



Validating fatty acid intake as estimated by an FFQ: how does the 24 h recall perform as reference method compared with the duplicate portion?

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1 VALIDATING FATTY ACID INTAKE AS ESTIMATED BY A FOOD FREQUENCY  
2 QUESTIONNAIRE: HOW DOES THE 24 HOUR RECALL PERFORM AS REFERENCE  
3 METHOD COMPARED TO THE DUPLICATE PORTION?  
4

5 Short title: VALIDATING FATTY ACID INTAKE  
6

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39

40 **Conflict of interest**

41 None

42

43 **Authorship**

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

51

52 **Ethical standards disclosure**

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

57

58 **Abstract**

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

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

68 Setting: Wageningen, The Netherlands.

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

70 Results: Validity coefficients for the fatty acid estimates by the FFQ tended to be lower when  
71 using the DP as reference method compared to the 24hR. Attenuation factors for the FFQ tended  
72 to be slightly higher based on the DP than those based on the 24hR as reference method.  
73 Furthermore, when using plasma fatty acids as reference, the DP showed comparable to slightly  
74 better ranking of participants according to their intake of n-3 fatty acids (0.33) and the  
75 n-3/LA ratio (0.34) than the 24hR (0.22 and 0.24 respectively).

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

79

80 Keywords: dietary assessment, validity, measurement errors, fatty acids, duplicate portion,  
81 biomarker

82 **Introduction**

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

107 Previous research concluded that the duplicate portion method (DP) is a suitable reference  
108 method and preferable over a 24hR for FFQ validation for nutrients for which no recovery  
109 biomarker is available <sup>(11)</sup>. The DP is a distinctive reference method as it does not depend on  
110 the availability and quality of the nutrient values in food composition databases, and also biases  
111 related to memory and estimation of portion sizes are less of a problem as compared to methods  
112 such as 24hR and FFQ. Altogether, the DP showed less proportional scaling bias and had a  
113 lower degree of correlated errors with the FFQ than the 24hR for protein, potassium and sodium  
114 <sup>(11)</sup>. In the present paper, we therefore compare the performance of the often used and more  
115 feasible 24hR as reference method for validation of fatty acid estimates from FFQ with the

116 more distinct DP as reference method. We additionally assessed the ability of DP and 24hR to  
117 rank individuals according to their intake of n-3 fatty acids, LA and the n-3/LA ratio using an  
118 objective biomarker (plasma fatty acids) as reference method.

119

## 120 **Subjects and Methods**

### 121 **Subjects and study design**

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

132

### 133 **24-hour recalls and FFQ**

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

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

143

### 144 **Duplicate portion collection and analytical methods**

145 Participants got verbal and written instructions preceding the collection of the DP. Participants  
146 collected all edible foods and drinks consumed over a 24-hour period in collection baskets and  
147 stored them in a cool box (5°C). At the study center, DPs were weighed, homogenized in a  
148 blender (Waring Commercial model 34BL22) and 2.5 mL 0.02% tert-butylhydrochinon (BHQ)  
149 in ethanol was added per kg of DP as antioxidant. For each DP, an aliquot of the homogenized

150 sample was stored within 1 hour at  $-20^{\circ}\text{C}$ , until further analysis. Total fat was measured  
151 gravimetrically by acid hydrolysis (AOAC method 14.019) <sup>(18)</sup>.

152

### 153 **Blood sampling and fatty acid assessment**

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

158

### 159 **Statistical analysis and measurement error models**

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

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

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

180

181 Reference method X (24hR or DP):  $X_{ij} = T + \varepsilon_{xij}$  (1)

182 Food Frequency Questionnaire:  $Q_{ij} = \alpha_Q + \beta_Q T + w_{Qi} + \varepsilon_{Qij}$  (2)

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Validity coefficients ( $\rho_{XT}$ , formula 3) were estimated to assess the ability of the dietary assessment method to rank participants according to their intake:

$$\rho_{XT} = \sqrt{\frac{\beta_X^2 \text{var}T}{\beta_X^2 \text{var}T + \frac{\text{var}\varepsilon_{Xij}}{k} + \text{var}w_{Xi}}} \quad (3)$$

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Where  $\text{var}T$  is the variance of the true nutrient intake;  $\text{var}\varepsilon_{Xij}$  the variance of the random error of method X and  $\text{var}w_{Xi}$  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:

$$\lambda_X = \frac{\rho_{XT}^2}{\beta_X} \quad (4)$$

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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<sup>(7)</sup> 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.

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Biomarker:  $M_{ij} = T + \varepsilon_{Mij}$  (5)

Method X (24hR or DP):  $X_{ij} = \alpha_X + \beta_X T + w_{Xi} + \varepsilon_{Xij}$  (6)

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

## 211 **Results**

### 212 **Baseline characteristics of the study population**



213 At baseline, mean age of the study population was 55.7 (SD 10.2) years and mean BMI was  
214 25.1 (SD 3.7) kg/m<sup>2</sup>. 52.5 percent completed a high level (university or college) and 18.7  
215 percent a low level of education (primary or lower education).

216

### 217 **Mean intakes of fatty acids**

218 Mean intakes and the lower (2.5) and higher (97.5) percentiles of the specific fatty acids in  
219 grams and expressed as percentages of the total amount of fatty acids are shown in Table 1.  
220 SFA intake by the DP (31.2 g) and the 24hR (30.1 g) were both higher than by the FFQ (26.9  
221 g). Also, MUFA and n-3 intakes were highest when assessed by the DP (32.3 g and 2.5 g),  
222 while intakes by the 24hR (27.9 g and 2.0 g) tended to be even lower than those by the FFQ  
223 (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  
224 g) intake tended to be slightly lower. n-3/LA ratios were rather similar. SFA intake as  
225 percentage of total fatty acids was highest when assessed by the 24hR (40.2%), followed by the  
226 DP (37.4%) and FFQ (35.5%). The MUFA intake percentage was highest when assessed by the  
227 DP (38.4%), followed by the FFQ (37.8%) and 24hR (36.8%). The LA intake percentage was  
228 highest when assessed by the FFQ (19.2%), with the 24hR (18.0%) being slightly higher than  
229 the DP (17.2%). For n-3 fatty acids and the n-3/LA ratio, percentages were rather similar for  
230 the three dietary assessment methods.

231

### 232 **DP and 24hR as reference methods for FFQ validation**

233 Validity coefficients for the FFQ were lower when the DP was used as reference method than  
234 when the 24hR was used as reference method when fatty acids were expressed as percentages  
235 of total fatty acids. This was especially true for MUFA (0.37 for DP, 0.65 for 24hR), LA (0.64  
236 for DP, 0.80 for 24hR) and the n-3/LA ratio (0.33 for DP, 0.76 for 24hR, Table 2).

237 For SFA and MUFA the attenuation factor was slightly higher when the DP was used as the  
238 reference method than when the 24hR was used. The other attenuation factors for the FFQ were  
239 rather similar when the DP was used as the reference method compared to the 24hR (Table 2).

240 Also, for fatty acids expressed in grams validity coefficients for the FFQ were lower when the  
241 DP was used as reference method than when the 24hR was used as reference method. This was  
242 especially true for n-3 fatty acids (0.44 for DP, 0.74 for 24hR) and LA (0.49 for DP, 0.69 for  
243 24hR, Table 3). Attenuation factors for the FFQ were higher when the 24hR was used as the  
244 reference method for SFA (0.30 for DP, 0.42 for 24hR), MUFA (0.17 for DP, 0.29 for 24hR)  
245 and LA (0.29 for DP, 0.48 for 24hR).

246 Validity coefficients and attenuation factors for the FFQ were similar, whether they were  
247 expressed in grams or as a percentage of total fatty acids. However, a few values were lower  
248 when expressed in grams: for SFA and LA, both validity coefficients and attenuation factors  
249 for both the DP and 24hR as the reference method. Also for MUFA and the n-3/LA ratio for  
250 the validity coefficient with the 24hR as the reference method values were lower when  
251 expressed in grams (0.47 vs 0.65 and 0.48 vs 0.76 respectively, Table 3).

### 252

### 253 **Ranking ability of DP and 24hR**

254 To additionally compare the performance of the DP and 24hR for ranking in an objective way,  
255 concentration biomarker measurements were used as reference method. Validity coefficients  
256 were used to assess the ability of both methods to rank individuals according to their fatty acid  
257 intake. The validity coefficient for the ranking based on a single DP (k=1) for the n-3 fatty acids  
258 (0.33) was slightly higher than for a single 24hR (0.22, Table 4). For LA and the n-3/LA ratio,  
259 validity coefficients were similar. A similar pattern was observed for validity coefficients based  
260 on two DP and two 24hR measurements as shown in table 4 (k=2).

### 261

### 262 **Discussion**

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

277

278 Intakes of fatty acids in our study population were comparable with those of the general Dutch  
279 population based on the 2007-2010 Dutch National Food Consumption Survey (DNFCS) <sup>(20)</sup>.

280 The DNFCS intake data are based on two telephone-based 24hRs and the same FCD (2011) as  
281 we used to calculate nutrient intakes. Assessment of nutrient intake is among others limited by  
282 the availability and quality of the data in the FCD. Fatty acid composition of foods may change  
283 over time and vary amongst different brands. However, a study comparing calculated and  
284 analysed test diets for controlled dietary interventions found a reasonable agreement between  
285 the two for SFA and MUFA <sup>(21)</sup> indicating the Dutch FCD performs reasonably well for these  
286 fatty acids.

287 Published data on validity coefficients for FFQs for fatty acids intake estimates are scarce. One  
288 study, using the method of triads with the biomarker and weighed food records as reference  
289 method, found a validity coefficient of 0.50 for n-3 fatty acids assessed by FFQ <sup>(22)</sup>, which is  
290 comparable to our results. A study by Kabagambe *et al*, also using the method of triads, found  
291 validity coefficients for the FFQ for LA between 0.77 and 0.89 <sup>(23)</sup>, using the biomarker and  
292 24hR as reference methods. This is in line with our findings for LA when using the 24hR as  
293 reference method. A recent study in Brazilian adults, also using the method of triads with a  
294 biomarker, FFQ and 24hR, reported validity coefficients for the FFQ for SFA (0.28) and LA  
295 (0.31), which are lower than our results<sup>(24)</sup>. Although differences in the statistical method to  
296 assess validity coefficients, adjustment for different covariates, study population, validity of the  
297 FCD and characteristics of the FFQ may hamper comparability of studies, our findings were in  
298 the same order of magnitude as the results previously published.

299 To be able to estimate model parameters, assumptions have to be made. These assumptions are  
300 universally made when the 24hR is used as reference method and are not specifically related to  
301 the use of measurement error models. In our first model we made the assumption of negligible  
302 error correlation between FFQ and DP or 24hR and between replicates of the reference  
303 methods, and the absence of proportional scaling bias for the DP and 24hR. Previous research  
304 showed that correlated errors between FFQ and 24hR and also between FFQ and DP were  
305 present and so was proportional scaling bias for the DP and 24hR for energy, protein, potassium  
306 and sodium intake <sup>(8; 9; 11)</sup>. It would thus be likely that correlated errors and proportional scaling  
307 bias are also present when assessing fatty acid intake. The presence of correlated errors between  
308 FFQ and reference method will lead to an overestimation of validity coefficients and attenuation  
309 factors for the FFQ when using DP or 24hR as reference method <sup>(25)</sup>. We previously showed  
310 that less correlated errors were present between DP and FFQ than between 24hR and FFQ <sup>(11)</sup>.  
311 This would imply that the validity coefficients of the FFQ obtained with the DP as the reference  
312 method would show less overestimation. We indeed observed lower validity coefficients for  
313 fatty acid estimates by the FFQ when the DP was used as reference method than when the 24hR

314 was used. Correlation of errors between replicates would cause the validity coefficient to be  
315 underestimated <sup>(25)</sup>. We carefully designed the study in such a way that replicates were taken  
316 independently with enough time in between. However, this does not remove correlated errors  
317 due to e.g. underreporting because of social desirability. For attenuation factors the influence  
318 of the proportional scaling bias also needs to be taken into account. Assuming this bias is mostly  
319 smaller than one <sup>(8; 11; 26)</sup>, the attenuation factor will be overestimated.

320 In our second model we assumed negligible error correlation between biomarker and DP or  
321 24hR and between replicates of the biomarker. In addition, absence of proportional scaling bias  
322 for the biomarker was assumed, however if this assumption is not met this does not affect the  
323 comparability of validity coefficients for DP and 24hR. The assumption of uncorrelated errors  
324 between biomarker and DP or 24hR is likely to hold since the errors in the biomarker  
325 measurement are assumed to be mostly physiological where the errors in DP and 24hR are due  
326 to the reporting of dietary intake, although complete absence of error correlation cannot be  
327 assumed. However, an individual's digestion, absorption and metabolism are likely to influence  
328 concentration biomarker measurements <sup>(27)</sup>, causing error correlations between replicates of the  
329 biomarker. Due to this error correlation, validity coefficients for the DP and 24hR will be  
330 underestimated which limits their interpretation as the calculated values should be interpreted  
331 as lower limit of the range of potential validity coefficient estimates. However, errors in the  
332 biomarker estimates are assumed to influence the validity coefficients for DP and 24hR equally,  
333 therefore the finding that the DP had comparable or slightly better ranking abilities than the  
334 24hR is sound. Lastly, given that the collection of DP is expensive and labour intensive our  
335 sample size is relatively large, but compared to other validation studies, like the OPEN study<sup>(8)</sup>,  
336 the sample size of this study is relatively small.

337 Using DP or 24hR as reference methods for FFQ validation enables to assess the validity of a  
338 wide range of fatty acids, while plasma fatty acids can only be used to evaluate ranking based  
339 on intakes of fatty acids that are not endogenously produced. Furthermore, DPs and 24hRs can  
340 be used to assess the validity of absolute FFQ fatty acid intakes, while the plasma fatty acids  
341 can only be expressed as percentage of total fatty acids. Using 24hR as reference method has  
342 previously been found to reduce but not eliminate the bias in diet-health associations with  
343 intakes on a continuous scale and is recommended to be used when no recovery biomarker is  
344 available <sup>(10)</sup>. DPs are assumed to be superior as they are not affected by errors originating from  
345 the FCD, while also portion size estimation bias and the influence of memory are expected to  
346 be small<sup>(11)</sup>. However DP are expensive to collect and less feasible to include in validation  
347 studies. Also, 24hR with other software or instructions and DP with other instructions, or in

348 other study populations can yield other results, therefore possible extrapolation of our results  
349 has to be done carefully.

350

351 In conclusion, taking into account that the assumptions made in our models prevent us from  
352 drawing firm conclusions, validity of assessment of fatty acid intake by FFQ differs slightly  
353 when the conventionally used 24hR is the reference method as compared to the DP. The 24hR  
354 seems to perform slightly worse than the DP when used to obtain validity coefficients for the  
355 FFQ, where for attenuation factors for the FFQ the use of DP or 24hR as reference method  
356 seem comparable. Therefore, the 24hR seems an acceptable reference method, given it is less  
357 burdensome for participants and researcher, for FFQ validation of fatty acid intake.

358

359 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  
 360 and FFQ

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

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

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365 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  
 366 methods

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

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

369 \*Models were adjusted for BMI and gender

370 †Estimates were obtained using model 1 (equation 1 and 2) and formula 3

371 ‡Estimates were obtained using model 1 (equation 1 and 2) and formula 4

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374 Table 3: Validity coefficients and attenuation factors of the FFQ for fatty acids (in grams) with DP or 24hR as reference methods

Ref method	N	SFA		MUFA		n-3		LA		n-3/LA ratio	
			CI		CI		CI		CI		CI
<b>Validity coefficient*†</b>											
<b>DP</b>	198	0.56	0.43-0.70	0.37	0.23-0.51	0.44	0.30-0.58	0.49	0.35-0.64	0.33	0.17-0.48
<b>24hR</b>	196	0.62	0.51-0.73	0.47	0.34-0.60	0.74	0.63-0.83	0.69	0.59-0.79	0.48	0.29-0.66
<b>Attenuation factor*‡</b>											
<b>DP</b>	198	0.30	0.21-0.40	0.17	0.08-0.25	0.44	0.28-0.59	0.29	0.19-0.39	0.49	0.25-0.73
<b>24hR</b>	196	0.42	0.32-0.52	0.29	0.19-0.39	0.53	0.42-0.64	0.48	0.38-0.58	0.39	0.22-0.56

375 SFA=saturated fatty acids, MUFA= mono-unsaturated fatty acids, n-3=n-3 fatty acids, LA=linoleic acid, CI=confidence interval,

376 DP=duplicate portion, 24hR= 24hour recall

377 \*Models were adjusted for BMI and gender

378 †Estimates were obtained using model 1 (equation 1 and 2) and formula 3

379 ‡Estimates were obtained using model 1 (equation 1 and 2) and formula 4

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383 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

384 % of total fatty acids) were used as reference method

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

385 n-3=n-3 fatty acids, LA=linoleic acid, k = number of measurements,

386 CI=confidence interval, DP=duplicate portion, 24hR= 24hour recall

387 \*Models were adjusted for BMI and gender

388 †Estimates were obtained using model 2 (equation 5 and 6) and formula 3

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