

[e] Fruits and vegetables

[f] eicosapentaenoic acid (C20:5 n-3)

[g] docosahexaenoic acid (C22:6 n-3)

Supplemental Table 1c. Spearman's correlation coefficients between food intakes and respective biomarkers, according to BMI category, NutriNet-Santé Dietary Validation Study

Men												
	Crude [a]						Adjusted [b]					
	BMI<25 n=63			BMI≥25 n=40			BMI<25 n=63			BMI≥25 n=40		
	r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]	
F&Vs [e] and vitamin C	0.40	0.17	0.59	0.44	0.15	0.66	0.42	0.17	0.61	0.40	0.07	0.65
Fruits and vitamin C	0.51	0.30	0.67	0.55	0.29	0.74	0.49	0.27	0.67	0.52	0.22	0.73
Vegetables and vitamin C	0.13	-0.13	0.37	0.13	-0.19	0.42	0.19	-0.08	0.43	0.17	-0.18	0.48
F&Vs [e] and β-carotene	0.52	0.32	0.68	0.38	0.08	0.62	0.40	0.15	0.60	0.54	0.25	0.74
Fruits and β-carotene	0.49	0.27	0.65	0.29	-0.02	0.55	0.35	0.10	0.56	0.36	0.02	0.62
Vegetables and β-carotene	0.36	0.12	0.56	0.39	0.08	0.62	0.32	0.06	0.53	0.54	0.25	0.74
Fish and EPA [f]	0.45	0.23	0.63	0.16	-0.16	0.45	0.53	0.32	0.69	0.22	-0.12	0.52
Fish and DHA [g]	0.49	0.28	0.66	0.36	0.06	0.61	0.60	0.40	0.74	0.49	0.18	0.71
Fish and EPA+DHA [f,g]	0.50	0.29	0.67	0.24	-0.08	0.51	0.61	0.43	0.75	0.40	0.08	0.65
Fatty fish and EPA [f]	0.43	0.21	0.62	0.18	-0.14	0.46	0.46	0.23	0.64	0.23	-0.11	0.52
Fatty fish and DHA [g]	0.39	0.16	0.58	0.27	-0.04	0.54	0.44	0.21	0.62	0.45	0.13	0.68
Fatty fish and EPA+ DHA [f,g]	0.43	0.21	0.62	0.21	-0.11	0.49	0.50	0.28	0.67	0.41	0.09	0.65
Women												
	BMI<25 n=67			BMI≥25 n=28			BMI<25 n=67			BMI≥25 n=28		
F&Vs [e] and vitamin C	0.19	-0.05	0.41	0.46	0.10	0.71	0.18	-0.07	0.42	0.49	0.09	0.76
Fruits and vitamin C	0.25	0.01	0.46	0.46	0.11	0.71	0.24	-0.01	0.46	0.39	-0.04	0.70
Vegetables and vitamin C	0.00	-0.24	0.24	0.31	-0.07	0.61	0.02	-0.23	0.27	0.47	0.06	0.75
F&Vs [e] and β-carotene	0.34	0.11	0.54	0.37	-0.01	0.65	0.36	0.12	0.56	0.51	0.12	0.76
Fruits and β-carotene	0.38	0.15	0.57	0.46	0.11	0.71	0.29	0.05	0.51	0.46	0.06	0.73
Vegetables and β-carotene	0.22	-0.02	0.43	0.21	-0.17	0.54	0.32	0.07	0.52	0.47	0.07	0.74
Fish and EPA [f]	0.19	-0.05	0.42	0.53	0.19	0.75	0.30	0.05	0.51	0.72	0.45	0.88
Fish and DHA [g]	0.35	0.12	0.54	0.48	0.14	0.73	0.41	0.18	0.60	0.62	0.27	0.82
Fish and EPA+DHA [f,g]	0.27	0.03	0.48	0.54	0.21	0.76	0.40	0.17	0.59	0.68	0.37	0.85
Fatty fish and EPA [f]	0.03	-0.21	0.27	0.29	-0.10	0.59	0.11	-0.15	0.35	0.51	0.13	0.76
Fatty fish and DHA [g]	0.23	-0.02	0.44	0.22	-0.17	0.55	0.26	0.01	0.48	0.41	0.00	0.70
Fatty fish and EPA+ DHA [f,g]	0.13	-0.11	0.36	0.26	-0.12	0.58	0.19	-0.06	0.42	0.47	0.07	0.74

[a] Spearman’s correlation coefficient on crude nutrient intake and mean biomarker value (log-transformed values)

[b] Spearman’s correlation coefficient for usual intake, i.e. de-attenuated for within-person variation, and adjusted for energy (residuals method), age, BMI, smoking status, alcohol consumption and specific dietary supplement use. Further adjustment for cholesterol for analyses with β-carotene.

[c] correlation coefficient

[d] 95% confidence interval;

[e] Fruits and vegetables

[f] eicosapentaenoic acid (C20:5 n-3)

[g] docosahexaenoic acid (C22:6 n-3)

Supplemental Table 1d. Spearman's correlation coefficients between food intakes and respective biomarkers, according to smoking category, NutriNet-Santé Dietary Validation Study

	Men											
	Crude [a]						Adjusted [b]					
	Non smoker n=91			Current Smoker n=12			Non smoker n=91			Current Smoker n=12		
	r [c]			95% CI [d]			r [c]			95% CI [d]		
F&Vs [e] and vitamin C	0.39	0.20	0.55	0.32	-0.31	0.76	0.38	0.19	0.55	0.54	-0.26	0.90
Fruits and vitamin C	0.46	0.28	0.61	0.42	-0.20	0.80	0.48	0.29	0.62	0.43	-0.39	0.87
Vegetables and vitamin C	0.17	-0.03	0.37	-0.24	-0.71	0.39	0.18	-0.04	0.38	0.25	-0.55	0.81
F&Vs [e] and β-carotene	0.45	0.27	0.60	0.43	-0.20	0.80	0.40	0.21	0.57	0.44	-0.39	0.87
Fruits and β-carotene	0.37	0.17	0.53	0.21	-0.42	0.70	0.33	0.13	0.51	0.33	-0.49	0.84
Vegetables and β-carotene	0.36	0.17	0.53	0.37	-0.26	0.78	0.32	0.12	0.50	0.85	0.37	0.97
Fish and EPA [f]	0.34	0.14	0.51	0.24	-0.38	0.72	0.40	0.20	0.56	0.26	-0.49	0.79
Fish and DHA [g]	0.40	0.22	0.56	0.53	-0.06	0.85	0.47	0.29	0.62	0.71	0.10	0.93
Fish and EPA+DHA [f,g]	0.38	0.19	0.54	0.49	-0.12	0.83	0.46	0.28	0.61	0.59	-0.13	0.90
Fatty fish and EPA [f]	0.32	0.13	0.50	0.38	-0.24	0.78	0.43	0.24	0.59	0.12	-0.59	0.73
Fatty fish and DHA [g]	0.34	0.14	0.51	0.31	-0.32	0.75	0.38	0.19	0.55	0.60	-0.10	0.90
Fatty fish and EPA+ DHA [f,g]	0.34	0.14	0.51	0.38	-0.24	0.78	0.46	0.28	0.61	0.45	-0.31	0.86
	Women											
	Non smoker n=79			Current Smoker n=16			Non smoker n=79			Current Smoker n=16		
F&Vs [e] and vitamin C	0.28	0.07	0.48	0.20	-0.33	0.63	0.26	0.04	0.46	0.50	-0.14	0.85
Fruits and vitamin C	0.32	0.10	0.50	0.37	-0.16	0.73	0.31	0.09	0.51	0.50	-0.14	0.85
Vegetables and vitamin C	0.12	-0.10	0.33	0.15	-0.37	0.60	0.08	-0.15	0.30	0.33	-0.33	0.78
F&Vs [e] and β-carotene	0.38	0.17	0.55	0.43	-0.08	0.76	0.31	0.08	0.50	0.46	-0.20	0.83
Fruits and β-carotene	0.38	0.18	0.56	0.57	0.10	0.83	0.25	0.02	0.45	0.76	0.30	0.93
Vegetables and β-carotene	0.25	0.03	0.45	0.40	-0.12	0.75	0.24	0.02	0.45	0.13	-0.51	0.68
Fish and EPA [f]	0.33	0.12	0.52	0.21	-0.32	0.64	0.47	0.27	0.63	0.35	-0.28	0.77
Fish and DHA [g]	0.37	0.16	0.54	0.60	0.15	0.84	0.47	0.28	0.63	0.55	-0.04	0.85
Fish and EPA+DHA [f,g]	0.37	0.17	0.55	0.44	-0.07	0.77	0.51	0.32	0.66	0.52	-0.07	0.84
Fatty fish and EPA [f]	0.11	-0.11	0.32	0.02	-0.48	0.51	0.26	0.04	0.46	0.54	-0.05	0.85
Fatty fish and DHA [g]	0.16	-0.06	0.37	0.54	0.07	0.82	0.29	0.07	0.49	0.76	0.33	0.93
Fatty fish and EPA+ DHA [f,g]	0.15	-0.08	0.36	0.25	-0.28	0.66	0.28	0.05	0.47	0.78	0.38	0.94

[a] Spearman’s correlation coefficient on crude nutrient intake and mean biomarker value (log-transformed values)

[b] Spearman’s correlation coefficient for usual intake, i.e. de-attenuated for within-person variation, and adjusted for energy (residuals method), age, BMI, smoking status, alcohol consumption and specific dietary supplement use. Further adjustment for cholesterol for analyses with β-carotene.

[c] correlation coefficient

[d] 95% confidence interval;

[e] Fruits and vegetables

[f] eicosapentaenoic acid (C20:5 n-3)

[g] docosahexaenoic acid (C22:6 n-3)

Supplemental Table 1e. Spearman's correlation coefficients between food intakes and respective biomarkers, according to supplement use, NutriNet-Santé Dietary Validation Study

	Men											
	Crude [a]						Adjusted [b]					
	Non consumer n=78			Consumer n=25			Non consumer n=78			Consumer n=25		
	r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]		r [c]	95% CI [d]	
F&Vs [e] and vitamin C	0.41	0.21	0.58	0.44	0.05	0.72	0.42	0.21	0.59	0.72	0.41	0.88
Fruits and vitamin C	0.48	0.28	0.63	0.53	0.16	0.77	0.50	0.31	0.66	0.75	0.47	0.90
Vegetables and vitamin C	0.11	-0.12	0.32	0.19	-0.23	0.55	0.08	-0.15	0.31	0.51	0.08	0.78
F&Vs [e] and β-carotene	0.48	0.29	0.64	0.45	0.06	0.72	0.42	0.21	0.59	0.36	-0.09	0.69
Fruits and β-carotene	0.30	0.08	0.49	0.53	0.18	0.77	0.32	0.10	0.51	0.44	0.00	0.74
Vegetables and β-carotene	0.43	0.23	0.60	0.19	-0.22	0.54	0.26	0.03	0.46	0.26	-0.20	0.63
Fish and EPA [f]	0.25	0.03	0.45	0.65	0.35	0.83	0.33	0.11	0.52	0.62	0.26	0.83
Fish and DHA [g]	0.44	0.24	0.60	0.47	0.09	0.73	0.50	0.30	0.65	0.57	0.18	0.80
Fish and EPA+DHA [f,g]	0.32	0.11	0.51	0.63	0.31	0.82	0.41	0.20	0.59	0.71	0.40	0.87
Fatty fish and EPA [f]	0.29	0.07	0.48	0.49	0.12	0.74	0.39	0.18	0.57	0.48	0.06	0.76
Fatty fish and DHA [g]	0.38	0.18	0.56	0.24	-0.17	0.58	0.42	0.22	0.59	0.44	0.00	0.73
Fatty fish and EPA+ DHA [f,g]	0.32	0.11	0.51	0.43	0.04	0.70	0.44	0.23	0.60	0.56	0.17	0.80
	Women											
	Non consumer n=61			Consumer n=34			Non consumer n=61			Consumer n=34		
F&Vs [e] and vitamin C	0.23	-0.03	0.45	0.28	-0.07	0.56	0.27	0.00	0.49	0.08	-0.29	0.44
Fruits and vitamin C	0.29	0.05	0.51	0.31	-0.03	0.59	0.32	0.06	0.53	0.20	-0.18	0.53
Vegetables and vitamin C	0.09	-0.17	0.33	0.17	-0.18	0.48	0.10	-0.17	0.36	-0.06	-0.42	0.31
F&Vs [e] and β-carotene	0.43	0.19	0.61	0.32	-0.02	0.60	0.38	0.13	0.59	0.11	-0.27	0.46
Fruits and β-carotene	0.43	0.19	0.61	0.40	0.07	0.65	0.35	0.10	0.56	0.21	-0.17	0.54
Vegetables and β-carotene	0.27	0.02	0.49	0.21	-0.14	0.51	0.26	-0.01	0.49	-0.02	-0.38	0.35
Fish and EPA [f]	0.32	0.07	0.53	0.37	0.04	0.63	0.54	0.33	0.70	0.48	0.14	0.71
Fish and DHA [g]	0.40	0.17	0.60	0.40	0.07	0.65	0.51	0.29	0.68	0.44	0.09	0.69
Fish and EPA+DHA [f,g]	0.37	0.13	0.57	0.43	0.11	0.67	0.55	0.34	0.71	0.50	0.17	0.73
Fatty fish and EPA [f]	0.05	-0.20	0.30	0.18	-0.17	0.49	0.35	0.10	0.56	0.36	0.00	0.64
Fatty fish and DHA [g]	0.27	0.01	0.48	0.16	-0.19	0.47	0.43	0.19	0.62	0.31	-0.06	0.60
Fatty fish and EPA+ DHA [f,g]	0.15	-0.10	0.39	0.21	-0.14	0.51	0.40	0.15	0.60	0.36	0.00	0.64

[a] Spearman’s correlation coefficient on crude nutrient intake and mean biomarker value (log-transformed values)

[b] Spearman’s correlation coefficient for usual intake, i.e. de-attenuated for within-person variation, and adjusted for energy (residuals method), age, BMI, smoking status, alcohol consumption and specific dietary supplement use. Further adjustment for cholesterol for analyses with β-carotene.

[c] correlation coefficient

[d] 95% confidence interval;

[e] Fruits and vegetables

[f] eicosapentaenoic acid (C20:5 n-3)

[g] docosahexaenoic acid (C22:6 n-3)

Table 1. Characteristics of the Participants in the NutriNet-Santé Dietary Validation Study, France, 2012-2013 [a]

	Men n=103		Women n=95		
	Mean	SD	Mean	SD	P-value [b]
Age (y)	50.2	16.2	50.7	16.8	0.82
BMI [c] (kg/m²)	24.1	2.9	23.9	4.2	0.59
LTPA [d] (MET-h/week)	35.6	30.0	21.4	21.9	0.0002
HDL [e] (mg/dL)	54.7	11.1	66.4	13.8	<.0001
LDL [f] (mg/dL)	125.0	32.7	123.4	30.3	0.68
Cholesterol (mg/dL) [g]	199.0	38.3	207.2	35.5	0.12
Dietary intake [h]					
Energy (kcal/day)	2408.1	585.5	1714.2	414.9	<.0001
Carbohydrate density [i]	42.2	6.7	41.2	6.9	0.31
Protein density [i]	16.6	3.5	17.8	3.8	0.03
Total fat density [i]	40.9	6.6	40.7	6.9	0.84
Alcohol (g/day)	13.9	16.4	7.3	8.6	0.001
Dietary fiber (g/day)	24.7	9.6	20.0	6.0	<.0001
	N	%	n	%	P-value [b]
Use of dietary supplement	25	24.3	34	35.8	0.07
Alcohol use	71	68.9	63	66.3	0.69
Fish consumer [j]	65	63.1	68	71.6	0.20
Fatty fish consumer [j]	49	47.6	42	44.2	0.64
Fruit consumer [j]	92	89.3	94	99.0	0.005
BMI category					0.001
Underweight (<18.5)	1	1.0	7	7.4	
Normal (18.5-24.9)	62	60.2	60	63.2	
Overweight (25-29.9)	37	35.6	17	17.9	
Obese (≥30)	3	2.9	11	11.6	
Tobacco smoking					0.39
Smoker - regularly	9	8.7	10	10.5	
Smoker - occasionally	3	2.9	6	6.3	
Former smoker	38	36.9	26	27.4	
Never smoker	53	51.5	53	55.8	
Living with a partner	68	66.0	53	55.8	0.14
Education					0.58
Up to high school	21	20.4	18	18.9	
Some college	33	32.0	25	26.3	
University graduate	49	47.6	52	54.7	

[a] Adapted with permission from Lassale C, Castetbon K, Laporte F et al. *Br J Nutr*. 2015;113:953-962
[b] P-value for the difference between men and women, t-test or chi² tests as appropriate
[c] BMI, body mass index
[d] LTPA, leisure time physical activity;
[e] HDL, high density lipoprotein cholesterol. To convert mg/dL HDL to mmol/L, multiply mg/dL by 0.0259. To convert mmol/L HDL to mg/dL, multiply mmol/L by 38.68. HDL of 54.8 mg/dL= 1.40 mmol/L.

[f] LDL, low density lipoprotein cholesterol. To convert mg/dL LDL to mmol/L, multiply mg/dL by 0.0259. To convert mmol/L LDL to mg/dL, multiply mmol/L by 38.68. LDL of 126.0 mg/dL= 3.26 mmol/L.

[g] To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.0259. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.68. Cholesterol of 200.0 mg/dL= 5.17 mmol/L.

[h] Mean intake calculated from 3 DRs

[i] % of energy intake (excluding alcohol)

[j] Based on 3 DRs: non-consumers have not consumed the food at any of the 3 DR

Table 2. Food and nutrient intake based on three diet records, NutriNet-Santé Dietary Validation Study, France, 2012-2013

	Men n=103		Women n=95		
	Mean (95% CI)	Adjusted mean (95% CI) [a]	Mean (95% CI)	Adjusted mean (95% CI) [a]	P-value [b]
Food groups					
Fruits (g/day)	207.6 (178.3 - 236.8)	205.1(177.5- 232.8) [c]	185.8 (155.4 - 216.2)	192.5(166.5- 218.5) [c]	0.08
Vegetables (g/day)	244.9 (220.9 - 268.9)	247.0(230.1- 263.9) [c]	228.8 (203.8 - 253.8)	235.1(219.2- 251.0) [c]	0.16
Fish (g/day)	34.5 (26.4 - 42.6)	32.7(28.5- 36.9) [c]	38.9 (30.4 - 47.3)	36.0(32.1- 40.0) [c]	0.56
Fatty fish (g/day)	19.9 (13.9 - 25.9)	20.1(17.8- 22.5) [c]	17.1 (10.9 - 23.4)	15.7(13.5- 17.9) [c]	0.40
Nutrients	Mean (95% CI)	Adjusted mean (95% CI) [a]	Mean (95% CI)	Adjusted mean (95% CI) [a]	P-value [b]
Vitamin C (mg/d)	127.2 (114.5 - 139.8)	123.1(111.5- 134.7)	111.8 (98.7 - 125.0)	111.6(100.7- 122.6)	0.09
β-carotene (µg/d)	4175.6 (3594.5 - 4756.8)	4133.5(3768.5-4498.6)	3562.5 (2957.3 - 4167.6)	3523.2(3179.6-3866.8)	0.07
Total n-3 PUFA (mg/d) [d]	1880.6 (1691.7 - 2069.6)	1883.0(1786.9-1979.0)	1514.9 (1318.1 - 1711.6)	1449.0(1358.6-1539.4)	0.0001
EPA (c20:5 n-3) (mg/d) [e]	213.6 (158.8 - 268.3)	129.4(103.1- 155.7)	176.5 (119.5 - 233.6)	136.9(112.1- 161.6)	0.38
DPA (c22:5 n-3) (mg/d) [f]	124.5 (71.3 - 177.8)	68.8(59.2- 78.4)	145.8 (90.4 - 201.3)	78.7(69.6- 87.7)	0.17
DHA (c22:6 n-3) (mg/d) [g]	288.0 (225.5 - 350.6)	208.9(171.8- 246.0)	242.9 (177.8 - 308.0)	213.7(178.8- 248.6)	0.51

[a] Usual intake calculated with the %MIXTRAN and %INDIVINT macro, using sex, age, BMI and educational level as covariates in a one-part model unless otherwise stated. Means presented here are further adjusted for tobacco smoking and specific dietary supplement use.

[b] P-value of the effect of sex from the "amount" part of model calculated with %MIXTRAN.

[c] Variance-reduced means calculated using a two-part model where the first part considers the probability of consumption, using the variable "frequency of consumption" from the food propensity questionnaire. The following other covariates were used in both parts of the model: sex, age, BMI and educational level.

[d] n-3 polyunsaturated fatty acid

[e] Eicosapentaenoic acid

[f] Docosapentaenoic acid

[g] Docosahexaenoic acid

Table 3. Plasma biomarkers based on two fasting blood draws, 3 weeks apart, NutriNet-Santé Dietary Validation Study, France, 2012-2013

	Men n=103		Women n=95		
	Geometric unadjusted mean (95% CI)	Adjusted Mean (95% CI) [a]	Geometric unadjusted mean (95% CI)	Adjusted Mean (95% CI) [a]	P-value [b]
Vitamin C (mg/dL) [c]	0.968 (0.913 - 1.023)	0.943 (0.889 - 1)	1.107 (1.051 - 1.163)	1.088 (1.035 - 1.14)	<.0001
β-carotene (µg/dL) [d]	40.01 (34.42 - 45.59)	40.92 (34.05 - 47.79)	45.16 (39.31 - 50.96)	46.02 (39.52 - 52.46)	0.19
Total n-3 PUFA [e] (mg/dL)	18.13 (16.99 - 19.27)	17.87 (16.61 - 19.14)	20.17 (18.98 - 21.35)	20.06 (18.87 - 21.25)	0.003
EPA (c20:5 n-3) (mg/dL) [f]	5.02 (4.47 - 5.57)	4.9 (4.35 - 5.45)	5.54 (4.97 - 6.11)	5.44 (4.92 - 5.96)	0.08
DPA (c22:5 n-3) (mg/dL) [g]	1.85 (1.77 - 1.94)	1.84 (1.74 - 1.94)	1.83 (1.74 - 1.92)	1.82 (1.73 - 1.92)	0.75
DHA (c22:6 n-3) (mg/dL) [h]	8.52 (7.98 - 9.07)	8.17 (7.51 - 8.84)	10.23 (9.67 - 10.8)	10 (9.38 - 10.63)	<.0001

[a] Variance-reduced mean biomarker value, calculated with the %MIXTRAN and %INDIVINT macro, using sex, age, BMI and educational level as covariates in a one-part model. Means presented here are further adjusted for tobacco smoking and specific dietary supplement use.

[b] P-value of the analysis of covariance on the adjusted means.

[c] To convert mg/dL vitamin C to µmol/L, multiply µmol/L by 56.78

[d] To convert µg/dL β-carotene to µmol/L, multiply µmol/L by 0.0186

[e] n-3 polyunsaturated fatty acid

[f] To convert mg/dL eicosapentaenoic acid (EPA) to µmol/L, multiply µmol/L by 33.11

[g] To convert mg/dL docosapentaenoic acid (DPA) to µmol/L, multiply µmol/L by 30.30

[h] To convert mg/dL docosahexaenoic acid (DHA) to µmol/L, multiply µmol/L by 30.49