

Prediction of fruit and vegetable intake from biomarkers using individual participant data of diet-controlled intervention studies

Olga W. Souverein¹, Jeanne H.M. de Vries¹, Riitta Freese², Bernhard Watzl³, Achim Bub³, Edgar R. Miller III⁴, Jacqueline J.M. Castenmiller⁵, Wilrike J. Pasman⁶, Karin van het Hof⁷, **Mridula Chopra**⁸, Anette Karlsen⁹, Lars O. Dragsted¹⁰, Renate Winkels¹, Catherine Itsiopoulos¹¹, Laima Brazionis¹², Kerin O'Dea¹³, Carolien A. van Loo-Bouwman¹⁴, Ton HJ Naber¹⁵, Hilko van der Voet¹⁶, Hendriek C. Boshuizen¹

- 1) Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV, Wageningen, the Netherlands;
- 2) Division of Nutrition, Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland;
- 3) Department of Physiology and Biochemistry of Nutrition, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany;
- 4) Johns Hopkins University, Baltimore, USA;
- 5) Netherlands Food and Consumer Product Safety Authority, Utrecht, the Netherlands;
- 6) TNO, Zeist, the Netherlands;
- 7) Unilever, Vlaardingen, the Netherlands;
- 8) School of Pharmacy and Biomedical Science, University of Portsmouth, Portsmouth, UK;
- 9) Department of Nutrition, Faculty of Medicine, Institute of Basic Medical Sciences, University of Oslo, Blindern, Oslo, Norway;
- 10) Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Frederiksberg C, Denmark;
- 11) Faculty of Health Sciences, Latrobe University, Bundoora, Victoria 3086, Australia;
- 12) Department of Medicine, University of Melbourne, St Vincent's Hospital, Victoria 3065, Australia;
- 13) Sansom Institute of Health Research, University of South Australia, South Australia 5001, Australia;
- 14) Department of Gastroenterology and Hepatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands;
- 15) Department of Internal Medicine and Gastroenterology, Tergooi, Hilversum, the Netherlands;
- 16) Biometris, Wageningen University and Research Centre, Wageningen, the Netherlands

Corresponding author: Dr. Olga W Souverein, Wageningen University, Division of Human Nutrition, PO Box 8129, 6700 EV, Wageningen, The Netherlands. T: + 31 317 483312. F: + 31 317 482782. E-mail: Olga.Souverein@gmail.com

Running title: Predicting fruit and vegetable intake

Keywords: fruit and vegetables; prediction model; vitamin C; folate; carotenoids

1 ABSTRACT

2 Fruit and vegetable consumption produces changes in several biomarkers in blood. This study
3 aims to examine the dose-response curve between fruit and vegetable consumption and
4 carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lycopene, lutein, zeaxanthin), folate and
5 vitamin C concentrations. Furthermore, a prediction model of fruit and vegetable intake based on
6 these biomarkers and subject characteristics (i.e., age, gender, BMI, smoking status) was
7 established. Data from 12 diet-controlled intervention studies were obtained to develop a
8 prediction model for fruit and vegetable intake (including and excluding fruit and vegetable
9 juices). The study population in this individual participant data meta-analysis consisted of 526
10 men and women. Carotenoid, folate and vitamin C concentrations showed a positive relationship
11 with fruit and vegetable intake. Measures of performance for the prediction model were
12 calculated using cross-validation. For the prediction model of fruit, vegetable and juice intake the
13 root mean squared error (RMSE) was 258.0 g, the correlation between observed and predicted
14 intake was 0.78, and the mean difference between observed and predicted intake was -1.7 g
15 (limits of agreement: -466.3; 462.8 g). For the prediction of fruit and vegetable intake (excluding
16 juices) the RMSE was 201.1 g, the correlation was 0.65 and the mean bias was 2.4 g (limits of
17 agreement: -368.2; 373.0 g). The prediction models which include the biomarkers and subject
18 characteristics may be used to estimate average intake at the group level and to investigate
19 ranking of individuals with regard to their intake of fruit and vegetables when validating
20 questionnaires that measure intake.

21 INTRODUCTION

22 A high consumption of fruit and vegetables has been associated with a reduced risk of several
23 chronic diseases such as cancer and cardiovascular disease⁽¹⁻³⁾. Therefore, intervention studies
24 that aim to increase the consumption of fruit and vegetables using advice or counseling are often
25 conducted. To investigate the success of the intervention, the subjects are asked to report or
26 recall their consumption of fruit and vegetables. However, as it is highly likely that the subject is
27 aware of the intervention (i.e., the advice or counseling), the report or recall is likely to be
28 biased. Objective measures such as serum/plasma concentrations of carotenoids have been used
29 to investigate whether the intervention led to an increase in fruit and vegetable consumption
30 compared to the control group⁽⁴⁻⁶⁾, but these biomarkers do not quantify the increase in fruit and
31 vegetable intake caused by the intervention.

32 The validation of fruit and vegetable intake relies at this moment on self-reporting instruments.
33 However, self-reported dietary intake instruments are found to be biased and to have correlated
34 errors when compared to recovery biomarkers such as doubly labeled water and urinary nitrogen
35 excretion⁽⁷⁻¹⁰⁾. Therefore, if we were able to quantify fruit and vegetable intake based on
36 biomarkers rather than on self-reporting, the comparison of self-reported intake with this
37 biomarker-based intake estimate will give a better idea of true validity. No recovery biomarker is
38 available for fruit and vegetable intake. Therefore, it would be useful to find a predictive
39 biomarker that can be related to true intake of fruit and vegetables^(11, 12).

40 It is not straightforward to relate an increase in for instance β -carotene concentration to an exact
41 increase in fruit and vegetable consumption. Single biomarkers or the sum of carotenoids have
42 previously been shown to have low correlations with self-reported intake of fruit and
43 vegetables⁽¹³⁻²¹⁾. Therefore, to ascertain the full range of fruit and vegetable intake it is
44 worthwhile to investigate whether a combination of biomarkers, possibly in combination with
45 other factors, can provide more reliable results. Baldrick et al.⁽²²⁾ found that the carotenoids and
46 vitamin C are the most consistently responsive biomarkers for fruit and vegetable intake. In
47 addition, serum/plasma folate may be used as a biomarker of fruit and vegetable intake, even
48 though this is a less sensitive marker especially in countries where fortification with folate is
49 mandatory^(23, 24). To be able to use biomarkers to quantify the consumption of fruit and
50 vegetables, the dose-response relationship between fruit and vegetable intake and the respective
51 biomarkers must be present. As dietary intake recorded by subjects is often biased, a cross-

52 sectional study with such data will not provide us with an unbiased estimate of the dose-response
53 curve. In contrast, for diet-controlled intervention studies where fruit and vegetables are provided
54 to the participants the intake data does not rely solely on self-reporting. In these studies the
55 combined information on the amount provided, the information from supervised consumption
56 and the self-reported information on compliance, may lead to a less biased estimate of the intake
57 of fruit and vegetables. We therefore conducted an individual participant data (IPD) meta-
58 analysis of such studies, covering a wide range of fruit and vegetable intakes. The first aim of
59 this study is to investigate the dose-response curve between fruit and vegetable consumption and
60 biomarkers, namely serum carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lycopene,
61 lutein, zeaxanthin), serum/plasma folate and serum/plasma vitamin C. The second aim is to
62 establish a prediction model of fruit and vegetable intake based on these biomarkers which may
63 be used to estimate group-level intake or as a predictive biomarker.

64

65 **METHODS**

66 **Search strategy**

67 The aim of the literature search was to find diet-controlled intervention studies (i.e., food
68 provision studies or partly supervised feeding studies) conducted in adult subjects where reports
69 on the amount of consumed fruits and vegetables were supported by information on the amount
70 provided and where significant efforts were made to maximise compliance. The following diet-
71 controlled intervention studies were included: i) all foods and drinks were provided to the
72 subjects during the intervention, or ii) all fruits and vegetables consumed were provided to the
73 subjects. In addition, carotenoids or folate concentrations in blood after intervention were
74 measured and papers were published in the English language. The search was conducted in
75 Scopus, Pubmed and by manual search of reference lists. Search terms in title and abstract were
76 'fruit' and 'vegetables' combined with 'intervention', 'trial' and 'feeding study', which was then
77 combined with 'biomarkers', 'biological markers', 'carotenoids', 'alpha-carotene', 'beta-
78 carotene', 'beta-cryptoxanthin', 'zeaxanthin', 'lycopene', 'lutein', 'folate' and 'bioavailability'.
79 The search included publications until October 2012.
80 Papers were first screened based on the title and abstracts. Then, the full text of the papers that
81 were considered potentially relevant were read and judged for relevancy. Next, the full text of
82 the papers was retrieved and judged using inclusion and exclusion criteria. The exclusion

83 criteria were: i) intervention study where the intervention consisted of dietary advice or
84 counseling (and therefore foods were not provided to the subjects by the investigators); ii)
85 intervention study where not all fruits and vegetables were provided (i.e., the provision consisted
86 of additional fruit and vegetables on top of normal fruit and vegetable consumption), or where
87 fruit and vegetables were provided as supplements (e.g., capsules), juices, or extracts; iii)
88 intervention study where the intervention involved a single ingestion of the intervention food(s)
89 or an intervention period of 6 days or less; and iv) the study was conducted in children,
90 adolescents, institutionalized elderly, or pregnant or lactating women.

91

92 **Data**

93 Current contact details of corresponding author, first author or other authors were searched on
94 the internet. Authors were contacted by email and asked whether they were willing to send the
95 original data of the study. These authors were offered a co-authorship on the present paper. We
96 requested individual participant data (where available) of subject characteristics (gender, age,
97 height, weight (or BMI), smoking status), serum/plasma values of biomarkers, and intake of
98 fruits and vegetables (or intervention group coding).

99 In addition, we collected information on: i) the study design (parallel or cross-over study,
100 whether a run-in period was included, and where applicable whether a wash-out period was
101 included); ii) the dietary intervention (duration of the dietary intervention, daily intake of fruit
102 and vegetables, carotenoids or folate); iii) the serum/plasma measurements (whether blood was
103 drawn after a fasting period, which methods were used for sample analysis).

104

105 **Statistical analysis**

106 Outliers, defined as all observations above $[Q3+4*IQR]$ (where Q3 refers to the third quartile
107 and IQR is the inter-quartile range), were removed from the dataset. The median number of
108 outliers per biomarker was 1 (range: 0-7).

109

110 *Dose-response curves*

111 The dose-response curve between log-transformed biomarker concentrations (dependent
112 variable) and fruit and vegetable intake (independent variable) and between biomarker
113 concentrations and the corresponding micronutrient was estimated using fractional polynomials

114 (FP)^(25, 26). To account for the one cross-over study and the between study heterogeneity the final
115 parameter estimates were calculated using mixed models using study and subjects as random
116 effects. Therefore, the estimated variance components refer to differences between studies,
117 differences between individuals (to account for the cross-over study) and residual variance.
118 To obtain predictions on the original scale rather than on the logarithmic scale, we applied the
119 following back-transformation: $E(Y) = \exp\left(\beta_0 + \sum_{k=1}^p \beta_k X_k + \frac{1}{2}\sigma^2\right)$, where Y is the biomarker
120 concentration on the original scale, X is the fruit and vegetable intake, and σ^2 is the sum of the
121 variance components estimated in the mixed model.

122 Several covariates were tested to see whether they statistically significantly predicted the
123 biomarker concentrations. Covariates that were tested were age, BMI, gender, and smoking. In
124 addition, the interaction between fruit and vegetable intake and these covariates was tested. The
125 covariates and interactions were tested by including them one at a time in separate fractional
126 polynomial regression models.

127

128 *Prediction models of fruit and vegetable intake*

129 We developed three different prediction models based on what we learned from the dose-
130 response curves. The models were estimated using linear regression: 1) a pre-specified model
131 where all continuous variables were added as linear terms, 2) a pre-specified model where the
132 shape of all continuous variables was established using multivariable fractional polynomials
133 (MFP; referred to as MFP model), and 3) a reduced model including only the statistically
134 significant predictors selected using MFP (referred to as reduced MFP model). The MFP models
135 were analyzed using STATA/SE 11.0 for Windows. Interactions between the subject
136 characteristics (age, BMI, gender and smoking status) and the biomarkers (α -carotene, β -
137 carotene, lutein+zeaxanthin, lycopene, β -cryptoxanthin) were tested for inclusion in the model in
138 four separate models (i.e., i. main effects + age*biomarkers; ii. main effects + BMI*biomarkers,
139 iii. main effects + gender*biomarkers; iv. main effects + smoking status*biomarkers). All
140 interactions were included as linear terms. Interactions with $p < 0.05$ were considered relevant for
141 inclusion in the prediction model. These interactions were then tested together in the model and a
142 backward selection was applied until all interactions included in the model had a p-value < 0.05 .
143 Because data on predictors and outcomes were not complete, we used a multiple imputation
144 approach where 10 multiple imputed data sets were created. The power and selection of the

145 predictors was established in all 10 imputed data sets separately and the final model was
146 established by majority voting⁽²⁷⁾.
147 The validation of the fruit, vegetable and juice intake (FVJ) and fruit and vegetable intake
148 (excluding juices; FV) prediction models was assessed using 10-fold cross-validation. First the
149 data was imputed as before, after which the data was randomly separated into 10 parts. One part
150 was left out to construct the training set (i.e., the remaining nine parts) and the prediction models
151 were fitted to each of the imputed data sets using linear regression models. The regression
152 coefficients were combined using normal procedures to obtain the regression coefficients for the
153 test data. The out-of-sample data (the test set) was used to calculate the predicted values for each
154 individual by multiplying the regression coefficients with the observed values of the predictors in
155 each of the imputed test sets. The final predicted values were calculated by averaging the
156 predicted values over the 10 imputed test sets. Each of the parts was left out once, so the
157 procedure was repeated 10 times. These predicted values were compared to the observed values
158 as an estimate of the model performance using three different measures: 1) the Root Mean
159 Square Error (MSE) = $\sqrt{\frac{1}{n} \sum (Y - \hat{Y})^2}$, 2) the correlation between observed intake and predicted
160 intake, and 3) the mean difference (observed intake minus predicted intake) with the
161 corresponding limits of agreement at the individual level (i.e, mean difference \pm
162 $1.96 * SD_{\text{difference}}$). Unless otherwise indicated, all analyses were performed using SAS version
163 9.2.

164

165 **RESULTS**

166 **Search and data retrieval**

167 A total of 1002 studies were found of which 27 qualified for inclusion in the present meta-
168 analysis⁽²⁸⁻⁵⁴⁾. Of these 27 papers, eight publications described a study population that was also
169 described in another publication. Therefore, the authors of a total of 19 unique diet-controlled
170 intervention studies were contacted for cooperation in retrieving individual data. The flowchart
171 of the selection of studies is shown in Figure 1. A total of 12 authors responded positively to the
172 request and made their data available for our analysis. A summary of study characteristics of
173 these studies is given in Table 1 and an overview of the data of these studies is presented in

174 Table 2. The data of four studies were unfortunately unavailable, and three authors did not
175 respond to our request. Information from these studies is available in Supplemental Table A.
176 For four studies specific groups were not useful in the present analysis^(36, 38, 41, 49, 50, 52), and for
177 one study data of a subset of participants was received⁽⁴⁴⁾. For the study of Miller II *et al.*⁽⁴⁴⁾,
178 intake of fruit and vegetables in servings was converted to grams per day by multiplying the
179 number of servings by 80 g. For the study of Itsiopoulos et al.⁽⁴⁰⁾ intake of fruit and vegetables
180 was known for 15 subjects. For the remaining 12 subjects the vegetable intake was imputed as
181 the mean of the intake as reported in the paper (i.e., 466 g/d vegetables and 162 g/d fruit). Where
182 needed α -carotene, β -carotene and lycopene were converted from $\mu\text{g/mL}$ to $\mu\text{mol/L}$.

183

184 **Dose-response analysis**

185 The estimated dose-response curves between the different biomarkers and fruit, vegetable and
186 juice intake are shown in Figure 2, and the dose-response curves between the biomarkers and
187 fruit and vegetable intake (excluding juices) are shown in Figure 3. All biomarkers show a
188 positive dose-response relationship with fruit and vegetable intake. The regression equations that
189 were obtained are shown in Supplemental Table B.

190 The p-values of the covariate and interaction analysis are shown in Supplemental Table C. Age
191 and smoking were significant predictors for all carotenoids, but not for plasma folate. BMI was a
192 significant predictor for α -carotene, β -carotene, lutein, β -cryptoxanthin and lycopene. Gender
193 was only a significant predictor for lutein, zeaxanthin and lutein+zeaxanthin. The interactions
194 between these covariates and the intake of fruits and vegetables were relevant ($p < 0.1$) in most
195 instances. The smoking*fruit and vegetable interaction was only a significant predictor for about
196 half of the biomarkers, but this may be due to the relatively low number of smokers included in
197 this sample.

198 Where possible, the dose-response relationship between the biomarkers and the intake of the
199 micronutrient was also investigated (Supplemental Figure A). The available sample size was
200 largest for β -carotene ($n=316$) and smallest for lutein+zeaxanthin ($n=35$). The sample size of
201 zeaxanthin was too low to warrant analysis. All curves showed a positive relationship between
202 intake and serum or plasma concentrations except lutein at high intakes. There is no biological
203 evidence for the drop that is visible in the lutein curve. As there were very few data available for
204 lutein intake above 15 mg/day, this part of the curve is not considered reliable.

205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234

Prediction model

The regression coefficients of the final prediction model are presented in Table 3 and the performance measures are presented in Table 4. The power and variable selection process of the MFP and the reduced MFP model is shown in Supplemental Tables D and E. For fruit, vegetable and juice intake, the reduced MFP model showed the lowest RMSE (i.e., 258.0 g) and the highest correlation between observed and predicted (i.e., 0.78) compared to the linear model and the full pre-specified MFP model. The mean difference of the reduced MFP model (-1.7 g) was a little higher than of the other two models (linear model: -1.6 g; MFP model: -1.5 g), but the limits of agreement were markedly smaller than those of the other two models. Bland-Altman plots are presented in Supplemental Figure B.

For fruit and vegetable intake (excluding juices) the MFP model was the best model. It showed the lowest RMSE (201.1 g), the highest correlation (0.65) and the lowest mean bias (2.4 g) with the smallest limits of agreement (-368.2; 373.0 g).

The prediction model for fruit and vegetable intake (excluding juices) showed a somewhat lower correlation and higher absolute mean difference than the model of fruit and vegetable intake which included juices. Therefore, we investigated whether a model including a predictor variable that represented the juice intake (in g/d) would improve the prediction for fruit and vegetable intake when juices were excluded. However, this did not markedly change the results. The MFP model including juice as a predictor variable had a RMSE of 202.8 g, a correlation of 0.64, mean bias of 0.2 g (limits of agreement: -374.1; 374.6 g). Therefore, the more simple model without juice as a predictor variable is preferred as a prediction model for fruit and vegetable intake (excluding juices).

To be able to compare the performance of the prediction model with the current practice of using the sum of carotenoids or any of the single biomarkers, we calculated the correlation coefficients between the observed intakes and the sum of carotenoids and between observed intakes and the single biomarkers (Table 5). For fruit, vegetable and juice intake the correlations range between 0.04 and 0.32, which is much lower than the 0.65 of the prediction model. Also for fruit and vegetable intake (excluding juices) the correlations (between 0.15 and 0.38) are lower than that of the prediction model (0.64).

235 To indicate the value of the prediction model for individual studies, an additional cross-
236 validation was performed by leaving one entire study out of the training set. The study that was
237 left out comprised the test set. Table 6 shows the RMSE and mean difference with the limits of
238 agreement for the reduced MFP model for fruit, vegetable and juice intake and the MFP model
239 for fruit and vegetable intake (excluding juices). These show that there is a difference on how
240 well the prediction models perform per study. The study of Karlsen *et al.*⁽⁴¹⁾ shows a worse
241 performance for fruit, vegetable and juice intake, but not for fruit and vegetable intake
242 (excluding juices). This is most likely caused by the relatively high intake of fruits, vegetables
243 and juices in this study (see Table 1).

244

245 **DISCUSSION**

246 The first part of this research showed that all investigated biomarkers (carotenoids and folate)
247 showed a positive relationship with fruit and vegetable intake, and are therefore useful for
248 predicting fruit and vegetable intake. Several covariates were significantly associated with the
249 biomarkers. The next aim was to develop a prediction model for fruit and vegetable intake based
250 on objective variables such as biomarkers and subject characteristics. Among the three
251 investigated models for predicting fruit, vegetable and juice intake the reduced MFP model
252 showed the best performance in cross-validation, and for fruit and vegetable intake (excluding
253 juices) the MFP model showed the best performance.

254 The sum of carotenoids has been used in an attempt to combine biomarkers into a single estimate
255 for fruit and vegetable intake in various studies. The sum of carotenoids was positively
256 correlated with self-reported fruit and vegetable intake^(14-21, 55, 56). In the present study, the
257 correlations between our predicted values, which can easily be calculated in future research by
258 multiplying observed values from biomarkers and subject characteristics with the corresponding
259 beta coefficients from Table 3 and then adding these together, and the observed fruit and
260 vegetable intake (both including and excluding juices) is markedly higher than the correlations
261 between the observed intakes and the sum of carotenoids or any of the single biomarkers.

262 Despite the models good performance on average, there is quite some residual variation as well
263 as an overestimation of low fruit and vegetable intake and an underestimation of high fruit and
264 vegetable intake. Not all fruits and vegetables contain the same concentration of carotenoids and
265 folate, and also other foods in the diet will contain these nutrients. Therefore, the type of fruits

266 and vegetables eaten as well as the diet as a whole will influence the final biomarker
267 concentrations in the blood. The current study tried to capture ‘normal’ diet effects as much as
268 possible by excluding those studies that provided only on a single fruit or vegetable, and
269 including intervention arms that focused on carotenoid-rich or folate-rich as well as those that
270 focused on carotenoid-poor or folate-poor fruits and vegetables. To obtain the large-sample
271 benefits of a meta-analysis these different study types were grouped together. This was done
272 under the assumption that since quite a number of studies were included, the applied regression
273 analysis will average out effects of individual studies, which resulted in an assumption that at
274 least this first approximation does not depend on the type of fruit and vegetables. Obviously the
275 assumption is not true in an absolute sense, as for example carrots contain more carotenoids than
276 some other vegetables, and this will require further investigation.

277 Another source of variability may come from the different intervention durations. We excluded
278 studies with a duration of less than seven days under the assumption that it would take
279 approximately a week to obtain a new steady-state for the carotenoids after the change in diet
280 induced by the intervention⁽⁵⁷⁾. The actual duration of the studies included in the prediction
281 models was much longer (Table 1).

282 Differences in analytical methods used in the different studies may be another source of residual
283 variation. In particular, folate levels were analysed using different assays, e.g. immunoassay,
284 radioassay. Also, among many other possible sources, laboratory variability may be caused by
285 different specimen collection and storage⁽⁵⁸⁾.

286 Gender, age, BMI and smoking impact on serum carotenoids, serum vitamin C and plasma folate
287 levels, and several other covariates such as serum cholesterol, serum triglycerides and
288 consumption of alcohol, fat and energy may also be related to the biomarkers⁽⁵⁹⁻⁶³⁾. It may be of
289 interest to investigate whether these covariates could significantly improve the prediction model.
290 However, current data did not allow us to investigate this thoroughly.

291 **Although significant efforts were made in all individual studies to encourage compliance to the**
292 **study protocol (e.g. supervised consumption of meals; see Table 1) the true intake of fruit and**
293 **vegetables cannot always be determined with absolute certainty when they rely on self-reports of**
294 **compliance. In quite a number of the individual studies the compliance was investigated with**
295 **e.g. questionnaires or diaries, and most often this self-reported compliance was high.**

296 Unfortunately, no external validation data was available for the prediction model. We chose to
297 use all the data from the diet-controlled intervention studies that were available to us to develop
298 the models. To perform an external validation, data from other or new diet-controlled
299 intervention studies would have to be obtained. As this is very complicated and the data from
300 these studies would then preferably be used to develop or improve the model rather than to just
301 validate it, we mimicked independent data by using cross-validation to calculate the measures of
302 performance ⁽⁶⁴⁾.

303 The use of individual participant data from diet-controlled intervention studies made it possible
304 to model the dose-response curves and the prediction models for a large range of fruit and
305 vegetable intake with a relatively large number of subjects using a more objective assessment of
306 intake. However, between-study differences may have influenced the study results. In the dose-
307 response analysis we took clustering into account by using mixed effect models⁽⁶⁵⁾. For the
308 prediction model, the marginal predictions (i.e., using only the fixed effects as the (unknown)
309 random effect cannot be used in predictions for new subjects) from the random intercept linear
310 regression model performed somewhat worse in cross-validation than the predictions from the
311 standard regression model (data not shown), and therefore we chose to present the standard
312 regression model. Bouwmeester et al.⁽⁶⁶⁾ found similar performance measures for a standard
313 logistic regression model and a random intercept logistic regression model in a study on surgical
314 patients that were clustered per anesthesiologist. Recently, Debray et al.⁽⁶⁷⁾ have developed an
315 approach to deal with risk prediction in new patients taking into account the random-intercept
316 after the model has been developed using IPD meta-analysis with mixed effects modeling. In the
317 present study, the performance of the conditional predictions was not considerably better than the
318 performance of the standard predictions in an apparent validation (i.e., an internal validation
319 based on the entire data, so not using cross-validation) (data not shown).

320 In conclusion, the relatively strong correlations between predictions and actual intake indicate
321 that our prediction models may be used to investigate ranking of individuals with regard to their
322 intake of fruit and vegetables when validating questionnaires that measure intake (e.g. FFQ or
323 24-hour recall). Furthermore, the low mean bias show the models have good potential to be used
324 to estimate average fruit and vegetable intake on a group level. The large limits of agreement
325 indicate that the prediction models should not be used to estimate individual fruit and vegetable
326 intake.

327

328 ACKNOWLEDGEMENTS

329

330 FINANCIAL SUPPORT

331 This research was financially supported by ZonMW (project number 200400014). ZonMW had
332 no role in the design, analysis or writing of this article.

333

334 CONFLICT OF INTEREST

335 None

336

337 AUTHORSHIP

338 The authors' responsibilities were as follows: HCB designed research. RF, BW, AB, ERM,
339 JJMC, WJP, KvdH, MC, AK, LOD, RW, CI, LB, KO, CAvL-B, THJN provided essential data
340 that was used for this study. JHMdV and HvdV provided essential advice. OWS performed
341 statistical analysis. OWS and HCB wrote the paper. OWS and HCB had primary responsibility
342 for final content. All authors read and approved the final manuscript.

REFERENCES

1. Boeing H, Bechthold A, Bub A *et al.* (2012) Critical review: vegetables and fruit in the prevention of chronic diseases. *Eur J Nutr* **51**, 637-663.
2. Hung HC, Joshipura KJ, Jiang R *et al.* (2004) Fruit and vegetable intake and risk of major chronic disease. *J Natl Cancer Inst* **96**, 1577-1584.
3. Riboli E & Norat T (2003) Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr* **78**, Suppl. 3, 559S-569S.
4. Macdonald HM, Hardcastle AC, Duthie GG *et al.* (2009) Changes in vitamin biomarkers during a 2-year intervention trial involving increased fruit and vegetable consumption by free-living volunteers. *Br J Nutr* **102**, 1477-1486.
5. Newman VA, Flatt SW, Pierce JP (2008) Telephone counseling promotes dietary change in healthy adults: results of a pilot trial. *J Am Diet Assoc* **108**, 1350-1354.
6. Rock CL, Moskowitz A, Huizar B *et al.* (2001) High vegetable and fruit diet intervention in premenopausal women with cervical intraepithelial neoplasia. *J Am Diet Assoc* **101**, 1167-1174.
7. Day N, McKeown N, Wong M *et al.* (2001) Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. *Int J Epidemiol* **30**, 309-317.
8. Kipnis V, Midthune D, Freedman L *et al.* (2002) Bias in dietary-report instruments and its implications for nutritional epidemiology. *Public Health Nutr* **5**, 915-923.
9. Kipnis V, Midthune D, Freedman LS *et al.* (2001) Empirical evidence of correlated biases in dietary assessment instruments and its implications. *Am J Epidemiol* **153**, 394-403.
10. Kipnis V, Subar AF, Midthune D *et al.* (2003) Structure of dietary measurement error: results of the OPEN biomarker study. *Am J Epidemiol* **158**, 14-21; discussion 22-16.
11. Tasevska N, Midthune D, Potischman N *et al.* (2011) Use of the predictive sugars biomarker to evaluate self-reported total sugars intake in the Observing Protein and Energy Nutrition (OPEN) study. *Cancer Epidemiol Biomarkers Prev* **20**, 490-500.
12. Tasevska N, Runswick SA, McTaggart A *et al.* (2005) Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiol Biomarkers Prev* **14**, 1287-1294.
13. Andersen LF, Veierod MB, Johansson L *et al.* (2005) Evaluation of three dietary assessment methods and serum biomarkers as measures of fruit and vegetable intake, using the method of triads. *Br J Nutr* **93**, 519-527.

14. Bogers RP, Dagnelie PC, Westerterp KR *et al.* (2003) Using a correction factor to correct for overreporting in a food-frequency questionnaire does not improve biomarker-assessed validity of estimates for fruit and vegetable consumption. *J Nutr* **133**, 1213-1219.
15. Bogers RP, Van Assema P, Kester AD *et al.* (2004) Reproducibility, validity, and responsiveness to change of a short questionnaire for measuring fruit and vegetable intake. *Am J Epidemiol* **159**, 900-909.
16. Brantsaeter AL, Haugen M, Rasmussen SE *et al.* (2007) Urine flavonoids and plasma carotenoids in the validation of fruit, vegetable and tea intake during pregnancy in the Norwegian Mother and Child Cohort Study (MoBa). *Public Health Nutr* **10**, 838-847.
17. Carlsen MH, Karlsen A, Lillegaard IT *et al.* (2011) Relative validity of fruit and vegetable intake estimated from an FFQ, using carotenoid and flavonoid biomarkers and the method of triads. *Br J Nutr* **105**, 1530-1538.
18. Jansen MC, Van Kappel AL, Ocke MC *et al.* (2004) Plasma carotenoid levels in Dutch men and women, and the relation with vegetable and fruit consumption. *Eur J Clin Nutr* **58**, 1386-1395.
19. Jilcott SB, Keyserling TC, Samuel-Hodge CD *et al.* (2007) Validation of a brief dietary assessment to guide counseling for cardiovascular disease risk reduction in an underserved population. *J Am Diet Assoc* **107**, 246-255.
20. Resnicow K, Odom E, Wang T *et al.* (2000) Validation of three food frequency questionnaires and 24-hour recalls with serum carotenoid levels in a sample of African-American adults. *Am J Epidemiol* **152**, 1072-1080.
21. Toft U, Kristoffersen L, Ladelund S *et al.* (2008) Relative validity of a food frequency questionnaire used in the Inter99 study. *Eur J Clin Nutr* **62**, 1038-1046.
22. Baldrick FR, Woodside JV, Elborn JS *et al.* (2011) Biomarkers of fruit and vegetable intake in human intervention studies: a systematic review. *Crit Rev Food Sci Nutr* **51**, 795-815.
23. Brevik A, Vollset SE, Tell GS *et al.* (2005) Plasma concentration of folate as a biomarker for the intake of fruit and vegetables: the Hordaland Homocysteine Study. *Am J Clin Nutr* **81**, 434-439.
24. Willett WC (2013) *Nutritional Epidemiology*, 3rd ed. Oxford: Oxford University Press.
25. Royston P & Altman DG (1994) Regression using fractional polynomials of continuous covariates - parsimonious parametric modeling. *Appl Statist* **43**, 429-467.
26. Sauerbrei W & Royston P (1999) Building multivariable prognostic and diagnostic models: transformation of the predictors by using fractional polynomials. *J R Stat Soc Ser A Stat Soc* **162**, 71-94.
27. Vergouwe Y, Royston P, Moons KGM *et al.* (2010) Development and validation of a prediction model with missing predictor data: a practical approach. *J Clin Epidemiol* **63**, 205-214.

28. Appel LJ, Miller III ER, Jee SH *et al.* (2000) Effect of dietary patterns on serum homocysteine: Results of a randomized, controlled feeding study. *Circulation* **102**, 852-857.
29. Bøhn SK, Myhrstad MC, Thoresen M *et al.* (2010) Blood cell gene expression associated with cellular stress defense is modulated by antioxidant-rich food in a randomised controlled clinical trial of male smokers. *BMC Med* **8**.
30. Bowen PE, Garg V, Stacewicz-Sapuntzakis M *et al.* (1993) Variability of serum carotenoids in response to controlled diets containing six servings of fruits and vegetables per day. *Ann N Y Acad Sci* **691**, 241-243.
31. Brevik A, Andersen LF, Karlsen A *et al.* (2004) Six carotenoids in plasma used to assess recommended intake of fruits and vegetables in a controlled feeding study. *Eur J Clin Nutr* **58**, 1166-1173.
32. Briviba K, Bub A, Möseneder J *et al.* (2008) No differences in DNA damage and antioxidant capacity between intervention groups of healthy, nonsmoking men receiving 2, 5, or 8 servings/day of vegetables and fruit. *Nutr Cancer* **60**, 164-170.
33. Broekmans WMR, Klöpping-Ketelaars IAA, Schuurman CRWC *et al.* (2000) Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. *J Nutr* **130**, 1578-1583.
34. Brouwer IA, Van Dusseldorp M, West CE *et al.* (1999) Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. *J Nutr* **129**, 1135-1139.
35. Castenmiller JJ, van de Poll CJ, West CE *et al.* (2000) Bioavailability of folate from processed spinach in humans. Effect of food matrix and interaction with carotenoids. *Ann Nutr Metab* **44**, 163-169.
36. Castenmiller JJ, West CE, Linssen JP *et al.* (1999) The food matrix of spinach is a limiting factor in determining the bioavailability of beta-carotene and to a lesser extent of lutein in humans. *J Nutr* **129**, 349-355.
37. Chopra M, O'Neill ME, Keogh N *et al.* (2000) Influence of increased fruit and vegetable intake on plasma and lipoprotein carotenoids and LDL oxidation in smokers and nonsmokers. *Clin Chem* **46**, 1818-1829.
38. Dragsted LO, Pedersen A, Hermetter A *et al.* (2004) The 6-a-day study: Effects of fruit and vegetables on markers of oxidative stress and antioxidative defense in healthy nonsmokers. *Am J Clin Nutr* **79**, 1060-1072.

39. Freese R, Alfthan G, Jauhiainen M *et al.* (2002) High intakes of vegetables, berries, and apples combined with a high intake of linoleic or oleic acid only slightly affect markers of lipid peroxidation and lipoprotein metabolism in healthy subjects. *Am J Clin Nutr* **76**, 950-960.
40. Itsiopoulos C, Brazionis L, Kaimakamis M *et al.* (2011) Can the Mediterranean diet lower HbA1c in type 2 diabetes? Results from a randomized cross-over study. *Nutr Metab Cardiovasc Dis* **21**, 740-747.
41. Karlsen A, Svendsen M, Seljeflot I *et al.* (2011) Compliance, tolerability and safety of two antioxidant-rich diets: a randomised controlled trial in male smokers. *Br J Nutr* **106**, 557-571.
42. Martini MC, Campbell DR, Gross MD *et al.* (1995) Plasma carotenoids as biomarkers of vegetable intake: The University of Minnesota cancer prevention research unit feeding studies. *Cancer Epidemiol Biomarkers Prev* **4**, 491-496.
43. Miller III ER, Appel LJ, Risby TH (1998) Effect of dietary patterns on measures of lipid peroxidation: Results from a randomized clinical trial. *Circulation* **98**, 2390-2395.
44. Miller III ER, Erlinger TP, Sacks FM *et al.* (2005) A dietary pattern that lowers oxidative stress increases antibodies to oxidized LDL: Results from a randomized controlled feeding study. *Atherosclerosis* **183**, 175-182.
45. Misikangas M, Freese R, Turpeinen AM *et al.* (2001) High linoleic acid, low vegetable, and high oleic acid, high vegetable diets affect platelet activation similarly in healthy women and men. *J Nutr* **131**, 1700-1705.
46. Moller P, Vogel U, Pedersen A *et al.* (2003) No effect of 600 grams fruit and vegetables per day on oxidative DNA damage and repair in healthy nonsmokers. *Cancer Epidemiol Biomarkers Prev* **12**, 1016-1022.
47. Silaste ML, Rantala M, Alfthan G *et al.* (2003) Plasma homocysteine concentration is decreased by dietary intervention. *Br J Nutr* **89**, 295-301.
48. Silaste ML, Rantala M, Alfthan G *et al.* (2004) Changes in dietary fat intake alter plasma levels of oxidized, low-density lipoprotein and lipoprotein(a). *Arterioscler Thromb Vasc Biol* **24**, 498-503.
49. van het Hof KH, Brouwer IA, West CE *et al.* (1999) Bioavailability of lutein from vegetables is 5 times higher than that of beta-carotene. *Am J Clin Nutr* **70**, 261-268.
50. Van Loo-Bouwman CA, West CE, Van Breemen RB *et al.* (2009) Vitamin A equivalency of beta-carotene in healthy adults: Limitation of the extrinsic dual-isotope dilution technique to measure matrix effect. *Br J Nutr* **101**, 1837-1845.

51. Watzl B, Kulling SE, Möseneder J *et al.* (2005) A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am J Clin Nutr* **82**, 1052-1058.
52. Winkels RM, Brouwer IA, Siebelink E *et al.* (2007) Bioavailability of food folates is 80% of that of folic acid. *Am J Clin Nutr* **85**, 465-473.
53. Yeon JY, Kim HS, Sung MK (2012) Diets rich in fruits and vegetables suppress blood biomarkers of metabolic stress in overweight women. *Prev Med* **54**, S109-S115.
54. Yeum KJ, Booth SL, Sadowski JA *et al.* (1996) Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am J Clin Nutr* **64**, 594-602.
55. Crispim SP, Geelen A, Souverein OW *et al.* (2011) Biomarker-based evaluation of two 24-h recalls for comparing usual fish, fruit and vegetable intakes across European centers in the EFCOVAL Study. *Eur J Clin Nutr* **65**, Suppl 1, S38-47.
56. Kristal AR, Vizenor NC, Patterson RE *et al.* (2000) Precision and bias of food frequency-based measures of fruit and vegetable intakes. *Cancer Epidemiol Biomarkers Prev* **9**, 939-944.
57. Chopra M, McLoone U, O'Neill M *et al.* (1996) Fruit and vegetable supplementation - effect on ex vivo LDL oxidation in humans. In: *Natural antioxidants and food quality in atherosclerosis and cancer prevention*, pp. 150-155 [JT Kumpulainen and JT Salonen, editors]. London: The Royal Society of Chemistry.
58. Blanck HM, Bowman BA, Cooper GR *et al.* (2003) Laboratory issues: use of nutritional biomarkers. *J Nutr* **133**, Suppl 3, 888S-894S.
59. Brady WE, Mares-Perlman JA, Bowen P *et al.* (1996) Human serum carotenoid concentrations are related to physiologic and lifestyle factors. *J Nutr* **126**, 129-137.
60. Drewnowski A, Rock CL, Henderson SA *et al.* (1997) Serum beta-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. *Am J Clin Nutr* **65**, 1796-1802.
61. Maiani G, Caston MJ, Catasta G *et al.* (2009) Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Mol Nutr Food Res* **53**, Suppl 2, S194-S218.
62. Tucker KL, Selhub J, Wilson PW *et al.* (1996) Dietary intake pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. *J Nutr* **126**, 3025-3031.

63. van Kappel AL, Steghens JP, Zeleniuch-Jacquotte A *et al.* (2001) Serum carotenoids as biomarkers of fruit and vegetable consumption in the New York Women's Health Study. *Public Health Nutr* **4**, 829-835.
64. Efron B (1983) Estimating the error rate of a prediction rule: improvement on cross-validation. *J Am Statist Assoc* **78**, 382, 316-331.
65. Abo-Zaid G, Guo B, Deeks JJ *et al.* (2013) Individual participant data meta-analyses should not ignore clustering. *J Clin Epidemiol* **66**, 865-873 e864.
66. Bouwmeester W, Twisk JW, Kappen TH *et al.* (2013) Prediction models for clustered data: comparison of a random intercept and standard regression model. *BMC Med Res Methodol* **13**, 19.
67. Debray TP, Moons KG, Ahmed I *et al.* (2013) A framework for developing, implementing, and evaluating clinical prediction models in an individual participant data meta-analysis. *Stat Med* **32**, 3158-3180.

Figure 1. Flow diagram of study selection process.

Figure 2. Dose-response curves between serum carotenoids, plasma/serum folate and vitamin C and fruit, vegetable and juice intake. The circles indicate the individual data points, the size is proportional to the number of individuals for that specific intake (i.e., the larger the circle the more individuals were available for analysis).

Figure 3. Dose-response curves between serum carotenoids, plasma/serum folate and vitamin C and fruit and vegetable intake (excluding juices). The circles indicate the individual data points, the size is proportional to the number of individuals for that specific intake (i.e., the larger the circle the more individuals were available for analysis).

.

Table 1. Overview of study characteristics of included studies

Author	Year	N*	Study design and dietary intervention	Checks on compliance / intake	Duration (days)	Fruit and vegetable intake of included groups (g/d)				
						Group‡	FV		FVJ	
							Mean	SD	Mean	SD
Broekmans <i>et al.</i> ⁽³³⁾	2000	47 (47)	Complete diet; parallel intervention	Evening meal under supervision at the institute, remaining parts were weighed and recorded. The remainder of the daily diet was handed out to the volunteers. Consumption was checked through a questionnaire.	28	A: Low (P)	100		100	
						B: High (P)	565		765	
Castenmiller <i>et al.</i> ^(35, 36)	1999	58 (72)	Complete diet with list of free choice; parallel intervention	Subjects received a hot meal at the university and foods for their other meals and snacks were packed to be taken home. The daily selection of free choice foods was recorded in a diary.	21	A: Control (P)	491	137	728	172
						B: Whole leaf spinach (P)	484	117	722	146
						C: Minced spinach (P)	471	108	712	135
						D: Liquefied spinach (P)	473	100	711	129
						E: Liquefied spinach plus dietary fibre (P)	468	90	711	122
Chopra <i>et al.</i> ⁽³⁷⁾	2000	34 (32)	Fruits and vegetables provided; cross-over intervention	Participants were provided with food items. Most of these were consumed during lunch at the University on the weekdays. Researchers relied on participants for extra consumption during the rest of the day and at weekends.	7	A: Red week (P)	350		350	
						B: Green week (P)	350		350	
Dragsted <i>et al.</i> ⁽³⁸⁾ ; Moller <i>et al.</i> ⁽⁴⁶⁾	2003	31 (43)	Complete diet; parallel intervention	All the food were provided free of charge throughout the intervention. In addition plasma alpha- and beta carotene and ascorbate were used as markers to assure that the groups differed.	24	A: Fruveg (P)	480		600	
						B: Placebo (P)	0		0	

Freese <i>et al.</i> ⁽³⁹⁾ ; Misikangas <i>et al.</i> ⁽⁴⁵⁾	2001	77 (77)	Complete diet with list of free choice; parallel intervention	In the intervention food consumption was controlled by serving the lunch at the department on weekdays and by asking the volunteers to mark in their study diaries if any study foods were not eaten. Also biomarkers were used to check compliance.	42	A: PUFA – low FBV (P) B: PUFA – high FBV (P) C: MUFA – low FBV (P) D: MUFA – high FBV (P)	217 807 235 809	32 166 51 138	505 1057 549 1059	73 217 119 181
Itsiopoulos <i>et al.</i> ⁽⁴⁰⁾	2011	27 (27)	Diet provided in excess of intake; cross-over intervention	Compliance was checked with 7 day diet diaries and participants were interviewed every 2 weeks when they returned to pick up supplies of foods. Participants were asked to tick off the foods they ate over the previous 2 weeks in a booklet. Plasma fatty acids and carotenoids, and body weight were measured as markers of compliance.	84	Mediterranean diet (R)	768	216	768	216
Karlsen <i>et al.</i> ⁽⁴¹⁾ ; Bohn <i>et al.</i> ⁽²⁹⁾	2010	33 (33)	Diet provided in excess of energy requirements; parallel intervention	A detailed questionnaire was completed at each weekly follow-up to record compliance. All participants were instructed to bring the remaining food items to the weekly follow-up. Individual counselling was given to the participants to help them consume the provided food items. Dietary intake during the intervention period, was recorded using a 7 d food record with a picture book, which was completed in the last week of the intervention	56	Antioxidant-rich diet (R)	525	242	1491	509

				period.							
Miller III <i>et al.</i> ⁽⁴⁴⁾	2005	60 (103)	Complete diet; parallel intervention	Meals were prepared in a metabolic kitchen and served in an outpatient dining facility. Throughout the 3 months of feeding, participants agreed to eat only the food provided to them and nothing else.	90	A: DASH diet (P) B: control diet (P)	- -			768 288	
Van het Hof <i>et al.</i> ^{(49)†}	1999	43 (54)	Complete diet with list of free choice; parallel intervention	The hot meals were provided at lunch time under supervision from Monday-Friday. Other foods during these days and during the weekends were eaten at home and compliance was checked via diaries. Volunteers were carefully instructed how to prepare the foods.	28	A: Low-vegetable diet (P) B: High vegetable diet (P)	255 605			455 805	
Van Loo–Bouwman <i>et al.</i> ⁽⁵⁰⁾	2009	24 (24)	Complete diet with list of free choice; cross- over intervention	The hot meals were provided at lunch time under supervision from Monday-Friday. Other foods during these days and during the weekends were eaten at home and compliance was checked via diaries.	21	Mixed diet (vegetables and fruit high in β - carotene) (P)	329	100	654	182	
Watzl <i>et al.</i> ⁽⁵¹⁾ ; Briviba <i>et al.</i> ⁽³²⁾	2005	63 (63)	Fruits and vegetables provided; parallel intervention	Each study participant was provided with a box with F&V. F&V which were not consumed during the study period had to be returned. Daily intake of F&V was assessed via a specific F&V protocol throughout the study period. During two 4-day periods the whole food intake was assessed via diary.	28	A: 2 servings/day (P) B: 5 servings/day (P) C: 8 servings/day (P)	- - -			250 565 955	
Winkels <i>et al.</i> ⁽⁵²⁾	2007	29	Complete diet	All foods were provided. Participants	28	Food folate group (P)	476			876	

(72)	with list of free choice; parallel intervention	were asked to report all free-choice items and any deviations in a diary.
------	---	---

FV, fruit and vegetable intake excluding juices; FVJ, fruit, vegetable and juice intake; FBV, fruit, berries and vegetables.

* The number of individuals used in the present analysis. Within brackets the number of individuals reported in the original publication. For several studies specific intervention groups were not useful in the present analysis^(36, 38, 41, 49, 50, 52), and for one study data of a subset of participants was received⁽⁴⁴⁾.

† The folate data of this study were no longer available⁽³⁴⁾.

‡ Between brackets it is indicated whether the amount of fruit and vegetables reported in the table and used in the analysis is the amount provided to the subjects (indicated by 'P') or that the amount relies partly on self-reporting (indicated by 'R').

Table 2a. Baseline characteristics of the included studies

Study	N	Age (y)		BMI (kg/m ²)		Gender % male	Smoking % smoking	Plasma folate (nmol/l)		Vitamin C (μmol/l)	
		mean	SD	mean	SD			mean	SD	mean	SD
Broekmans <i>et al.</i> ⁽³³⁾	47	49.3*	5.1*	25.7	3.1	51.1	25.5	13.7	7.1	49.4	18.6
Castenmiller <i>et al.</i> ^(35, 36)	58	22.8	7.7	22.1	2.1	39.7	0	15.3	4.2	-	-
Chopra <i>et al.</i> ⁽³⁷⁾	34	37.2	8.7	-	-	0	-	-	-	-	-
Dragsted <i>et al.</i> ^(38, 46)	31	27.3	7.3	23.1	2.3	48.4	0	10.8	4.0	-	-
Freese <i>et al.</i> ^(39, 45)	77	25.1	6.6	22.6	3.2	26.0	5.2	10.0	4.1	51.9	16.5
Itsiopoulos <i>et al.</i> ⁽⁴⁰⁾	27	59.1	7.1	30.2	3.7	59.3	-	-	-	-	-
Karlsen <i>et al.</i> ^(29, 41)	33	56.7	6.4	24.8	2.7	100	100	-	-	46.7	17.0
Miller III <i>et al.</i> ⁽⁴⁴⁾	60	52.0*	10.0*	29.6*	4.4*	44*	14*	-	-	-	-
Van het Hof <i>et al.</i> ⁽⁴⁹⁾	43	22.4	6.4	22.4	2.1	27.9	0	-	-	66.6	17.4
Van Loo – Bouwman <i>et al.</i> ⁽⁵⁰⁾	24	22.0	4.0	21.8	2.2	41.7	0	-	-	-	-
Watzl <i>et al.</i> ^(32, 51)	63	31.2	9.0	23.7	2.7	100	0	-	-	83.7	16.6
Winkels <i>et al.</i> ⁽⁵²⁾	29	23.3	4.8	22.6	2.8	24.1	13.8	12.1	-	-	-
Total population	526	30.9	13.8	23.6	3.4	47.9	13.1	12.1	5.2	60.8	22.2

Table 2b. Baseline characteristics of the included studies

Study	α -carotene ($\mu\text{mol/l}$)		β -carotene ($\mu\text{mol/l}$)		β -cryptoxanthin ($\mu\text{mol/l}$)		Lycopene ($\mu\text{mol/l}$)		Lutein ($\mu\text{mol/l}$)		Zeaxanthin ($\mu\text{mol/l}$)		Lutein+zeaxanthin ($\mu\text{mol/l}$)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Broekmans <i>et al.</i>	0.06	0.04	0.40	0.21	0.14	0.10	0.45	0.27	0.34	0.15	0.04	0.03	0.37	0.16
Castenmiller <i>et al.</i>	0.07	0.03	0.27	0.14	0.28	0.12	0.19	0.10	0.22	0.07	0.03	0.01	0.25	0.08
Chopra <i>et al.</i>	0.10	0.07	0.38	0.29	0.13	0.09	0.34	0.16	0.23	0.10	-	-	-	-
Dragsted <i>et al.</i>	-	-	0.36	0.23	-	-	-	-	0.26	0.12	-	-	-	-
Freese <i>et al.</i>	0.20	0.10	0.60	0.30	0.10	0.05	0.62	0.19	0.26	0.10	-	-	-	-
Itsiopoulos <i>et al.</i>	0.08	0.05	0.31	0.20	0.16	0.14	0.43	0.20	-	-	-	-	0.35	0.13
Karlsen <i>et al.</i>	0.07	0.06	0.35	0.29	0.15	0.10	0.56	0.26	0.16	0.07	0.04	0.02	0.20	0.08
Miller III <i>et al.</i>	0.05	0.05	0.23	0.13	0.07	0.04	0.28	0.15	0.16	0.06	0.04	0.02	0.19	0.07
Van het Hof <i>et al.</i>	0.08	0.06	0.40	0.19	0.34	0.21	0.27	0.12	0.17	0.07	0.04	0.02	0.20	0.09
Van Loo – Bouwman <i>et al.</i>	0.10	0.06	0.75	0.36	0.34	0.14	-	-	-	-	-	-	-	-
Watzl <i>et al.</i>	0.13	0.08	0.55	0.31	0.23	0.12	0.55	0.25	0.26	0.10	0.06	0.02	0.33	0.14
Winkels <i>et al.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total population	0.10	0.08	0.42	0.28	0.18	0.15	0.42	0.24	0.23	0.11	0.04	0.02	0.27	0.13

* These data are taken from the original publication, but were not available for the present analysis

Table 3. Regression coefficients, standard errors, and powers for the predictors on the multiple completed* data sets (N=492† in each completed data set) from a linear regression analysis

Predictors	Linear model		MFP model			Reduced MFP model			
	β	SE	β	SE	Power	β	SE	Power	
FVJ									
Constant		-172.8	158.9	-1691.4	526.9	-	1043.2	180.0	-
α -carotene	$\mu\text{mol/l}$	479.8	142.2	607.8	133.4	0.5	674.1	90.1	0.5
β -carotene	$\mu\text{mol/l}$	123.1	53.1	101.5	50.9	1	-	-	-
Lutein + zeaxanthin	$\mu\text{mol/l}$	193.2	68.8	154.6	70.6	1	-153.7	36.8	-0.5
β -cryptoxanthin	$\mu\text{mol/l}$	162.1	138.5	141.2	138.3	1	-	-	-
Lycopene	$\mu\text{mol/l}$	-13.8	87.4	-78.0	82.2	1	-	-	-
Folate‡	nmol/l	158.9	38.9	49.9	11.1	2	48.9	10.9	2
Vitamin C	$\mu\text{mol/l}$	0.91	0.93	0.78	0.96	1	-	-	-
BMI	kg/m^2	7.6	7.9	10.2	7.2	1	-	-	-
Female gender		-40.2	27.3	-55.3	28.1		-63.5	29.2	x
Age§	yr	39.4	24.4	-1711.6	596.0	0	-992.9	341.0	0
				1982.9	676.6	0.5	470.2	149.4	0
Smoking		-367.4	248.6	-278.6	195.3		-232.2	187.4	x
Smoking* folate		38.1	13.7	31.3	10.5	1	28.4	10.3	1
FV									
Constant		-274.2	166.5	-304.9	164.2	-	-85.5	141.5	
α -carotene	$\mu\text{mol/l}$	939.2	205.0	830.9	219.9	1	-	-	-
β -carotene	$\mu\text{mol/l}$	104.1	45.9	95.4	45.1	1	300.2	65.2	1
Lutein + zeaxanthin	$\mu\text{mol/l}$	276.8	69.5	414.4	90.2	1	-158.3	29.3	-0.5
				-562.4	140.2	1			
β -cryptoxanthin	$\mu\text{mol/l}$	146.1	105.7	74.4	100.7	1	-	-	-
Lycopene	$\mu\text{mol/l}$	-764.1	306.0	-782.8	295.8	1	-	-	-
Folate‡	nmol/l	74.0	34.7	59.6	33.0	1	62.5	33.4	1
Vitamin C	$\mu\text{mol/l}$	1.7	0.7	1.4	0.6	1	1.6	0.6	1
BMI	kg/m^2	4.9	6.6	5.6	6.2	1	16.4	3.8	1
Female gender		42.0	41.5	-57.3	21.8		-42.8	22.4	x
Age§	yr	63.6	12.4	1.1	0.2	3	53.1	14.4	1
Smoking		8.5	45.5	19.8	43.8		-	-	-
Age* α -carotene		-22.0	5.3	-19.1	5.4	1	-	-	-
BMI*lycopene		29.0	11.9	28.6	11.6	1	-	-	-
Gender*lut+zeax		-215.0	82.2	-	-	-	-	-	-
Age* β -carotene		-	-	-	-	-	-5.0	2.1	1

FVJ, fruit, vegetable and juice intake, FV, fruit and vegetable intake excluding juices.

* Completed data sets refers to the data after multiple imputation

† The study of Chopra⁽³⁷⁾ could not be used in this analysis due to estimation problem

‡ Folate is scaled as folate/10

§ Age is scaled as age/10

Table 4. Performance measures of the different prediction models as calculated by cross-validation

	FVJ				FV			
	RMSE	correlation	mean difference between observed and predicted	limits of agreement	RMSE	correlation	mean difference between observed and predicted	limits of agreement
Linear model	265.7	0.77	-1.6	-478.4; 475.2	205.6	0.64	4.4	-372.3; 381.1
MFP model	260.0	0.78	-1.5	-467.6; 464.7	201.1	0.65	2.4	-368.2; 373.0
Reduced MFP model	258.0	0.78	-1.7	-466.3; 462.8	205.2	0.61	6.8	-382.3; 396.0

FVJ, fruit, vegetable and juice intake; FV, fruit and vegetable intake excluding juices

Table 5. Pearson correlations between fruit and vegetable intake and biomarkers

Biomarker	FVJ	FV
α -carotene at follow-up ($\mu\text{mol/l}$)	0.29	0.26
β -carotene at follow-up ($\mu\text{mol/l}$)	0.27	0.24
Cryptoxanthin at follow-up ($\mu\text{mol/l}$)	0.08	0.16
Lycopene at follow-up ($\mu\text{mol/l}$)	0.19	0.24
Combined lutein and zeaxanthin at follow-up ($\mu\text{mol/l}$)	0.08	0.15
Sum of carotenoids ($\mu\text{mol/l}$)	0.23	0.33
Serum/plasma folate at follow-up (nmol/l)	0.32	0.26
Serum/plasma vitamin C at follow-up ($\mu\text{mol/l}$)	0.04	0.38

FVJ, fruit, vegetable and juice intake; FV, fruit and vegetable intake excluding juices.

Table 6. Performance measures of the best performing prediction models per study as calculated by cross-validation

	FVJ (reduced MFP model)			FV (MFP model)		
	RMSE	mean difference between observed and predicted	limits of agreement	RMSE	mean difference between observed and predicted	limits of agreement
Broekmans <i>et al.</i> ⁽³³⁾	340.9	-127.9	-743.2; 487.5	209.8	-88.3	-457.4; 280.8
Castenmiller <i>et al.</i> ^(35, 36)	188.2	10.1	-358.4; 378.6	126.8	17.0	-224.7; 258.8
Dragsted <i>et al.</i> ^(38, 46)	303.4	-198.9	-631.7; 233.9	191.9	-80.1	-407.9; 247.6
Freese <i>et al.</i> ^(39, 45)	274.7	94.7	-410.3; 599.7	304.0	150.2	-368.1; 668.5
Itsiopoulos <i>et al.</i> ⁽⁴⁰⁾	271.0	4.8	-492.4; 502.0	253.6	129.6	-289.5; 548.8
Karlsen <i>et al.</i> ^(29, 41)	673.8	555.8	-159.4; 1271.0	228.7	33.0	-408.2; 474.2
Miller III <i>et al.</i> ⁽⁴⁴⁾	242.0	46.7	-326.1; 419.6	236.4	50.4	-370.8; 471.7
Van het Hof <i>et al.</i> ⁽⁴⁹⁾	125.5	27.0	-170.3; 224.2	88.9	16.0	-146.1; 178.0
Van Loo – Bouwman <i>et al.</i> ⁽⁵⁰⁾	181.4	0.48	-305.9; 306.9	195.1	-156.1	-331.4; 19.2
Watzl <i>et al.</i> ^(32, 51)	270.1	-141.1	-576.3; 294.1	210.6	-64.8	-441.2; 311.7
Winkels <i>et al.</i> ⁽⁵²⁾	241.1	145.9	-121.3; 413.0	133.5	7.5	-101.4; 116.5

FVJ, fruit, vegetable and juice intake; FV, fruit and vegetable intake excluding juices

Figure 2

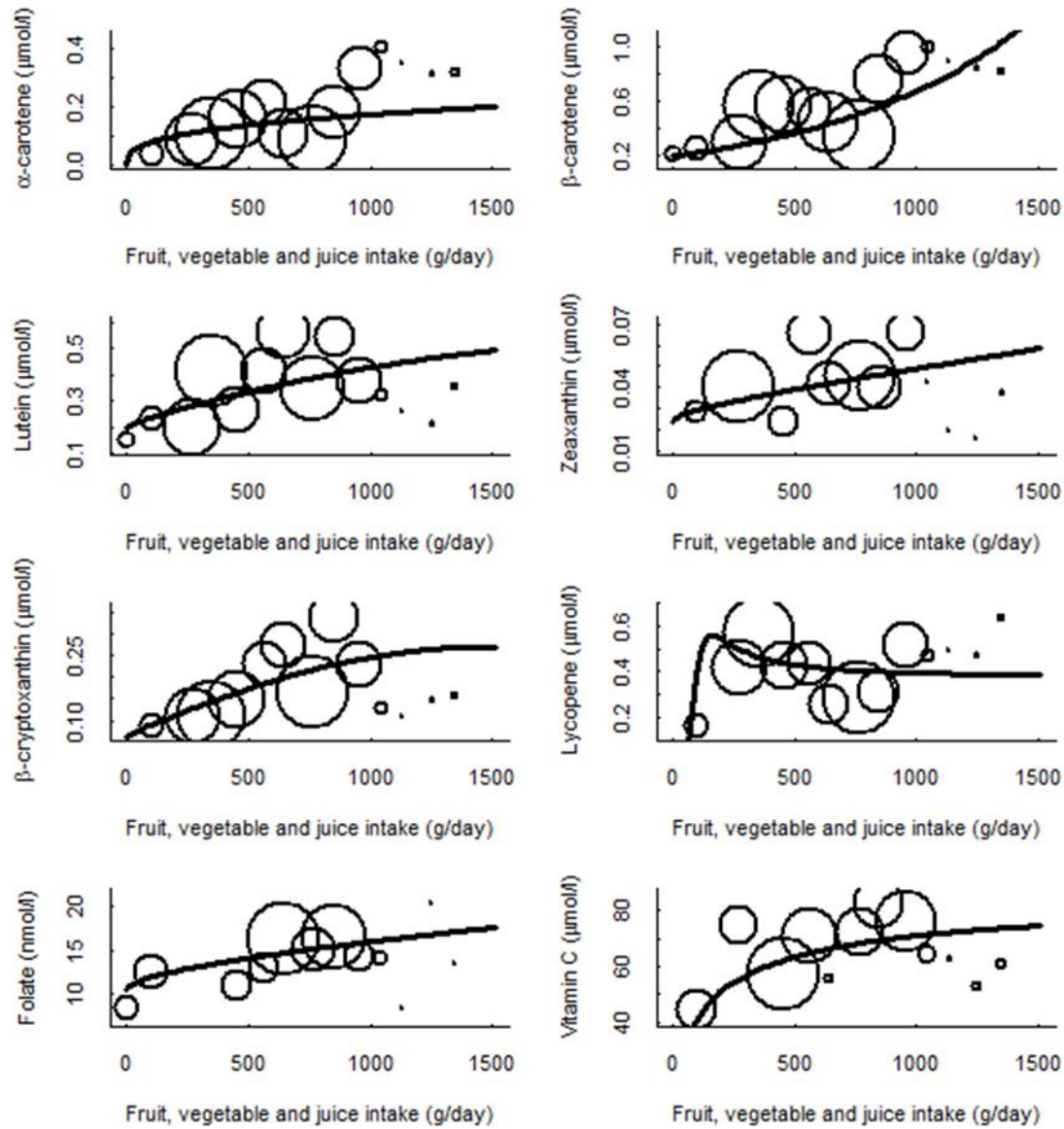


Figure 3

