VALIDATING FATTY ACID INTAKE AS ESTIMATED BY A FOOD FREQUENCY QUESTIONNAIRE: HOW DOES THE 24 HOUR RECALL PERFORM AS REFERENCE METHOD COMPARED TO THE DUPLICATE PORTION?

Short title: VALIDATING FATTY ACID INTAKE

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Conflict of interest

None

Authorship

The authors’ contributions are as follows: LT collected the data and contributed to the study design, data analysis and interpretation of findings and wrote the manuscript. JHMdV, PvtV and AG contributed to the study design, interpretation of findings and revised the earlier versions of the manuscript. HCB contributed to the data analysis, interpretation of findings and revised the earlier versions of the manuscript. PJMH and PCHH contributed to the study design and revised the earlier versions of the manuscript. All authors read and approved the final version of the manuscript.

Ethical standards disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the medical ethical committee of Wageningen University. Written informed consent was obtained from all subjects/patients.
Abstract

Objective: To compare the performance of the commonly used 24 hour recall (24hR) with the more distinct duplicate portion (DP) as reference method for validation of fatty acid intake estimated with food frequency questionnaires (FFQ).

Design: Intakes of saturated (SFA), monounsaturated (MUFA) and n-3 fatty acids and linoleic acid (LA) were estimated by chemical analysis of two DPs and by on average five 24hRs and two FFQs. Plasma n-3 fatty acids and LA were used to objectively compare ranking of individuals based on DP and 24hR. Multivariate measurement error models were used to estimate validity coefficients and attenuation factors for the FFQ with the DP and 24hR as reference methods.

Setting: Wageningen, The Netherlands.

Subjects: Ninety-two men and 106 women (aged 20-70).

Results: Validity coefficients for the fatty acid estimates by the FFQ tended to be lower when using the DP as reference method compared to the 24hR. Attenuation factors for the FFQ tended to be slightly higher based on the DP than those based on the 24hR as reference method.

Furthermore, when using plasma fatty acids as reference, the DP showed comparable to slightly better ranking of participants according to their intake of n-3 fatty acids (0.33) and the n-3/LA ratio (0.34) than the 24hR (0.22 and 0.24 respectively).

Conclusions: The 24hR gives only slightly different results compared to the distinctive but less feasible DP, therefore the use of the 24hR seems appropriate as reference method for FFQ validation of fatty acid intake.

Keywords: dietary assessment, validity, measurement errors, fatty acids, duplicate portion, biomarker
**Introduction**

Inconclusive results about the risks of intake of total fat and various fatty acids on diseases such as breast cancer \(^1; 2\) and coronary diseases \(^3; 4\) plague epidemiological research. This inconclusiveness may originate from limitations and errors in food composition databases and dietary assessment methods to assess total fat and fatty acid intake. Food frequency questionnaires (FFQs) are often used in epidemiological studies, since they are relatively cheap and pose a low burden on the participants. However, they are suspected to be affected by systematic and random errors that together obscure the true variation in fat intake between subjects. The observed association between fat intake and disease can be adjusted for these measurement errors by an attenuation factor derived from a validation study. The reference method used in the validation study should generate unbiased dietary intake data (i.e. no proportional scaling bias should be present) and have uncorrelated errors with the FFQ \(^5; 6\). However for most nutrients, including fatty acids, only imperfect reference methods are available, e.g. 24-hour recalls (24hRs) or concentration biomarkers. Unfortunately, concentration biomarkers are only informative on ranking of individuals according to their intakes and not on their absolute levels of intake. Furthermore, use of plasma fatty acids as biomarkers of intake is limited to fatty acids that are not endogenously produced (i.e. n-3 and n-6 fatty acids) \(^7\). 24hRs are able to assess the intake of a wide array of fatty acids, but are biased and showed correlated errors with FFQs for energy and protein \(^8; 9\). Freedman et al.\(^10\) recently recommended using regression calibration based on 24hRs to adjust diet-health associations when no recovery biomarkers are available. However, based on their investigation on intakes of energy, protein, potassium and sodium, they showed that the 24hR was certainly not a perfect reference method given the presence of intake related bias and errors correlated with those of the FFQ. It is unclear how these limitations affect the use of 24hR as reference method for validation of fatty acid estimates from FFQ.

Previous research concluded that the duplicate portion method (DP) is a suitable reference method and preferable over a 24hR for FFQ validation for nutrients for which no recovery biomarker is available \(^11\). The DP is a distinctive reference method as it does not depend on the availability and quality of the nutrient values in food composition databases, and also biases related to memory and estimation of portion sizes are less of a problem as compared to methods such as 24hR and FFQ. Altogether, the DP showed less proportional scaling bias and had a lower degree of correlated errors with the FFQ than the 24hR for protein, potassium and sodium \(^11\). In the present paper, we therefore compare the performance of the often used and more feasible 24hR as reference method for validation of fatty acid estimates from FFQ with the
more distinct DP as reference method. We additionally assessed the ability of DP and 24hR to rank individuals according to their intake of n-3 fatty acids, LA and the n-3/LA ratio using an objective biomarker (plasma fatty acids) as reference method.

**Subjects and Methods**

**Subjects and study design**

In this Dutch validation study called DuPLO, which is part of the National Dietary Assessment Reference Database (NDARD) (12), 200 Dutch adults (92 men, 108 women) were enrolled. The recruitment and study procedures are described elsewhere (11). Briefly, between July 2011 and July 2014 each participant collected two DPs (~5 months apart), and two blood samples (~13 months apart). Also two FFQs (~7 months apart) were filled out. An average of five 24hRs per subject was administrated by a telephone interview by a dietician (~4 months apart). A varying number of 24hRs per person (between 0 and 8 measurements) was collected because participants were enrolled in different sub-studies of the NDARD study. Participants with missing data for one or more of the methods were included in the analysis because they provided information for the other dietary assessment methods.

**24-hour recalls and FFQ**

The 24hR administration followed a standardized protocol based on the 5-step multiple pass method (13). Participants got an unannounced phone call from a trained dietician. Portion sizes of foods or recipes were reported using household measures, standard portion sizes, weight in grams, or volume in liters (14).

The 180 item FFQ (15; 16) was administered via the web using the online open-source survey tool Limesurvey™. The reference period for the FFQ was one month and frequencies of intake were combined with standard portion sizes and household measures to assess amounts of intake (14). Self-reported dietary intake data from 24hR and FFQ were converted into nutrient data using the Dutch food composition database (FCD) of 2011 (17).

**Duplicate portion collection and analytical methods**

Participants got verbal and written instructions preceding the collection of the DP. Participants collected all edible foods and drinks consumed over a 24-hour period in collection baskets and stored them in a cool box (5°C). At the study center, DPs were weighed, homogenized in a blender (Waring Commercial model 34BL22) and 2.5 mL 0.02% tert-butylhydrochinon (BHQ) in ethanol was added per kg of DP as antioxidant. For each DP, an aliquot of the homogenized
sample was stored within 1 hour at -20°C, until further analysis. Total fat was measured
gravimetrically by acid hydrolysis (AOAC method 14.019)\(^{(18)}\).

**Blood sampling and fatty acid assessment**

Blood samples were collected from the participants in a fasting state. EDTA plasma was stored
at -80°C until further analysis. Cholesteryl esters from plasma were isolated using solid phase
extraction silica columns and fatty acid profiles of the plasma cholesteryl esters were analyzed
by gas chromatography as previously described\(^{(19)}\).

**Statistical analysis and measurement error models**

In total 198 participants were included for analysis, 92 males and 106 females. Two participants
got pregnant during the study. As it was expected that they had altered their habitual dietary
intake they were excluded from analysis. Means and 95% confidence intervals were estimated
for SFA, MUFA, n-3 fatty acids, and LA in grams and as a percentage of the total amount of
fatty acids for DP, 24hR and FFQ. An n-3/LA ratio (LA is an n-6 fatty acid) closer to one
indicates a healthier distribution and this ratio is therefore included as an additional outcome
measure in this research. Because of their skewed distribution, a log transformation was used
for all variables to obtain a normal distribution.

Our measurement error models assumed a linear relationship between the log(intake) according
to DP, 24hR, FFQ or biomarker and the true unknown intake \(T\), with intakes of the specific
fatty acids expressed as percentages of the total fatty acid intake. Measurement error models
were adjusted for BMI and gender. In our measurement error models \(i\) indicates the person and
\(j\) the occasion. Furthermore, in all measurement error models \(\alpha\) expresses the constant bias and
\(\beta\) the proportional scaling bias. The person specific bias for the method is given by \(w_{xi}\) and the
random error by \(\epsilon_{xij}\) with mean zero and constant variance.

To evaluate the comparability of the 24hR and the DP as reference methods for the FFQ (for
both level of intake and ranking), model 1 (with equations 1 and 2) is defined as below. In this
model the assumptions of negligible error correlation between reference method and FFQ and
between replicates of the reference method, and absence of proportional scaling bias in the
reference method (\(\beta_{x} = 1\)) were made to enable estimation of the model parameters.

\begin{align*}
\text{Reference method X (24hR or DP):} & \quad X_{ij} = T + \epsilon_{xij} \quad (1) \\
\text{Food Frequency Questionnaire:} & \quad Q_{ij} = \alpha_{Q} + \beta_{Q}T + w_{Qi} + \epsilon_{Qij} \quad (2)
\end{align*}
Validity coefficients ($\rho_{XT}$, formula 3) were estimated to assess the ability of the dietary assessment method to rank participants according to their intake:

$$\rho_{XT} = \frac{\beta_x^2 \text{var} T}{\beta_x^2 \text{var} T + \frac{\text{var} \varepsilon_{Xij}}{k} + \text{var} w_{Xi}}$$  \hspace{1cm} (3)

Where var\text{T} is the variance of the true nutrient intake; var\varepsilon_{Xij} the variance of the random error of method X and var\text{wXi} the variance of the person specific bias for method X.

The attenuation factor ($\lambda_x$, formula 4) provides information about the extent to which diet-health associations are affected by measurement error:

$$\lambda_x = \frac{\rho_{XT}^2}{\beta_x}$$  \hspace{1cm} (4)

As an additional check of the performance of the two reference methods, we used the biomarker to objectively compare the ranking based on individual fatty acid intakes when using the DP and the 24hR. Since the biomarker is only valid for n-3 and n-6 fatty acids (7) this was only done for the n-3 fatty acids, LA and the n-3/LA ratio. Therefore we specified measurement error model 2 (with equations 5 and 6) as given below. In this model the assumptions of negligible error correlation between biomarker and DP or 24hR and between replicates of the biomarker and absence of proportional scaling bias for the biomarker ($\beta_M = 1$) were made to enable estimation of the model parameters.

Biomarker:

$$M_{ij} = T + \varepsilon_{M_{ij}}$$  \hspace{1cm} (5)

Method X (24hR or DP):

$$X_{ij} = \alpha_x + \beta_x T + w_{Xi} + \varepsilon_{X_{ij}}$$  \hspace{1cm} (6)

All statistical tests were performed in SAS version 9.3 (SAS Institute Inc. Cary, NC, USA, 2012).

Results

Baseline characteristics of the study population
At baseline, mean age of the study population was 55.7 (SD 10.2) years and mean BMI was 25.1 (SD 3.7) kg/m². 52.5 percent completed a high level (university or college) and 18.7 percent a low level of education (primary or lower education).

Mean intakes of fatty acids

Mean intakes and the lower (2.5) and higher (97.5) percentiles of the specific fatty acids in grams and expressed as percentages of the total amount of fatty acids are shown in Table 1. SFA intake by the DP (31.2 g) and the 24hR (30.1 g) were both higher than by the FFQ (26.9 g). Also, MUFA and n-3 intakes were highest when assessed by the DP (32.3 g and 2.5 g), while intakes by the 24hR (27.9 g and 2.0 g) tended to be even lower than those by the FFQ (28.7 g and 2.3 g). For LA, DP (14.3 g) was rather similar to FFQ (14.6 g), while 24hR (13.5 g) intake tended to be slightly lower. n-3/LA ratios were rather similar. SFA intake as percentage of total fatty acids was highest when assessed by the 24hR (40.2%), followed by the DP (37.4%) and FFQ (35.5%). The MUFA intake percentage was highest when assessed by the DP (38.4%), followed by the FFQ (37.8%) and 24hR (36.8%). The LA intake percentage was highest when assessed by the FFQ (19.2%), with the 24hR (18.0%) being slightly higher than the DP (17.2%). For n-3 fatty acids and the n-3/LA ratio, percentages were rather similar for the three dietary assessment methods.

DP and 24hR as reference methods for FFQ validation

Validity coefficients for the FFQ were lower when the DP was used as reference method than when the 24hR was used as reference method when fatty acids were expressed as percentages of total fatty acids. This was especially true for MUFA (0.37 for DP, 0.65 for 24hR), LA (0.64 for DP, 0.80 for 24hR) and the n-3/LA ratio (0.33 for DP, 0.76 for 24hR, Table 2). For SFA and MUFA the attenuation factor was slightly higher when the DP was used as the reference method than when the 24hR was used. The other attenuation factors for the FFQ were rather similar when the DP was used as the reference method compared to the 24hR (Table 2). Also, for fatty acids expressed in grams validity coefficients for the FFQ were lower when the DP was used as reference method than when the 24hR was used as reference method. This was especially true for n-3 fatty acids (0.44 for DP, 0.74 for 24hR) and LA (0.49 for DP, 0.69 for 24hR, Table 3). Attenuation factors for the FFQ were higher when the 24hR was used as the reference method for SFA (0.30 for DP, 0.42 for 24hR), MUFA (0.17 for DP, 0.29 for 24hR) and LA (0.29 for DP, 0.48 for 24hR).
Validity coefficients and attenuation factors for the FFQ were similar, whether they were expressed in grams or as a percentage of total fatty acids. However, a few values were lower when expressed in grams: for SFA and LA, both validity coefficients and attenuation factors for both the DP and 24hR as the reference method. Also for MUFA and the n-3/LA ratio for the validity coefficient with the 24hR as the reference method values were lower when expressed in grams (0.47 vs 0.65 and 0.48 vs 0.76 respectively, Table 3).

 Ranking ability of DP and 24hR
To additionally compare the performance of the DP and 24hR for ranking in an objective way, concentration biomarker measurements were used as reference method. Validity coefficients were used to assess the ability of both methods to rank individuals according to their fatty acid intake. The validity coefficient for the ranking based on a single DP (k=1) for the n-3 fatty acids (0.33) was slightly higher than for a single 24hR (0.22, Table 4). For LA and the n-3/LA ratio, validity coefficients were similar. A similar pattern was observed for validity coefficients based on two DP and two 24hR measurements as shown in table 4 (k=2).

Discussion
To investigate to what extent the 24hR, often used as a reference method for FFQ, reduces the bias in estimated risk parameters for the intake of fatty acids we compared its performance to the DP as reference method. Fatty acid intakes expressed in grams were (slightly) lower when assessed by the 24hR as compared to the DP. For the fatty acid intakes expressed as percentages of total fatty acids, differences between the dietary assessment methods did not show a clear pattern. Validity coefficients for fatty acid estimates by the FFQ were higher or comparable when the 24hR was used as reference method than when the DP was used for data expressed in grams and percentages of total fatty acids. For attenuation factors, however, the 24hR as reference method showed a slightly lower value for MUFA for data expressed in percentages of total fatty acids and a higher value when expressed in grams. For data expressed in grams, higher attenuation factors were also observed for SFA and LA when the 24hR was used as the reference method. Using plasma fatty acids as reference method showed that the 24hR was able to rank participants according to their intake of n-3 fatty acids, LA and the n-3/LA ratio to a similar degree or slightly worse than the DP.

Intakes of fatty acids in our study population were comparable with those of the general Dutch population based on the 2007-2010 Dutch National Food Consumption Survey (DNFCS) (20).
The DNFCS intake data are based on two telephone-based 24hRs and the same FCD (2011) as
we used to calculate nutrient intakes. Assessment of nutrient intake is among others limited by
the availability and quality of the data in the FCD. Fatty acid composition of foods may change
over time and vary amongst different brands. However, a study comparing calculated and
analysed test diets for controlled dietary interventions found a reasonable agreement between
the two for SFA and MUFA (21) indicating the Dutch FCD performs reasonably well for these
fatty acids.

Published data on validity coefficients for FFQs for fatty acids intake estimates are scarce. One
study, using the method of triads with the biomarker and weighed food records as reference
method, found a validity coefficient of 0.50 for n-3 fatty acids assessed by FFQ (22), which is
comparable to our results. A study by Kabagambe et al., also using the method of triads, found
validity coefficients for the FFQ for LA between 0.77 and 0.89 (23), using the biomarker and
24hR as reference methods. This is in line with our findings for LA when using the 24hR as
reference method. A recent study in Brazilian adults, also using the method of triads with a
biomarker, FFQ and 24hR, reported validity coefficients for the FFQ for SFA (0.28) and LA
(0.31), which are lower than our results (24). Although differences in the statistical method to
assess validity coefficients, adjustment for different covariates, study population, validity of the
FCD and characteristics of the FFQ may hamper comparability of studies, our findings were in
the same order of magnitude as the results previously published.

To be able to estimate model parameters, assumptions have to be made. These assumptions are
universally made when the 24hR is used as reference method and are not specifically related to
the use of measurement error models. In our first model we made the assumption of negligible
error correlation between FFQ and DP or 24hR and between replicates of the reference
methods, and the absence of proportional scaling bias for the DP and 24hR. Previous research
showed that correlated errors between FFQ and DP and also between FFQ and 24hR were
present and so was proportional scaling bias for the DP and 24hR for energy, protein, potassium
and sodium intake (8; 9; 11). It would thus be likely that correlated errors and proportional scaling
bias are also present when assessing fatty acid intake. The presence of correlated errors between
FFQ and reference method will lead to an overestimation of validity coefficients and attenuation
factors for the FFQ when using DP or 24hR as reference method (25). We previously showed
that less correlated errors were present between DP and FFQ than between 24hR and FFQ (11).
This would imply that the validity coefficients of the FFQ obtained with the DP as the reference
method would show less overestimation. We indeed observed lower validity coefficients for
fatty acid estimates by the FFQ when the DP was used as reference method than when the 24hR
Correlation of errors between replicates would cause the validity coefficient to be underestimated \(^{(25)}\). We carefully designed the study in such a way that replicates were taken independently with enough time in between. However, this does not remove correlated errors due to e.g. underreporting because of social desirability. For attenuation factors the influence of the proportional scaling bias also needs to be taken into account. Assuming this bias is mostly smaller than one \(^{(8, 11, 26)}\), the attenuation factor will be overestimated.

In our second model we assumed negligible error correlation between biomarker and DP or 24hR and between replicates of the biomarker. In addition, absence of proportional scaling bias for the biomarker was assumed, however if this assumption is not met this does not affect the comparability of validity coefficients for DP and 24hR. The assumption of uncorrelated errors between biomarker and DP or 24hR is likely to hold since the errors in the biomarker measurement are assumed to be mostly physiological where the errors in DP and 24hR are due to the reporting of dietary intake, although complete absence of error correlation cannot be assumed. However, an individual’s digestion, absorption and metabolism are likely to influence concentration biomarker measurements \(^{(27)}\), causing error correlations between replicates of the biomarker. Due to this error correlation, validity coefficients for the DP and 24hR will be underestimated which limits their interpretation as the calculated values should be interpreted as lower limit of the range of potential validity coefficient estimates. However, errors in the biomarker estimates are assumed to influence the validity coefficients for DP and 24hR equally, therefore the finding that the DP had comparable or slightly better ranking abilities than the 24hR is sound. Lastly, given that the collection of DP is expensive and labour intensive our sample size is relatively large, but compared to other validation studies, like the OPEN study\(^{(8)}\), the sample size of this study is relatively small.

Using DP or 24hR as reference methods for FFQ validation enables to assess the validity of a wide range of fatty acids, while plasma fatty acids can only be used to evaluate ranking based on intakes of fatty acids that are not endogenously produced. Furthermore, DPs and 24hRs can be used to assess the validity of absolute FFQ fatty acid intakes, while the plasma fatty acids can only be expressed as percentage of total fatty acids. Using 24hR as reference method has previously been found to reduce but not eliminate the bias in diet-health associations with intakes on a continuous scale and is recommended to be used when no recovery biomarker is available \(^{(10)}\). DPs are assumed to be superior as they are not affected by errors originating from the FCD, while also portion size estimation bias and the influence of memory are expected to be small \(^{(11)}\). However DP are expensive to collect and less feasible to include in validation studies. Also, 24hR with other software or instructions and DP with other instructions, or in
other study populations can yield other results, therefore possible extrapolation of our results has to be done carefully.

In conclusion, taking into account that the assumptions made in our models prevent us from drawing firm conclusions, validity of assessment of fatty acid intake by FFQ differs slightly when the conventionally used 24hR is the reference method as compared to the DP. The 24hR seems to perform slightly worse than the DP when used to obtain validity coefficients for the FFQ, where for attenuation factors for the FFQ the use of DP or 24hR as reference method seem comparable. Therefore, the 24hR seems an acceptable reference method, given it is less burdensome for participants and researcher, for FFQ validation of fatty acid intake.
Table 1: Mean intake of SFA, MUFA, n-3 fatty acids, LA, and n-3/LA ratio in grams and as a percentage of total fatty acids for the DP, 24hR and FFQ

<table>
<thead>
<tr>
<th>Intake in grams</th>
<th>N</th>
<th>SFA</th>
<th>CI</th>
<th>MUFA</th>
<th>CI</th>
<th>n-3</th>
<th>CI</th>
<th>LA</th>
<th>CI</th>
<th>n-3/LA ratio</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>198</td>
<td>31.2</td>
<td>29.9-32.6</td>
<td>32.3</td>
<td>31.0-33.7</td>
<td>2.49</td>
<td>2.26-2.71</td>
<td>14.3</td>
<td>13.5-15.2</td>
<td>0.18</td>
<td>0.17-0.20</td>
</tr>
<tr>
<td>24hR</td>
<td>155</td>
<td>30.1</td>
<td>28.7-31.5</td>
<td>27.9</td>
<td>26.6-29.2</td>
<td>2.02</td>
<td>1.89-2.15</td>
<td>13.5</td>
<td>12.7-14.2</td>
<td>0.17</td>
<td>0.16-0.18</td>
</tr>
<tr>
<td>FFQ</td>
<td>196</td>
<td>26.9</td>
<td>25.6-28.3</td>
<td>28.7</td>
<td>27.4-30.0</td>
<td>2.25</td>
<td>2.14-2.35</td>
<td>14.6</td>
<td>13.9-15.4</td>
<td>0.16</td>
<td>0.16-0.17</td>
</tr>
<tr>
<td>Intake in percentage of total FA</td>
<td>N</td>
<td>SFA</td>
<td>CI</td>
<td>MUFA</td>
<td>CI</td>
<td>n-3</td>
<td>CI</td>
<td>LA</td>
<td>CI</td>
<td>n-3/LA ratio</td>
<td>CI</td>
</tr>
<tr>
<td>DP</td>
<td>198</td>
<td>37.4</td>
<td>36.6-38.3</td>
<td>38.4</td>
<td>37.7-39.0</td>
<td>2.98</td>
<td>2.76-3.20</td>
<td>17.2</td>
<td>16.5-18.0</td>
<td>0.18</td>
<td>0.17-0.20</td>
</tr>
<tr>
<td>24hR</td>
<td>155</td>
<td>40.2</td>
<td>39.4-41.1</td>
<td>36.8</td>
<td>36.1-37.4</td>
<td>2.83</td>
<td>2.66-3.01</td>
<td>18.0</td>
<td>17.3-18.7</td>
<td>0.17</td>
<td>0.16-0.18</td>
</tr>
<tr>
<td>FFQ</td>
<td>196</td>
<td>35.5</td>
<td>34.7-36.2</td>
<td>37.8</td>
<td>37.4-38.1</td>
<td>3.04</td>
<td>2.93-3.14</td>
<td>19.2</td>
<td>18.7-19.7</td>
<td>0.16</td>
<td>0.16-0.17</td>
</tr>
</tbody>
</table>

SFA=saturated fatty acids, MUFA= mono-unsaturated fatty acids, n-3=n-3 fatty acids, LA=linoleic acid, CI=confidence interval, DP=duplicate portion, 24hR=24hour recall, FFQ=food frequency questionnaire, FA=fatty acids

Table 2: Validity coefficients and attenuation factors of the FFQ for fatty acids (expressed as % of total fatty acids) with DP or 24hR as reference methods

<table>
<thead>
<tr>
<th>Ref method</th>
<th>N</th>
<th>SFA</th>
<th>CI</th>
<th>MUFA</th>
<th>CI</th>
<th>n-3</th>
<th>CI</th>
<th>LA</th>
<th>CI</th>
<th>n-3/LA ratio</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validity coefficient*†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>198</td>
<td>0.76</td>
<td>0.63-0.89</td>
<td>0.37</td>
<td>0.19-0.54</td>
<td>0.47</td>
<td>0.32-0.62</td>
<td>0.64</td>
<td>0.48-0.79</td>
<td>0.33</td>
<td>0.17-0.48</td>
</tr>
<tr>
<td>24hR</td>
<td>196</td>
<td>0.82</td>
<td>0.77-0.86</td>
<td>0.65</td>
<td>0.56-0.74</td>
<td>0.62</td>
<td>0.48-0.76</td>
<td>0.80</td>
<td>0.75-0.85</td>
<td>0.76</td>
<td>0.70-0.82</td>
</tr>
<tr>
<td>Attenuation factor‡‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>198</td>
<td>0.57</td>
<td>0.46-0.68</td>
<td>0.34</td>
<td>0.17-0.50</td>
<td>0.63</td>
<td>0.41-0.85</td>
<td>0.60</td>
<td>0.45-0.76</td>
<td>0.49</td>
<td>0.25-0.73</td>
</tr>
<tr>
<td>24hR</td>
<td>196</td>
<td>0.46</td>
<td>0.38-0.53</td>
<td>0.21</td>
<td>0.15-0.27</td>
<td>0.56</td>
<td>0.41-0.71</td>
<td>0.55</td>
<td>0.44-0.66</td>
<td>0.45</td>
<td>0.32-0.58</td>
</tr>
</tbody>
</table>

SFA=saturated fatty acids, MUFA= mono-unsaturated fatty acids, n-3=n-3 fatty acids, LA=linoleic acid, CI=confidence interval, DP=duplicate portion, 24hR=24hour recall

*Models were adjusted for BMI and gender
†Estimates were obtained using model 1 (equation 1 and 2) and formula 3
‡Estimates were obtained using model 1 (equation 1 and 2) and formula 4
Table 3: Validity coefficients and attenuation factors of the FFQ for fatty acids (in grams) with DP or 24hR as reference methods

<table>
<thead>
<tr>
<th>Ref method</th>
<th>N</th>
<th>SFA</th>
<th>MUFA</th>
<th>n-3</th>
<th>LA</th>
<th>n-3/LA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
</tr>
<tr>
<td>Validity coefficient*†</td>
<td>198</td>
<td>0.56</td>
<td>0.43-0.70</td>
<td>0.37</td>
<td>0.23-0.51</td>
<td>0.44</td>
</tr>
<tr>
<td>DP</td>
<td>196</td>
<td>0.62</td>
<td>0.51-0.73</td>
<td>0.47</td>
<td>0.34-0.60</td>
<td>0.74</td>
</tr>
<tr>
<td>Attenuation factor‡</td>
<td>198</td>
<td>0.30</td>
<td>0.21-0.40</td>
<td>0.17</td>
<td>0.08-0.25</td>
<td>0.44</td>
</tr>
<tr>
<td>24hR</td>
<td>196</td>
<td>0.42</td>
<td>0.32-0.52</td>
<td>0.29</td>
<td>0.19-0.39</td>
<td>0.53</td>
</tr>
</tbody>
</table>

SFA=saturated fatty acids, MUFA=mono-unsaturated fatty acids, n-3=n-3 fatty acids, LA=linoleic acid, CI=confidence interval,
DP=duplicate portion, 24hR=24hour recall
*Models were adjusted for BMI and gender
†Estimates were obtained using model 1 (equation 1 and 2) and formula 3
‡Estimates were obtained using model 1 (equation 1 and 2) and formula 4

Table 4: Validity coefficients*† of the DP and 24hR for n-3, LA and n-3/LA ratio where the mean of two plasma fatty acid values (expressed as % of total fatty acids) were used as reference method

<table>
<thead>
<tr>
<th></th>
<th>k</th>
<th>n-3</th>
<th>CI</th>
<th>LA</th>
<th>CI</th>
<th>n-3/LA ratio</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>1</td>
<td>0.33</td>
<td>0.20-0.45</td>
<td>0.18</td>
<td>0.07-0.30</td>
<td>0.34</td>
<td>0.22-0.47</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.39</td>
<td>0.25-0.54</td>
<td>0.22</td>
<td>0.09-0.36</td>
<td>0.41</td>
<td>0.26-0.56</td>
</tr>
<tr>
<td>24hR</td>
<td>1</td>
<td>0.22</td>
<td>0.11-0.32</td>
<td>0.21</td>
<td>0.12-0.29</td>
<td>0.24</td>
<td>0.15-0.34</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.28</td>
<td>0.15-0.41</td>
<td>0.27</td>
<td>0.16-0.39</td>
<td>0.32</td>
<td>0.20-0.45</td>
</tr>
</tbody>
</table>

n-3=n-3 fatty acids, LA=linoleic acid, k = number of measurements, CI=confidence interval, DP=duplicate portion, 24hR=24hour recall
*Models were adjusted for BMI and gender
†Estimates were obtained using model 2 (equation 5 and 6) and formula 3
References