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ORIGINAL COMMUNICATION

Validation of a food-frequency questionnaire assessment of carotenoid and vitamin E intake using weighed food records and plasma biomarkers: The method of triads model

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Background: Reliability or validity studies are important for the evaluation of measurement error in dietary assessment methods. An approach to validation known as the method of triads uses triangulation techniques to calculate the validity coefficient of a food-frequency questionnaire (FFQ).

Objective: To assess the validity of an FFQ estimates of carotenoid and vitamin E intake against serum biomarker measurements and weighed food records (WFRs), by applying the method of triads.

Design: The study population was a sub-sample of adult participants in a randomised controlled trial of β-carotene and sunscreen in the prevention of skin cancer. Dietary intake was assessed by a self-administered FFQ and a WFR. Nonfasting blood samples were collected and plasma analysed for five carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene) and vitamin E. Correlation coefficients were calculated between each of the dietary methods and the validity coefficient was calculated using the method of triads. The 95% confidence intervals for the validity coefficients were estimated using bootstrap sampling.

Results: The validity coefficients of the FFQ were highest for α-carotene (0.85) and lycopene (0.62), followed by β-carotene (0.55) and total carotenoids (0.55), while the lowest validity coefficient was for lutein (0.19). The method of triads could not be used for β-cryptoxanthin and vitamin E, as one of the three underlying correlations was negative.

Conclusions: Results were similar to other studies of validity using biomarkers and the method of triads. For many dietary factors, the upper limit of the validity coefficients was less than 0.5 and therefore only strong relationships between dietary exposure and disease will be detected.

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Keywords: validity coefficient; dietary assessment; biomarkers; food-frequency questionnaire; weighed food record; method of triads; carotenoids; vitamin E

Introduction

Assessment of long-term dietary intake has generally been associated with significant measurement errors. Reliability or validity studies can be used to determine the validity coefficient and quantify the degree of measurement error in the dietary assessment method (Armstrong et al, 1992). Most studies assess validity through the comparison of two methods containing errors that are likely to be correlated, resulting in distorted measures of validity (Armstrong et al, 1992).
Kaaks (1997) describes an approach to measurement of validity using biomarkers. They recommend that when determining the validity coefficient of the questionnaire measurement ($\rho_{QT}$), at least two additional measurements of dietary intake are necessary, for example, biomarker measurements and weighed food records or 24-h recalls. The triangular approach to validation known as the ‘method of triads’ uses the correlations between each of the three methods to calculate the validity coefficient. The following equation is used to estimate the validity coefficient for the questionnaire:

$$\rho_{QT} = \sqrt{\rho_{QB} \times \rho_{QB}}$$

(1)

where: $\rho_{QT}$ is the validity coefficient for the questionnaire, $\rho_{QB}$ the correlation between the FFQ and the biomarker, $\rho_{QB}$ the correlation between weighed food record and biomarker and $\rho_{QB}$ the correlation between FFQ and the weighed food record.

This method has been described in further detail by Ocke and Kaaks (1997). The major assumption of this method is that the correlations between the three measurements are explained by the fact that they are all linearly correlated with the true intake and that their errors are independent. The errors of biomarkers and of questionnaire measurements or food records can be considered independent and uncorrelated, but since questionnaires and food records may have common sources of error, the errors may be positively correlated. Consequently, the correlation coefficient between the questionnaire and the food record will be over-estimated. It is suggested that the value calculated by the method of triads should be used as the upper limit of the true validity coefficient, that is, $\rho_{QT} < \rho_{QT(trib)}$ and a range for the validity coefficient can be established by using the correlation between the questionnaire measurement and the biomarker measurement as an estimate of the lower limit of the validity coefficient, that is $\rho_{QB} < \rho_{QT} < \rho_{QT(trib)}$ (Ocke and Kaaks, 1997).

Although the method of triads was described a number of years ago there have been few applications within dietary validation studies. Many studies have used biomarkers for validation of dietary intake; however, they have applied the traditional model of validation using only two dietary methods (for example, biomarkers vs an FFQ) and few studies have interpreted their results with respect to the validity coefficient. Currently, only three published studies have applied the method of triads to the validation of dietary data using serum/plasma biomarkers (Ocke and Kaaks, 1997, Daures et al, 2000, Kabagambe et al, 2001).

The objective of this study was to determine the validity of the estimates of carotenoids and vitamin E intake from the FFQ used in the Nambour Skin Cancer Study using data from serum biomarker measurements and WFRs applying the method of triads. This study is among the first to use this method to investigate carotenoids other than β-carotene and vitamin E.

**Methods**

**Subjects**

The main focus of the Nambour Skin Cancer Study was a randomised controlled field trial of β-carotene and sunscreen use for the prevention of keratinocytic skin cancer conducted in the township of Nambour, 100 km north of Brisbane (Green et al, 1994; 1999). In 1992, prior to the beginning of the trial (baseline), a subset of the Nambour Skin Cancer Study group was randomly selected to participate in a dietary intake validation study (Ashton et al, 1996). A total of 168 subjects were invited into the study, with 115 subjects completing both the FFQ and the WFRs, a response rate of 69%. A subset of these 115 subjects (selected randomly) also had blood samples taken and analysed. A sample of 30 subjects had blood samples analysed for carotenoids, vitamin E and total cholesterol and therefore had dietary exposure data available from all three methods. Subjects were excluded from this analysis if they did not respond to over 90% of items on the FFQ ($n = 1$) or if they completed less than 10 days of recording in the WFRs ($n = 1$).

**Food-frequency questionnaire**

Dietary intake was assessed by a self-administered, semi-quantitative FFQ at baseline in 1992. The FFQ was based on a questionnaire designed for use in the United States (Willett et al, 1985) and adapted for use in Queensland by the Nutrition Program, University of Queensland. The questionnaire consisted of 129 specific foods or food groups (including 20 fruit items and 26 vegetable and legume items) with nine response options ranging from ‘never’ to ‘4+ times per day’ for the frequency of consumption of specified serving sizes. Questions on cooking methods, specific types of fats, oils, margarines, breakfast cereals, takeaway foods and self-prescribed nutritional supplements were also included on the questionnaire. Subjects were asked to recall their frequency of consumption over the preceding six months.

**Weighed food records**

Subjects completed records on two nonconsecutive days, every 2 months for 1 year during 1993. Starting days were randomly allocated in the initial recording period and then the rest of the week was worked through in subsequent recording periods so that each day of the week was recorded at least once. Detailed instructions on use of the scales and the weighing and recording of food and drink consumed including leftovers were provided for the participants during the initial interview. Participants were asked to record information on time and place of food and meal preparation, brand names of products and recipes used. For meals eaten away from home, participants were asked to provide detailed descriptions and approximate amounts of what was
eaten. Details of any self-prescribed nutritional supplements consumed (other than the trial supplement) were also recorded.

**Serum biomarkers**

Nonfasting venous blood samples were collected at baseline in 1992 using standard venipuncture techniques performed by experienced phlebotomists. Blood samples were processed at the time of collection and serum samples were stored in approximately 1 ml aliquots at −70°C until analysis.

Measurement of carotenoids, vitamin E and cholesterol was conducted by Queensland Health Pathology Services at the Royal Brisbane Hospital. Measurement of carotenoids and vitamin E was conducted simultaneously using the method of Sowell et al. (1994). Serum was treated with ethanol to precipitate proteins, and the carotenoids and vitamin E were extracted using petroleum ether and analysed by reverse-phase high-performance liquid chromatography (HPLC) using acetonitrile:ethanol (60:40) with 0.1 ml/l triethylamine on a Waters Novapak column. Values labelled as lutein represent lutein and zeaxanthin as these were not separated in the HPLC analysis. Total cholesterol was measured using an enzymatic colorimetric test (Siedel et al., 1983; Katterman et al., 1984). This is based on the determination of Δ⁴-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of the cholesterol by cholesterol oxidase and measurement by the Trinder reaction of the hydrogen peroxide formed using photometric methods.

**Analysis**

Dietary intake of carotenoids and vitamin E was calculated using international food composition data (United States Department of Agriculture, 1998; United States Department of Agriculture, 1999) as these dietary factors were not available in the standard Australian database (National Food Authority, 1995). Nutrient intake from food and the consumption of dietary supplements was analysed using software and a supplements database (Ashton et al., 1997) developed by the Nutrition Program, University of Queensland.

Dietary intake was calculated as intake from diet alone and as intake from diet and supplements for those dietary factors affected by supplement use. Total carotenoids were calculated as the sum of the five individual carotenoids for both serum and dietary intakes. Energy intakes were investigated but no extreme values as defined by Willett (1998) were identified.

Intakes of dietary factors calculated from the FFQ and the WFRs were adjusted for energy intake using the nutrient residual method described by Willett (1998). It is recommended that if dietary intakes are to be adjusted for energy intake in studies of diet–disease relationships, measurements of validity should also be adjusted for energy intake (Willett, 2001). A similar method of adjustment commonly used to adjust serum carotenoids and vitamin E for cholesterol was applied in the current study due to the underlying relationship between serum carotenoids and vitamin E, and serum cholesterol (Stryker et al., 1990; Mandel et al., 1997; Hunter, 1998).

Spearman correlation coefficients were calculated between the three dietary assessment methods (i.e. FFQ and WFR, FFQ and biomarker, WFR and biomarker) for each individual dietary factor. The small number of subjects precluded the analysis being undertaken separately for males and females and across age groups or other potential confounding factors (e.g. smoking status). Analyses were performed using SPSS for Windows Version 10.0.5 (SPSS Inc., 1999).

The correlations between each of the three dietary methods were used to calculate the validity coefficient via the method of triads, using Equation (1). This is interpreted as the upper limit of the validity coefficient whereas the correlation between the biomarker and the FFQ is interpreted as the lower limit of the validity coefficient. The 95% confidence intervals for the validity coefficients were estimated using bootstrap sampling where 1000 samples of equal size (n = 28) were obtained with replacement from the study subjects (Ocke & Kaaks, 1997; Kabagambe et al., 2001).

**Results**

Data for carotenoids and vitamin E for all three dietary exposure measurements were available on 28 subjects. Table 1 provides details on the general characteristics of the study group including age, gender, body mass index (BMI), smoking status and supplement use.

Table 2 presents the mean and standard deviation of the serum biomarker measurements of each of the five carotenoids, vitamin E and cholesterol. Table 3 presents the mean, standard deviation and range of intakes of the dietary factors from the WFRs and the FFQs. The mean FFQ intakes were consistently higher than the mean intakes based on the

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 (39.3%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17 (60.7%)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>Mean ± s.d.</td>
<td>48.0 ± 10.5</td>
</tr>
<tr>
<td>Range</td>
<td>27.0–69.0</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean ± s.d.</td>
<td>25.7 ± 3.8</td>
</tr>
<tr>
<td>Range</td>
<td>19.6–34.7</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>Non-smoker</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>10 (35.7%)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>3 (10.7%)</td>
<td></td>
</tr>
<tr>
<td>Supplement use</td>
<td>Any supplement</td>
<td>11 (39.3%)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>2 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>9 (32.1%)</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index; s.d., standard deviation.

*Based on data collected as part of the FFQ.
WFR. Carotenoid intakes based on the FFQ varied from 1.6-fold higher for lycopene to 3.1-fold higher for lutein compared to intake on the WFR. Vitamin E intakes based on the FFQ were 1.6-fold higher than on the WFR.

Intake of dietary factors from self-prescribed supplements was also assessed and dietary intakes were calculated based on intake from diet alone and intakes based on diet and supplements. The number of subjects consuming supplements based on the FFQ is shown in Table 1. Supplement intake was also assessed on the WFR and eight subjects reported taking supplements containing vitamin E while no subjects reported taking supplements containing β-carotene. Table 3 includes the total intakes from diet and supplements for those dietary factors affected by supplement use.

The Spearman correlation coefficients between each of the three dietary assessment methods are shown in Table 4. Moderate correlations between the FFQ and the biomarker were shown for α-carotene, β-carotene (diet only), lycopene, total carotenoids and vitamin E (diet and supplements), while poor correlations were shown for β-cryptoxanthin, lutein and vitamin E (diet only). Correlations between the WFR and the biomarkers were moderate for α-carotene, β-carotene, β-cryptoxanthin, lutein, and total carotenoids, while poor correlations were shown for lycopene and vitamin E. The correlations between the WFR and the FFQ were more consistent (r > 0.29) except β-cryptoxanthin and lycopene, which were less than 0.14.

The correlations between each of the three dietary exposure measurements were used to calculate the validity coefficient for the FFQ using the method of triads approach and the results are shown in Table 4 along with the 95% confidence intervals. Table 4 also presents the range for the validity coefficient, where the upper limit is that calculated by the method triads and the lower limit is the correlation between the FFQ and the biomarker as described earlier. However, when using the method of triads, a validity coefficient cannot be calculated if one of the three correlations is negative (Ocke & Kaaks, 1997). Therefore, for β-cryptoxanthin and vitamin E, the method of triads could not be used and only a lower limit for the validity coefficient is presented. The validity coefficients for α-carotene and lycopene (0.85, 0.62) were the highest of all the dietary factors, followed by β-carotene (0.55) and total carotenoids (0.55) while the lowest validity coefficient was for lutein (0.19). The validity coefficients for the biomarker measurements and the WFR have also been presented in Table 4 for completeness. Overall, the validity coefficients for the FFQ were higher than those of the serum biomarkers, however the validity coefficients of the WFR tended to be higher than those of both the FFQ and biomarkers. The correlations between the three methods and the validity coefficients were also investigated when intakes were adjusted for body weight.

### Table 2
Mean, standard deviations and range of the serum biomarkers

<table>
<thead>
<tr>
<th>Serum biomarker (μmol/l)</th>
<th>Mean ± s.d.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-carotene</td>
<td>0.11±0.08</td>
<td>0.01-0.32</td>
</tr>
<tr>
<td>β-carotene</td>
<td>0.62±0.59</td>
<td>0.03-2.70</td>
</tr>
<tr>
<td>β-cryptoxanthin</td>
<td>0.25±0.35</td>
<td>0.02-1.88</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.36±0.45</td>
<td>0.03-2.31</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.17±0.12</td>
<td>0.02-0.61</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>1.51±1.23</td>
<td>0.24-5.22</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>34.8±11.4</td>
<td>20.0-55.0</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.35±1.18</td>
<td>3.61-8.02</td>
</tr>
</tbody>
</table>

Values labelled as lutein represent lutein and zeaxanthin as these were not separated in the HPLC analysis.

### Table 3
Mean, standard deviation and range of intakes of each dietary factor (diet only) as estimated by the WFRs and the FFQ

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>WFR</th>
<th>FFQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s.d.</td>
<td>Range</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>7946±2147</td>
<td>4765-1259</td>
</tr>
<tr>
<td>1601±856</td>
<td>122-3575</td>
<td>4234±2275</td>
</tr>
<tr>
<td>α-Carotene (μg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>4067±2271</td>
<td>623-9429</td>
</tr>
<tr>
<td>Diet + supplements</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>β-cryptoxanthin (μg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>213±214</td>
<td>9-802</td>
</tr>
<tr>
<td>Diet + supplements</td>
<td>523±264</td>
<td>151-1036</td>
</tr>
<tr>
<td>Lutein (μg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>2336±1464</td>
<td>66-7489</td>
</tr>
<tr>
<td>Diet + supplements</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Lycopene (μg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>8741±2937</td>
<td>2775-13956</td>
</tr>
<tr>
<td>Diet + supplements</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Total carotenoids (μg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>7.82±2.44</td>
<td>4.28-14.25</td>
</tr>
<tr>
<td>Diet + supplements</td>
<td>37.69±11.84</td>
<td>4.28-635.69</td>
</tr>
</tbody>
</table>

WFRs, weighed food records; FFQ, food-frequency questionnaire; s.d., standard deviation; N/A, not applicable.

*a* 1 kcal = 4.184 kJ.

*No change in intakes as no subject reported taking supplements containing β-carotene on the WFR.

Values labelled as lutein represent lutein and zeaxanthin as these were not separated in the HPLC analysis.
Table 4 Spearman correlation coefficients between each of the three dietary assessment methods and the validity coefficient as calculated by the method of triads

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Correlation coefficients</th>
<th>FFQ</th>
<th>WFR</th>
<th>Biomarker</th>
<th>Range for the validity coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_{FB}$</td>
<td>$r_{FS}$</td>
<td>$r_{FR}$</td>
<td>$p_{FB}$</td>
<td>$p_{FS}$</td>
</tr>
<tr>
<td>$a$-carotene</td>
<td>0.36</td>
<td>0.19</td>
<td>0.38$^*$</td>
<td>0.85</td>
<td>0.65 - 1.00</td>
</tr>
<tr>
<td>$\beta$-carotene</td>
<td>0.22</td>
<td>0.26</td>
<td>0.36</td>
<td>0.55</td>
<td>0.15 - 1.00</td>
</tr>
<tr>
<td>Diet only Diet + supplements</td>
<td>0.12</td>
<td>0.26$^*$</td>
<td>0.29</td>
<td>0.36</td>
<td>0.06 - 1.00</td>
</tr>
<tr>
<td>$\beta$-cryptoxanthin$^b$</td>
<td>-0.002</td>
<td>0.42$^*$</td>
<td>0.13</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Lutein$^b$</td>
<td>0.03</td>
<td>0.35</td>
<td>0.40$^*$</td>
<td>0.19</td>
<td>0.05 - 0.71</td>
</tr>
<tr>
<td>Lycoene$^c$</td>
<td>0.19</td>
<td>0.07</td>
<td>0.14</td>
<td>0.62</td>
<td>0.12 - 1.00</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>0.28</td>
<td>0.33</td>
<td>0.35</td>
<td>0.55</td>
<td>0.10 - 1.00</td>
</tr>
<tr>
<td>Diet only Diet + supplements</td>
<td>0.18</td>
<td>0.33$^*$</td>
<td>0.31</td>
<td>0.41</td>
<td>0.10 - 1.00</td>
</tr>
<tr>
<td>Vitamin E$^c$</td>
<td>0.05</td>
<td>-0.11</td>
<td>0.57$^*$</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Diet only Diet + supplements</td>
<td>0.31</td>
<td>-0.02</td>
<td>0.34</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

FFQ, food-frequency questionnaire; WFR, weighed food record; $r_{FB}$, correlation between the FFQ and the biomarker; $r_{FS}$, correlation between weighed food record and biomarker; $r_{FR}$, correlation between weighed food record and the FFQ; $p_{FB}$, validity coefficient of the questionnaire; $p_{FS}$, validity coefficient of the biomarker; $p_{FR}$, validity coefficient for the WFR; N/A, not applicable.

$^a$All values > 1.00 were truncated as this is the largest possible value.

$^b$The lower limit is $r_{FB}$ for the FFQ and the biomarker and $r_{FS}$ for the WFR, and the upper limit is calculated by the method of triads.

$^c$p < 0.05.

$^d$No change in correlation between diet only and diet and supplements as no subject reported taking supplements containing $\beta$-carotene on the weighed food records.

$^e$An upper limit for validity coefficient using the method of triads cannot be calculated, as one of the individual correlations is negative.

$^f$Values labelled as lutein represent lutein and zeaxanthin as these were not separated in the HPLC analysis.

Based on calculating intakes per kilogram of body weight. No consistent or substantial differences were shown compared to the energy-adjusted values (data not shown).

**Discussion**

The objective of this study was to determine the validity of the FFQ used in the Nambour Skin Cancer Study using dietary exposure data for five carotenoids and vitamin E available from WFRs and serum biomarkers. The method of triads was used to calculate validity coefficients for each of the dietary factors and provides an upper limit for the validity coefficient while the correlation between the biomarker and the FFQ provides a lower limit for the validity coefficient (Kaaks & Riboli, 1997).

The 95% confidence intervals for the validity coefficients in this study are wide, with the upper limits for the intervals, for all carotenoids except lutein, greater than one, which reflect the small sample size. Heywood cases are those validity coefficients which are greater than one, and occur if the product of two of the correlations between the dietary methods is greater than the third correlation (Ocke & Kaaks, 1997). Heywood cases may be due to either random sampling variations in the correlations between dietary methods or violations in the underlying model assumptions (Ocke & Kaaks, 1997, Daures et al, 2000). The confidence intervals in this study are similar to those shown in other studies using the method of triads (Ocke & Kaaks, 1997; Daures et al, 2000; Kabagambe et al, 2001). Most studies investigating the validity of dietary exposures do not investigate the confidence intervals of their estimates and therefore provide no indication of precision.

The measurement of serum biomarkers in this study was based on a single blood measurement. Van Kappel et al (2001) investigated the reproducibility of serum levels of carotenoids and $\alpha$-tocopherol by taking three blood samples at yearly intervals. They found that a single sample could accurately rank individuals for $a$-carotene, $\beta$-carotene, lutein and $\alpha$-tocopherol.

It is important to note the different reference periods or timeframes for each of the dietary exposure measures in this study. The FFQ and blood measurement were made at approximately the same time at the beginning of 1992. The FFQ asked respondents to consider intake over the previous 6 months and the serum biomarkers are likely to represent the preceding weeks and months of dietary exposure while the WFRs were completed approximately 1y later. Despite differing time frames, relatively good correlations have been shown between the WFRs and the other two dietary exposure measures for some dietary factors and the correlations are within the range found in other dietary validation studies (Willett & Lenart, 1998). However, the correlations shown between the WFRs and the biomarkers may be an underestimate of the true correlations due to the differing time frames.

One of the limitations of this study is the small sample size available for the analysis. Data for all three dietary assessments were available on 28 subjects for the five carotenoids and vitamin E. One of the implications of the small sample size is that it did not allow investigation of the validity across
important subject characteristics such as age, sex and smoking status. This is important as the validity of dietary intake measures has been shown to vary between males and females (Nelson, 1997). Similarly, studies have shown that the correlations between intake and plasma/serum biomarkers are generally lower among non-smokers and has been shown for β-carotene, total carotenoids, β-cryptoxanthin and lutein but not lycopene or vitamin E (Peng et al, 1995; Margetts & Jackson, 1996; EPIC Group of Spain, 1997).

**Carotenoids**

The correlations between the biomarkers and the two dietary assessment methods were similar to those seen in other studies of similar size, with correlations for β-carotene ranging from 0.16 to 0.50 (Le Marchand et al, 1994; Scott et al, 1996; Mandel et al, 1997; Kanetsky et al, 1998). Correlations for other carotenoids were 0.25-0.52 for x-carotene, 0.10-0.64 for lutein, −0.04-0.15 for β-cryptoxanthin and 0.47-0.55 for lycopene (Le Marchand et al, 1994; Scott et al, 1996; Kanetsky et al, 1998).

Comparisons with other studies using the method of triads is limited as only three published studies have applied the method of triads approach using serum/plasma biomarkers, however the results for β-carotene in our study are similar to those of previous studies (Ocke & Kaaks, 1997; Daures et al, 2000; Kabagambe et al, 2001). The study by Kabagambe et al (2001) is currently the only published study to investigate carotenoids other than β-carotene and showed that β-cryptoxanthin had the highest validity. However, no validity coefficient could be calculated for β-cryptoxanthin in the current study, as one of the underlying correlations was negative.

An alternative approach for calculating the upper limit of the validity coefficient for β-cryptoxanthin would be to use the square root of correlation between WFR and FFQ as an approximation of the upper limit as described in the model for validation studies proposed by Armstrong (Armstrong et al, 1992). This would provide a value of 0.36 as the upper limit of the validity coefficient of the FFQ, and therefore the range of the validity coefficient would be −0.002 to 0.36. This provides an advantage over just using the correlation between the FFQ and the biomarker as the lower limit, especially considering the lack of a meaningful correlation between the biomarker and the FFQ.

Serum carotenoid levels are not homeostatically controlled and therefore are largely dependent on the content of the diet (Olson, 1999). However, a range of physiologically related factors may impact on the concentrations in serum and the correlation between dietary intake and serum carotenoids. These include the efficiency of absorption of the carotenoids, metabolism of carotenoids, uptake by body tissues, release from tissues back into plasma and the rates of catabolism (Olson, 1999).

The absorption and bioavailability of carotenoids can vary considerably depending on the food source or food matrix, the presence of promoters or inhibitors and processing factors such as cooking. The carotenoids in orange fruits, sweet potato and pumpkin are present in lipid droplets and are more available than those in green leafy vegetables, which are complexed with proteins, and those in carrots and tomatoes, which are present as crystals (Castenmill & West, 1998; Huang et al, 2000; Ribaya-Mercado, 2002). The amount of dietary fat in the diet is known to impact on the absorption of carotenoids as the formation of micelles in the intestine requires the presence of dietary fat (Van Het Hof et al, 2000b). The presence of protein or lecithin can also improve carotenoid absorption through the enhancement of micelle formation (Castenmill & West, 1998) while fibre has an inhibitory effect (Torzonen et al, 1996). Processing of foods affects carotenoid bioavailability through disruption of cell walls (Van Het Hof et al, 2000b). Heating and homogenisation of tomatoes has been shown to increase the bioavailability of lycopene (Van Het Hof et al, 2000a) while homogenisation of spinach increased the serum response to β-carotene although not all carotenoids were affected equally (Castenmill et al, 1999).

Provitamin A carotenoids undergo metabolism to vitamin A in the intestinal mucosa, irrespective of vitamin A status, and are incorporated into chylomicrons for transport to the liver, whereas non provitamin A carotenoids are absorbed intact (Parker, 1997). The extent and efficiency of metabolism, including the proportion of the ingested carotenoid affected in this way, is not entirely clear. Therefore, serum levels of provitamin A carotenoids may only partially reflect their dietary intake (Crews et al, 2001).

The influence of all these factors means that while it is possible for the estimated intakes of subjects to be equal, the differing bioavailabilities due to the different sources of carotenoids and the processing of foods and the coconsumption of inhibitors and promoters of carotenoid absorption may result in differing observed serum responses between subjects. These differences in bioavailability may also explain the variability in the strength of the correlations between the different carotenoids shown in this study (Van Het Hof et al, 1999).

**Vitamin E**

Serum vitamin E showed good correlations with the FFQ but only when supplement intake was also considered. This is in agreement with other studies that have investigated correlations between serum vitamin E and other measures of dietary intake with correlations ranging from 0.31 to 0.88 in studies of similar size (Portini et al, 1995; Dixon et al, 1996; Booth et al, 1997; Mandel et al, 1997; Kanetsky et al, 1998). However, correlations between the biomarker and WFR were poor regardless of whether supplement intake was included. The difference in correlations between the FFQ and the biomarker, and the WFR and the biomarker suggests that the FFQ provides a better estimate of intake than the WFR, or,
more likely it reflects the congruence between the reference periods.

The method of triads could not be applied to vitamin E as one of the individual correlations was negative and therefore only a lower limit for the validity coefficient was established. As described earlier for β-cryptoxanthin, the square root of correlation between WFR and FFQ could be used as an upper limit of validity coefficient for the FFQ, which would provide an upper limit value of 0.75 for intake from diet alone and 0.58 for intake from diet and supplements.

There are a number of possible reasons for the poor correlations between intake from diet alone and serum vitamin E. Firstly, there were differences in the exposure measures in that dietary intake in this study was calculated as α-tocopherol equivalents, which are based on the amounts and relative activities of the various tocopherols and tocotrienols (United States Department of Agriculture, 1999); however, the biomarker measurement represents serum α-tocopherol only. Similarly, for the subjects consuming supplements, vitamin E from foods does not represent their true exposure. However, subanalysis of the 15 subjects who reported no use of supplements containing vitamin E (on either the FFQ or WFR) showed equally poor correlations (data not shown). An alternative explanation may relate to the underlying nature of the diet-serum relationship. Increased vitamin E intake does lead to increased serum levels over time however studies have tended to use intakes achievable only through use of supplements (Willett et al, 1983; Brown et al, 1994; Meydani et al, 1998). It is possible that there is a poor relationship between intake and serum levels at the range of intakes from diet alone and a good relationship at the range of intakes achieved through dietary supplements use. The strength of the relationship at levels of intake shown by supplement-users may be sufficient to overwhelm the lack of relationship in the range of intakes shown by non-supplement-users. Finally, although the inclusion of supplements results in an extension of the range of intakes, this would not have caused the improvement in correlations as Spearman correlation coefficients were used in the analysis rather than Pearson correlation coefficients which may be susceptible to this effect (Streiner and Norman, 1991; Delcourt et al, 1994).

Conclusions

Many studies of dietary validation compare test and reference measures that contain errors, which are correlated resulting in flawed estimates of validity. It is recognised that biomarkers provide advantages over other dietary assessment with respect to their independent errors and while many studies have used biomarkers as tools for validation, few studies interpret their results in terms of the validity coefficient and few studies have applied the method of triads model. This study is among the first to assess the validity of intake of carotenoids other than β-carotene and vitamin E using the method of triads.

The upper limit of the validity coefficients for the FFQ was less than 0.5 for β-carotene (diet and supplement), lutein, total carotenoids (diet and supplements) and β-cryptoxanthin (if the correlation between the FFQ and WFRs is used as the upper limit). The upper limit for the validity coefficient is above 0.5 for α and β-carotene (diet only), lycopene, total carotenoids (diet only), and vitamin E (if the correlation between the FFQ and WFRs is used as the upper limit). This suggests that for many dietary factors, only strong relationships between exposure and disease will be detected.

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References


