

## Applying the triads method in the validation of dietary intake using biomarkers

Método das tríades na validação do consumo alimentar com biomarcadores

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### Abstract

*The triads method is applied in validation studies of dietary intake to evaluate the correlation between three measurements (food frequency questionnaire, reference method and biomarker) and the true intake using validity coefficients ( $\rho$ ). The main advantage of this technique is the inclusion of the biomarker, which presents independent errors compared with those of the traditional methods. The method assumes the linearity between the three measurements and the true intake and independence between the three measurement errors. Limitations of this technique include the occurrence of  $\rho > 1$ , known as “Heywood case”, and the existence of negative correlations, which do not allow the calculation of  $\rho$ . The objective of this review is to present the concept of the method, describe its application and examine the validation studies of dietary intake that use the triads method. We also conceptualize the “bootstrap” method, used to estimate the confidence intervals of the validity coefficients.*

*Food Consumption; Nutrition Surveys; Validation Studies*

### Introduction

The food frequency questionnaire (FFQ) is the most widely used instrument for the assessment of habitual food intake of a population. A properly validated FFQ for the intended population allows for stratification according to nutritional intake at the reference time considered <sup>1</sup>. Biomarkers offer the possibility of further validation aiming to improve the accuracy of the instrument <sup>2</sup>.

In the process of validating a FFQ, multiple dietary records or 24-hour recalls (24hR) are often used as the method of reference <sup>3</sup>. The application of a reference method is important in order to estimate the errors associated with the FFQ, particularly the attenuation bias caused by random measurement errors of the instrument which will impact in the statistical power of the study <sup>1,3</sup>. The limitations of using dietary measures as the reference method is that both the instrument being tested (FFQ) and the reference method (24hR) are subject to the same random and systematic errors, because they rely on the memory of the interviewee and due to errors related to the estimation of the reported food intake <sup>4,5</sup>.

In this scenario, biological markers may offer advantages and be able to improve the estimates of dietary intake assessment, due to the independence of their random errors in relation to the errors inherent to the intake questionnaires <sup>5</sup>. However, the biomarkers do not replace the tradition-

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al methods of food intake<sup>6</sup>. They should be used as additional measures because not all nutrients have biological markers and many are influenced by factors other than intake, such as bioavailability, metabolism and genetic factors<sup>5,7</sup>. Moreover, most of the biomarker analyses are expensive and quite often it is not possible to perform them as part of a large epidemiological study<sup>6</sup>.

According to Kaaks<sup>4</sup>, when information from the FFQ, 24hR and biological markers measurements are available, the triangulation technique or the “method of triads” may be applied for the validation of the assessment methods of dietary intake. This method allows the comparison of food consumption estimated by the three methods with the true (but unknown) intake by calculating the validity coefficient ( $\rho$ )<sup>4</sup>.

The aim of this review is to present the concept of the method, describe its implementation and examine the studies published from 1995 to 2009 in which the triangulation technique was applied in the validation of nutrient intake. An overview of the “bootstrap” resampling method for the estimation of the 95% confidence intervals (95%CI) of validity coefficients will be presented, as well.

## Method

Considering that the triads method is a relatively recent technique, we chose to extend the search to chapters in books, in addition to the papers published in specialized journals. Concepts, application procedures and validation studies where triangulation method was used were sought. The “bootstrap” technique for the calculation of confidence intervals of the validity coefficients is also reviewed.

We performed a literature search in the MEDLINE and PubMed databases for studies published in English, Spanish and Portuguese between 1995 and 2009, using a database searching filter and manual selection as needed. The keywords “triads method” or “validity coefficient” was combined (AND) with the following terms (in parenthesis are, respectively, the number of titles found with the combination “triads method” AND term; “validity coefficient” AND term): dietary intake (34; 252); dietary assessment (24; 139); biomarkers (52; 125). The combination of the keywords “triads method” AND “validity coefficient” resulted in 21 articles. The search retrieved 647 titles. After eliminating duplicates, a total of 152 abstracts were identified. Using the final inclusion criteria which consisted of original articles that applied the triads method, we found 16 studies, all of which were in-

cluded in this review. For the quality assessment and discussion of the selected articles, we used part of the criteria to evaluate dietary intake validation studies (mainly the sample size criteria) proposed by Serra-Majem et al.<sup>8</sup>. Although it does not consider specific components used in the triads method, this is the first quality criteria tool developed for the assessment of validation studies of dietary questionnaires.

## Results and discussion

### Biomarkers of food intake

According to Kaaks et al.<sup>7</sup>, the biomarkers of dietary intake can be classified into markers based on recovery or on concentration.

- Recovery-based markers are based on precise and quantitative measures of the physiological balance between intake and excretion of a compound. Examples include 24-hour urinary nitrogen (for the intake of protein), urinary excretion of potassium (for potassium intake) and doubly labeled water (for energy expenditure)<sup>7</sup>. Recovery markers have a direct and quantitative relation to nutrient intake. For instance, it is known that for any individual in protein and energy balance, the 24-hour urinary nitrogen represents approximately 80% of the nitrogen intake. On the assumption that only protein contributes significantly to dietary nitrogen content and its concentration in different types of protein is relatively constant, it is possible to estimate the absolute protein consumption of an individual from the quantity of nitrogen excreted in the 24-hour urine samples<sup>7</sup>.
- Concentration-based markers are based on the concentration of a specific compound<sup>7</sup>. The concentration can be measured in biological materials such as blood plasma or serum (carotenoids and tocopherols)<sup>6,9,10</sup>, fractions of blood lipid (fatty acid in phospholipids)<sup>11</sup>, adipose tissue (carotenoids and tocopherols)<sup>6</sup> and urine (nitrogen)<sup>6</sup>. These markers are not expressed in terms of time units, and their quantitative relationship with intake may differ among individuals<sup>7</sup>. Thus, concentration-based biomarkers provide correlations with intake levels but can not be transformed into absolute measures of ingestion<sup>7</sup>. The triads method is being applied mostly in the validation studies that use concentration-based biomarkers.

### Triads method: definition

Kaaks<sup>4</sup> proposed the triads method as a way to validate dietary intake instruments when the

quantitative intake information from the three methods (FFQ, 24hR and biological markers) was available. This method is an application of factor analysis to this specific problem. The idea is that, although it is not possible to directly measure the true intake (the latent variable), it can be estimated by means of FFQ and 24hR indicators, and biological markers, also known as manifest variables <sup>4,12</sup>. The model assumes that the value of each indicator can be decomposed into two components, one associated with the actual intake and the other one to its own specificities. Mathematically, we can write:

$$\begin{aligned} \text{FFQ} &= b_{10} + b_{11} | + e_1; \\ \text{24hR} &= b_{20} + b_{21} | + e_2; \\ \text{Biological markers} &= b_{30} + b_{31} | + e_3. \end{aligned}$$

Where, b denotes coefficients that relates | to FFQ, 24hR and biological markers; and  $e_1, e_2$  and  $e_3$  are the specific factors of each indicator. If the factor of a particular indicator has little variation, it means that this indicator provides a good approximation to the actual intake, *ie*, the correlation between the true intake and the indicator is high <sup>4,12</sup>.

The assumptions for this technique are: the linearity of the relationship between the three variables and the true intake and independence of the specific factors <sup>12</sup>. The assumption of independence implies that the correlations between any pair of variables are due to the relationship between each variable and the actual intake and not due to errors inherent in each assessment instrument (FFQ, 24hR and biological markers) <sup>4,12,13</sup>.

The equations of this technique were generated by the factor analysis model, although they can also be calculated by the structural equation analysis. Figure 1 illustrates the concept of the triads method triads <sup>4,12</sup>.

The validity coefficients ( $\rho$ ) are calculated by the following equations:

$$(1) \rho_{QI} = \sqrt{\frac{r_{QR} \times r_{QB}}{r_{BR}}};$$

$$(2) \rho_{RI} = \sqrt{\frac{r_{QR} \times r_{BR}}{r_{QB}}};$$

$$(3) \rho_{BI} = \sqrt{\frac{r_{BR} \times r_{QB}}{r_{QR}}};$$

Where, B = biological markers; Q = food frequency questionnaire (FFQ); R: 24-hour recalls (24hR).

From these equations, the correlation coefficients ( $r$ ) between variables can be calculated:

$$\begin{aligned} (4) r_{QR} &= \rho_{QI} \times \rho_{RI}; \\ (5) r_{QB} &= \rho_{QI} \times \rho_{BI}; \\ (6) r_{BR} &= \rho_{RI} \times \rho_{BI}. \end{aligned}$$

Where,  $\rho_{QI}$  is the validity coefficient of FFQ in relation to true intake,  $\rho_{RI}$  is the validity coefficient of the reference method in relation to true intake,  $\rho_{BI}$  is the validity coefficient of the biomarker in relation to true intake;  $r_{QR}$  is the correlation coefficient between the estimated intake by FFQ and reference method;  $r_{QB}$  is the correlation coefficient between the estimated intake by FFQ and biomarker and  $r_{BR}$  is the correlation coefficient between the estimated intake by reference method and the biomarker <sup>4,12</sup>.

It should be noted that the correlation coefficient used to calculate the validity coefficient is the Pearson coefficient, when using numerical variables. The Spearman correlation coefficient can also be used when the interest is on the order of the variables <sup>14</sup>. The validity coefficient for the 3 variables ( $\rho_{QI}, \rho_{RI}$  and  $\rho_{BI}$ ) can be calculated using the formula described, with no need for a specific software <sup>12</sup>.

The validity coefficients vary from 0 to 1, which is different from correlations coefficients, which range from -1 to 1. There are no negative validity coefficients, because this calculation includes the square root. In general, the estimated validity coefficient for each variable ( $\rho_{QI}, \rho_{RI}$  and  $\rho_{BI}$ ) is equal to or greater than the correlations between the variables ( $r_{QR}, r_{RB}$  and  $r_{BQ}$ ) <sup>13</sup>.

When  $\rho_{QI}, \rho_{RI}$  and  $\rho_{BI}$  are high in relation to the true intake, it is expected that the correlations between the manifest variables ( $r_{QR}, r_{RB}$  and  $r_{BQ}$ ) are also relatively high. If the apparent correlation between two manifest variables is low, it suggests that at least one of the variables is not a good indicator of the true intake, resulting in low validity coefficient and wider confidence intervals <sup>4,12</sup>.

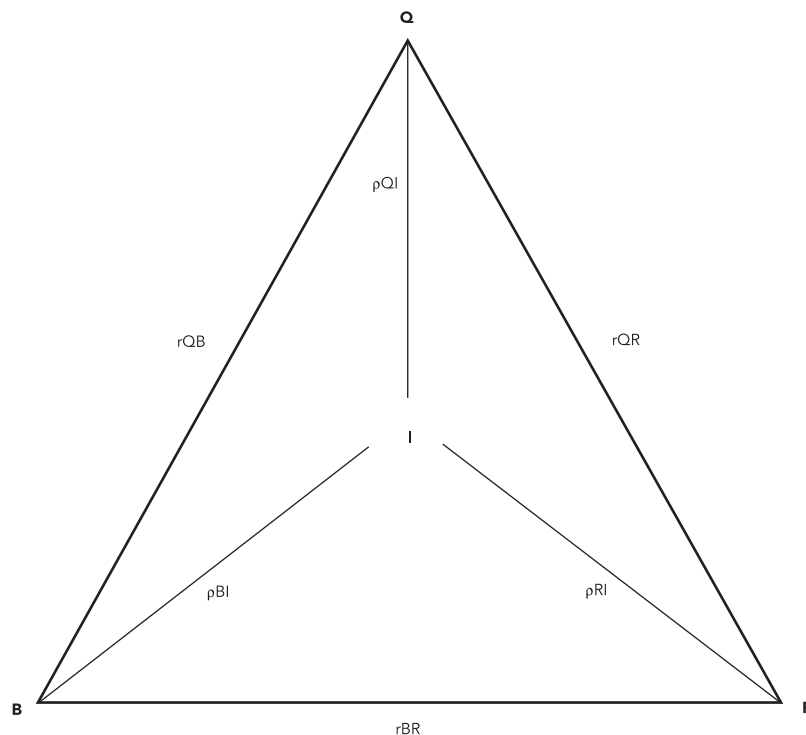
**The “bootstrap” method for calculating the 95%CI of the validity coefficient**

In the estimation of validity coefficients, the accuracy of the coefficient may be evaluated from the confidence interval of the parameters.

The confidence interval of the validity coefficients are often calculated with the “bootstrap” method <sup>6,10,15</sup>, originally proposed by Efron & Gong <sup>16</sup>. This is a resampling method in which hundreds or thousands of “bootstrap” samples are generated from the original sample with the purpose of deriving estimates of confidence interval or standard errors of a parameter <sup>14</sup>. Each “bootstrap” sample is a random sample with replacement from the same original sample. The “bootstrap” samples are used to build the “boot-

Figure 1

Triads method: triangular comparison between food frequency questionnaire, the reference method (24-hour recalls) and biological markers <sup>4</sup>.



Q: food frequency questionnaire; R: reference method, B: biomarker; I: true intake; rQB: correlation between food frequency questionnaire and biomarker; rQR: correlation between food frequency questionnaire and reference method, rRB: correlation between biomarker and the reference method; pQI: validity coefficient of food frequency questionnaire; pRI: validity coefficient of the reference method; pBI: validity coefficient of the biomarker.

strap” distribution of the validity coefficients, from which the confidence interval is calculated. This technique requires no prior knowledge of the estimated validity coefficient’s theoretical distribution <sup>4</sup>. The confidence interval of the validity coefficients can, then, be calculated by software programs such as SPSS (SPSS Inc., Chicago, USA) or Stata (Stata Corp., College Station, USA).

Thus, the “bootstrap” method provides an empirical distribution of the validity coefficients of the three (FFQ, 24hR and biological markers) variables. Large amplitudes of confidence interval will indicate low correlations between the variables <sup>4</sup>.

#### Limitations of the triads method

One of the limitations of the triangulation technique is the occurrence of validity coefficients

greater than one, known as the Heywood case <sup>12</sup>. For the three correlation coefficients ( $r_{QR}$ ,  $r_{RB}$  and  $r_{QB}$ ), the Heywood case occurs when the result of the multiplication of two of the three correlation coefficients is greater than the other correlation coefficient for the same nutrient (eg.:  $r_{QR} \times r_{RB} > r_{QB}$ ) <sup>12</sup>. For example, in the study by Verkleij-Hagoort et al. <sup>17</sup>, the validity coefficient between FFQ and the “true intake” for vitamin B<sub>12</sub> was greater than 1 ( $\rho_{QI} = 1.66$ ). Their results indicated a Heywood case, where the product of  $r_{QR}$  (0.66)  $\times$   $r_{QB}$  (0.21) was equal to 0.13, being larger than  $r_{RB}$  (0.05).

The main causes for the occurrence of the Heywood case include random sampling variations or violation of one or more assumptions of the triads method. In the first case, a validity coefficient above 1 is acceptable. However, in the second case, the estimated validity coefficient is

the result of systematic errors<sup>4</sup>. Violation of the assumption of independence of random errors between variables is more common, because FFQ and 24hR usually have correlated errors, particularly when 24hR or food records are used as reference methods. Therefore, some studies have considered the validity coefficient FFQ ( $\rho_{QI}$ ) as the upper limit and the correlation coefficient of FFQ and biological markers ( $r_{QB}$ ) as the lower limit of the validity coefficient between FFQ and the true intake<sup>10,17,18</sup>.

The existence of negative correlations for  $r_{QR}$ ,  $r_{RB}$  and  $r_{QB}$  is another limitation of this technique, because validity coefficients ( $\rho_{QI}$ ,  $\rho_{RI}$  and  $\rho_{BI}$ ) and  $\rho$  of the “bootstrap” samples cannot be calculated<sup>4,12</sup>. Empirical negative correlations occur when the true correlations are near zero, *ie*, the specific factors of the variable predominate over the latent variable. High incidence of negative correlation means less precise confidence intervals which is due to the low accuracy of the estimated validity coefficients<sup>4,12</sup>. Increasing the sample size and using more accurate reference methods and biomarkers should reduce the likelihood of negative correlations<sup>4</sup>. Therefore, in addition to using carefully chosen accurate reference methods, a minimum of 50 subjects is indicated for validation studies with biomarkers<sup>8,15</sup>.

The validity coefficients of a nutrient are not always comparable between studies. Food consumption is culture specific, thus validity of dietary intake measurement methods are estimated for different ethnic groups or study populations using reference methods for that population. Additionally, differences in the number of days of survey application, of the reference method used, sample size, the structure and number of food items of FFQ and the biomarker's intrinsic variability (such as bioavailability and metabolism of the nutrient being tested) and analytical errors are some of the other factors that limit the comparability among studies<sup>1,4</sup>.

#### **Food consumption validation studies using the triads method**

The nutrients investigated in the studies reviewed were: carotenoids (7)<sup>6,9,10,15,18,19,20</sup>, tocopherols (6)<sup>6,9,10,15,18,20</sup>, retinol (1)<sup>9</sup>, folic acid (4)<sup>9,17,21,22</sup>, fatty acids (4)<sup>6,11,23,24</sup>, vitamin B<sub>12</sub> (2)<sup>17,22</sup>, proteins (3)<sup>9,15,25</sup>, potassium (2)<sup>15,20</sup>, cholesterol (1)<sup>20</sup>, dithiocarbamate (1)<sup>26</sup>, phytoestrogens (1)<sup>27</sup> and flavonoids (1)<sup>25</sup>. Table 1 presents a summary of the studies in which the triangulation was used to analyze the correlation between methods of intake, biomarkers and the unknown “true” intake. Nutrients most studied were carotenoids and

tocopherols. The majority of the studies aimed to validate a food intake questionnaire but some intended to test the biomarkers as indicators of food intakes<sup>19,23,25,26</sup>.

The sample size of the studies varied from 27 to 161 individuals (Table 1). Recent guidelines by the European Micronutrient Recommendations Aligned Network of Excellence (EURRECA) consider satisfactory a sample size of 50 for validation studies with biomarkers as reference method<sup>8</sup>. Likewise, a sample of 100 individuals might be necessary for a validity coefficient with a 95%CI lower limit of at least 0.4, assuming correlation between FFQ and 24hR ( $r_{QR}$ ) of 0.6, 80% power and 5% significance level<sup>1</sup>. However, even this sample size may often be insufficient to estimate validity coefficients precisely<sup>4</sup>. Of the 16 published studies considered in this review, seven of them used sample sizes equal to or greater than 100<sup>6,9,18,20,23,24,25</sup>, while four had sample sizes below 50<sup>10,11,21,26</sup>. Indeed, larger sample sizes will reduce the chances of Heywood case events and the negative correlations between variables<sup>9,12</sup>. Validity coefficients greater than 1 and negative correlations were observed in the studies of various sample sizes reviewed. In one study, despite the moderate correlation ( $r_{QR} = 0.57$ ) obtained between 24hR and FFQ for vitamin E, the correlation between biological markers and 24hR was negative ( $r_{RB} = -0.11$ )<sup>9</sup>. Small sample size ( $n = 28$ ) may account for this result. On the other hand, even with larger sample sizes, negative correlation was observed for adipose tissue  $\alpha$ -carotene and palmitic acid<sup>6</sup>. Considering the sample size ( $n = 120$ ) the Heywood cases were attributable to random errors due to low specificity of the biomarker<sup>4</sup>. Therefore, sample sizes should consider a biomarker with good quantitative relation to intake and the necessary study power, in addition to the extra work imposed for the laboratory analyses.

Except for the dithiocarbamate<sup>26</sup>, that used urine as a sole biological material, 15 studies analyzed biomarkers in blood samples or in adipose tissue (Table 1). For carotenoids, tocopherol, folic acid and vitamin B<sub>12</sub> studies, serum and erythrocyte were analyzed. For fatty acid biomarkers analyses, plasma phospholipids<sup>11</sup>, erythrocyte membrane<sup>24</sup> and adipose tissue<sup>6,23</sup> were used. In Kabagambe et al.'s<sup>6</sup> study, plasma performed better than adipose tissue both for carotenoids and tocopherols. In the studies, blood collection was done in a fasting, fasting since midnight<sup>19</sup> or non-fasting state<sup>10,20</sup>. The effect of such differences in the biological material, the handling and analytical procedures adopted and natural fluctuation of the biomarker level in the body (due to the amount ingested, bioavailability and

Table 1

Validation studies where validity coefficients ( $\rho$ ) in relation to the "true" intake (I) were estimated for the triads method.

Reference	Country	n	Dietary intake: assessment method	Biological sample	Bootstrap samples (n)	Validity coefficients ( $\rho$ QI, $\rho$ RI, $\rho$ BI) and observations about the study
Carotenoids and tocopherol						
Daurès et al. <sup>15</sup> (2000)	France	87 adults	16 days WR 28 days WR 1 Q	Plasma	10,000	(0.39, 0.52, 0.85) for $\beta$ -carotene. Q and WR were evaluated against B
Kabagambe et al. <sup>6</sup> (2001)	Costa Rica	120 adults	7 R24 2 Q	Plasma, adipose tissue	1,000	(0.45, 1.0, 0.36) for $\beta$ -carotene (adipose tissue); (0.59, 0.81, 0.21) for $\alpha$ -tocopherol (adipose tissue) (0.76, 0.71, 0.50) for $\beta$ -carotene in plasma Q was evaluated against 24R and two B. Overall plasma results were better than the adipose tissue $\rho$ *
Shai et al. <sup>9</sup> (2005)	Israel	161 adults	6 R24 3 Q	Serum	NI	(0.67, 0.60, 0.67) for $\beta$ -carotene (0.56, 0.97, 0.34) for $\alpha$ -tocopherol Performance of $\rho$ QI were superior to $\rho$ BI
McNaughton et al. <sup>10</sup> (2005) **	Australia	28 adults	12 days WR 1 Q	Non-fasting serum	1,000	(0.55, 0.64, 0.51) for $\beta$ -carotene Not calculated for $\alpha$ -tocopherol (negative correlation). Q for carotenoid and vit E were evaluated against 24R and B
Andersen et al. <sup>19</sup> (2005) ***	Norway	86-100 military men	14 days WR 2 Q (180 and 27 items)	Serum	1,000	(0.54, 0.79, 0.47) for 180 item Q and (0.60, 0.91, 0.43) for 27 item Q, both for $\alpha$ -carotene. Q were evaluated for fruit and vegetable consumption
Dixon et al. <sup>18</sup> (2006) #	USA	130 adults	4 R24 1 DHQ	Serum (fasting from midnight)	NI	(0.61, 0.42, 0.64) - $\beta$ -carotene in women (0.80, 0.63, 0.71) - $\beta$ -carotene in men (0.74, 0.76, 0.14) for $\alpha$ -tocopherol DHQ was evaluated for carotenoid and tocopherol intake. $\rho$ were different according to gender and varied among nutrients tested
Mirmiran et al. <sup>20</sup> (2010)	Iran	132 adults	12 R24 2 Q	Non-fasting plasma	NI	(0.50, 0.48, 0.53) for $\beta$ -carotene; (0.38, 0.39, 0.73) for $\alpha$ -tocopherol; (0.55, 0.51, 0.38) for retinol. Blood samples were collected 4 times during a year
Fatty acids						
Kabagambe et al. <sup>6</sup> (2001)	Costa Rica	120 adults	7 R24 2 Q	Adipose tissue	1,000	(0.89, 0.82, 0.67) for 18:2n-6; (0.59, 0.99, 0.46) for 18:3n-3 Adipose tissue was a poor biomarker for saturated and monounsaturated fatty acids
Brevik et al. <sup>23</sup> (2005) ##	Norway	107-110 military men	14 days WR 1 Q	Serum and adipose tissue	1,000	(0.50, 0.94, 0.56) for 15:0 in adipose tissue and (0.58, 0.88, 0.49) for serum 15:0 Fatty acids 15:0 and 17:0 were tested as biomarkers of milk fat and dairy product intake. $\rho$ RI were superior to others
McNaughton et al. <sup>11</sup> (2007)	Australia	43 adults	12 days WR 1 Q	Plasma phospholipid	1,000	(0.45, 1.00, 0.29) for 20:4n-6; (0.62, 0.65, 0.35) for 20:5n-3; (0.62, 0.83, 0.52) for 22:6n-3
Zhang et al. <sup>24</sup> (2010)	China	125 adults	3DR 1 Q	Erythrocyte membrane	1,000	(0.61, 0.92, 0.17) for n-6; (0.65, 0.96, 0.29) for 18:3n-3; (0.75, 0.56, 0.50) for 20:5n-3; (0.67, 0.42, 0.24) for 22:6n-3

(continues)

Table 1 (continued)

Reference	Country	n	Dietary intake: assessment method	Biological sample	Bootstrap samples (n)	Validity coefficients (ρQI, ρRI, ρBI) and observations about the study
Folic acid and vitamin B <sub>12</sub>						
Pufulete et al. <sup>21</sup> (2002) ###	United Kingdom	36 adults	7 day WR 2 Q	Serum and erythrocyte	NI	(0.74, 0.72, 0.64) for serum folic acid (0.70, 0.78, 0.35) for erythrocyte folic acid. Validity coefficients were calculated using correlation values from the original work
Shai et al. <sup>9</sup> (2005)	Israel	161 adults	6 R24 3 Q	Serum	NI	(0.72, 0.39, 0.65) for folic acid
Verkleij-Hagoort et al. <sup>17</sup> (2007)	Netherlands	53 women	3 R24 1 Q	Serum and erythrocyte	1,000	(0.94, 1.00, 0.21) for serum folic acid (0.75, 1.30, 0.37) for erythrocyte folic acid (1.00, 0.39, 0.12) for serum vitamin B <sub>12</sub> Q for folate and vitamin B <sub>12</sub> was tested for women at reproductive age. ρRI, ρBI were calculated using correlation values in the original work
Shuaibi et al. <sup>22</sup> (2008)	USA	95 women	3 day FR 1 FCM	Serum	NI	(0.97, 0.79, 0.46) for folic acid and (0.95, 0.85, 0.46) for Vitamin B <sub>12</sub> Q for folate and vitamin B <sub>12</sub> was tested in college-aged women
Other food components						
Fowke et al. <sup>26</sup> (2002)	USA	27 women	3 R24 2 FVQ	Urine	1,000	(0.54, 1.00, 0.42) before the intervention, (0.67, 0.49, 0.57) after intervention Urinary dithiocarbamate was analyzed as marker of cruciferous vegetable intake in an intervention study aiming to increase its consumption
Bhakta et al. <sup>27</sup> (2005)	United Kingdom	58 women	12 R24 1 Q	Plasma	10,000	(0.76, 0.82, 0.65) for genistein (0.67, 0.83, 0.45) for daizhein Subjects were South Asian women living in England. Other 2 phytoestrogens were analyzed but results were less consistent
Shai et al. <sup>9</sup> (2005)	Israel	161 adults	6 R24 3 Q	24-hour urine	NI	(0.77, 0.68, 0.44) urinary nitrogen for protein intake
Brantsaeter et al. <sup>25</sup> (2007)	Norway	119 pregnant women	4 days WR 1 Q	24-hour urine, plasma	1,000	(0.59, 0.66, 0.47) for citrus fruit and juice, with zeaxanthin as biomarker, (0.65, 0.63, 0.43) for citrus fruit and juice, considering Q plus hesperetin and zeaxanthin as biomarkers. Urine was used for flavonoid analyses and plasma for carotenoids
Mirmiran et al. <sup>20</sup> (2010)	Iran	132 adults	12 R24 2 Q	24-hour urine, plasma	NI	(0.56, 1.16, 0.38) for protein (0.61, 0.63, 0.60) for potassium (0.95, 0.46, 0.32) for cholesterol Urine was used for nitrogen and potassium analyses. Plasma was used for cholesterol analysis

B: biological markers; DHQ: diet history questionnaire; n: sample size; FCM: "food choice map"; FR: food registry; FVQ: fruit and vegetable questionnaire; NI: not informed; Q: food frequency questionnaire; R24: 24-hours recall; WR: weighed record; ρQI: validity coefficient for food frequency questionnaire; ρRI: validity coefficient for reference intake method; ρBI: validity coefficient for biomarker.

\* Mean validity coefficients for the tocopherols and carotenoids (α-carotene, β-carotene, lycopene, zeaxanthin and lutein);

\*\* The study analyzed α and β-carotene, β-cryptoxanthin, lutein, lycopene;

\*\*\* The study analyzed lutein, zeaxanthin, lycopene, α and β-carotene;

# The study analyzed α and β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin, α and γ-tocopherol;

## Validity coefficient presented refers to dairy product as a whole;

### When validity coefficients were not available in the original work, they were calculated using Kaaks <sup>4</sup> equation.

metabolism), are potential confounders for the biomarker and may result in weaker correlations to the true intake<sup>1,4</sup>. The comparison between studies must incorporate these important differences among them.

For the intake reference method, studies used multiple 24hR<sup>6,9,18,20,26,27</sup>, weighed food records<sup>10,11,15,19,21,23</sup> or several days food registry<sup>22</sup> as the reference method. Taking the example of carotenoids and tocopherols, shown in Table 1, validity coefficients of the reference method and the questionnaire performed well, ranging from  $\rho_{RI}$  of 0.39 to 0.97 and  $\rho_{QI}$  from 0.38 to 0.80, respectively. Some Heywood cases were also observed<sup>6,11,16,17,26</sup> for the reference method, and potential causes are violation of the assumption of independence of variances<sup>4,12</sup>. The biomarker  $\rho_{BI}$  performed fairly well ranging from 0.36 to 0.71 for the carotenoids and 0.14 to 0.73 for tocopherols. Lower  $\rho_{BI}$  for tocopherol may indicate poor correlation with the latent variable or low specificity of the biomarker<sup>4</sup>.

Dietary fatty acids are nutrients that are of validation interest from an epidemiological standpoint, due to their relationship with chronic diseases<sup>28</sup>. Four studies applied the method of triads<sup>6,11,23,24</sup>, but comparison of the studies must be done with caution. The fatty acids were measured in different tissues (from blood and adipose tissue) and studies did not analyze same fatty acids. Overall,  $\rho_{BI}$  performance varied, ranging from 0.17 to 0.67 for essential fatty acids (18:2*n*-6 and 18:3*n*-3)<sup>6,24</sup> and odd number fatty acids<sup>23</sup>. Adipose tissue and serum showed comparable results for the odd number fatty acids but it was a poor biomarker for saturated and monounsaturated fatty acids<sup>6</sup>. The validity coefficients for the very long chain polyunsaturated fatty acids (20:5*n*-3 and 22:6*n*-3) varied among studies<sup>11,24</sup>. Apparently, fatty acids that are not synthesized in the body, such as the essential and odd number fatty acids, seems to perform better as biomarkers than those which are not derived solely from the diet (20:4*n*-6, 20:5*n*-3, 22:6*n*-3)<sup>11</sup>. This should be further investigated.

The method of triads was also used to compare different biomarkers for intakes of fruit and vegetables<sup>19,25</sup>. In the study conducted in Norway with male soldiers<sup>19</sup>, the evaluation of different types of carotenoids (lutein, zeaxanthin, lycopene,  $\alpha$  and  $\beta$  carotene) as biomarkers of fruit and vegetable intake showed  $\alpha$ -carotene as having the best validity coefficient ( $\rho_{BI} = 0.47$ ). In addition, the authors compared a 180 item FFQ with a 27 item one, for the fruit and vegetable consumption, using  $\alpha$ -carotene as the biomarker. Both FFQ had similar validity coefficients ( $\rho_{QI}$ 180 items = 0.54;  $\rho_{QI}$ 27 items = 0.60),

showing that a FFQ with 27 items was sufficient to categorize fruit and vegetable intake in that population<sup>19</sup>.

Overall, for folic acid and vitamin B<sub>12</sub>, the  $\rho_{QI}$  and  $\rho_{RI}$  performed better than the  $\rho_{BI}$  (Table 1). Still, these intake variables (FFQ and 24hR) have common sources of errors which violate the model assumption. Thus, this aspect needs to be taken in consideration when comparing the results with that of the  $\rho_{BI}$ .

Biomarkers do not always perform better than other methods of food intake assessment. Among the 17 nutrients examined by Kabagambe et al.<sup>6</sup>, the  $\alpha$ -tocopherol and  $\beta$ -carotene had higher correlation coefficients between the FFQ and 24hR than between the biological markers (adipose tissue  $\alpha$ -tocopherol and  $\beta$ -carotene) and FFQ ( $\alpha$ -tocopherol  $r_{QB} = 0.13$ ;  $\beta$ -carotene  $r_{QB} = 0.16$ ). Furthermore, the 95%CI for validity coefficients of FFQ ( $\rho_{QI} = 0.12$  to 1.00) and 24hR ( $\rho_{RI} = 0.40$  to 1.00) were better than the biomarker ( $\rho_{BI} = 0.02$  to 0.67). These results indicate that for some nutrients, the traditional methods of dietary assessment are better than the biomarker, hence biomarkers should be used in addition to and not in replacement of dietary surveys<sup>6</sup>. Furthermore, the possibility of violation of the method's assumption must be remembered for FFQ and 24hR.

Studies also used the method of triads to validate new biomarkers<sup>23,26,27</sup>. One study tested adipose and serum odd chain fatty acids as markers of dairy fat intake<sup>23</sup> and another one used urinary dithiocarbamates as biomarkers of cruciferous vegetable ingestion<sup>26</sup>. In the cruciferous study, authors conducted an intervention in which participants were encouraged to increase the consumption of cruciferous vegetables from 26.6g/day to 190.1g/day using a FFQ to estimate fruit and vegetable intake. They compared the consumption of these vegetables before and at the end of the intervention period using three 24hR as reference method and two fruit and vegetable intake questionnaires. Since the detection of urinary dithiocarbamate depends on the consumption of reasonable amount of cruciferous vegetables, the validity coefficient of the biomarker after intervention ( $\rho_{BI} = 0.65$ ) was higher than the pre-intervention value ( $\rho_{BI} = 0.42$ ). Although the authors recommend the use of this biomarker in the validation of cruciferous vegetable intake, the results need to be evaluated with caution, because the time reference of the FFQ was very short (7 days) and sample size differed between before ( $n = 27$ ) and after ( $n = 33$ ) the intervention<sup>26</sup>.

Most biomarkers reflect short term intake of nutrients, which can be a limiting factor be-



cause quite often validation studies are intended to relate food consumption over time with the development of chronic diseases. Bhakta et al.<sup>27</sup> conducted a study to validate a FFQ on phytoestrogen intake by Asian women living in the United Kingdom (Table 1). Biomarkers used in the study were plasma phytoestrogens, which reflect their short term intake. Four plasma samples were collected along a one year period. The biomarker validity coefficients ( $\rho_{BI}$ ) were the lowest for all phytoestrogens (0.11 for lignans and 0.45 for genistein). The results show that FFQ can be a better instrument than the biomarker in the estimation of phytoestrogen<sup>27</sup>. Shai et al.<sup>9</sup> used six repeated measures of 24hR, three FFQ, two blood samples and three urine samples, with the intent of obtaining a more precise measure of the attenuation factors for each variable studied. Also, in the study conducted in Iran, four urine (potassium and protein intake estimate) and blood ( $\beta$ -carotene,  $\alpha$ -tocopherol, retinol and cholesterol intake estimate) samples were collected<sup>20</sup>. Urinary nitrogen<sup>9,20</sup> and potassium<sup>20</sup> performed well as biomarkers of protein and potassium intakes, respectively. These results should be useful in the adjustment of diet-disease relative risk relationships in future studies.

The study by Brantsaeter et al.<sup>25</sup> used two independent biomarkers (from 24-hour urinary flavonoids and plasma carotenoids) to validate a FFQ focused on fruits, vegetables and tea intake. Despite being costly and laborious, the inclusion of two nutrient biomarkers, one serving as the reference method, may show advantages due to the three independent variable errors generated. In their study, the highest validity coefficients

were seen for FFQ of citrus fruit/juice intake ( $\rho_{QI} = 0.65$ ), using urine hesperetin and plasma zeaxanthin as independent biomarkers. Comparable results were obtained when the triads method was applied to two dietary estimates and one biomarker ( $\rho_{QI} = 0.59$ )<sup>25</sup>.

#### **Final considerations**

The triads method is a technique that has been used in recent dietary validation studies. This method adds a third variable – the biomarker – with an independent error from FFQ and the reference method, allowing the expansion of the parameters for validation. The use of this method does not exclude the need of correlation and Bland-Altman agreement analyses.

The number of published studies is still small and methodological differences related to population, the type of questionnaire and the reference method hamper the comparability of the results. Although most of the biomarkers behaved relatively well compared to the dietary estimates, the small sample size of some studies, together with other subject characteristics, like age, sex, supplement usage and smoking status (for carotenoids) may have interfered in some results, which tended to be less correlated to the true intake than the FFQ and 24hR methods.

It is interesting to observe the use of new biomarkers, and the key for their acceptance will be the sensitivity of these markers. Future studies should aim to refine the critical parameters of the method. Repeated measures of a biomarker or the use of two independent biomarkers are some of the new approaches being tested.

## Resumo

*O método das tríades vem sendo utilizado em estudos de validação do consumo alimentar para avaliação da correlação entre três variáveis (questionário de frequência alimentar, método de referência e biomarcador) e a ingestão real, por meio dos coeficientes de validade ( $\rho$ ). A principal vantagem deste método é a inclusão do biomarcador, que apresenta erros independentes dos métodos tradicionais. Os pressupostos desta técnica são a linearidade entre as três variáveis e a ingestão real, e a existência de erros independentes entre as variáveis. Entre as limitações deste método, destaca-se a existência de  $\rho > 1$ , conhecido como "Heywood case", e de correlações negativas, que não permitem o cálculo do  $\rho$ . O objetivo deste trabalho foi apresentar o conceito do método, descrever a sua aplicação e examinar estudos de validação do consumo alimentar que utilizaram o método das tríades, além de conceituar o método "bootstrap" para obtenção de intervalos de confiança dos coeficientes de validade.*

*Consumo de Alimentos; Inquéritos Nutricionais; Estudos de Validação*

## Contributors

R. T. C. Yokota drafted the manuscript. E. S. Miyazaki contributed with the drafting and critical review of the statistical aspects of the article. M. K. Ito helped with the drafting and contributed with the critical review of the article.

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