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

Olga W. Souverein[1], Jeanne H.M. de Vries[1], Riitta Freese[2], Bernhard Watzl[3], Achim Bub[3], Edgar R. Miller III[4], Jacqueline J.M. Castenmiller[5], Wilrike J. Pasman[6], Karin van het Hof[7], **Mridula Chopra[8]**, Anette Karlsen[9], Lars O. Dragsted[10], Renate Winkels[1], Catherine Itsiopoulos[11], Laima Brazionis[12], Kerin O'Dea[13], Carolien A. van Loo-Bouwman[14], Ton HJ Naber[15], Hilko van der Voet[16], Hendriek C. Boshuizen[1]

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

ABSTRACT

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

INTRODUCTION

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

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

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

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

METHODS

Search strategy

The aim of the literature search was to find diet-controlled intervention studies (i.e., food provision studies or partly supervised feeding studies) conducted in adult subjects where reports on the amount of consumed fruits and vegetables were supported by information on the amount provided and where significant efforts were made to maximise compliance. The following diet-controlled intervention studies were included: i) all foods and drinks were provided to the subjects during the intervention, or ii) all fruits and vegetables consumed were provided to the subjects. In addition, carotenoids or folate concentrations in blood after intervention were measured and papers were published in the English language. The search was conducted in Scopus, Pubmed and by manual search of reference lists. Search terms in title and abstract were ‘fruit’ and ‘vegetables’ combined with ‘intervention’, ‘trial’ and ‘feeding study’, which was then combined with ‘biomarkers’, ‘biological markers’, ‘carotenoids’, ‘alpha-carotene’, ‘beta-carotene’, ‘beta-cryptoxanthin’, ‘zeaxanthin’, ‘lycopene’, ‘lutein’, ‘folate’ and ‘bioavailability’. The search included publications until October 2012.

Papers were first screened based on the title and abstracts. Then, the full text of the papers that were considered potentially relevant were read and judged for relevancy. Next, the full text of the papers was retrieved and judged using inclusion and exclusion criteria. The exclusion

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

Data

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

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

Statistical analysis

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

Dose-response curves

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

(FP)[25, 26]. To account for the one cross-over study and the between study heterogeneity the final parameter estimates were calculated using mixed models using study and subjects as random effects. Therefore, the estimated variance components refer to differences between studies, differences between individuals (to account for the cross-over study) and residual variance.

To obtain predictions on the original scale rather than on the logarithmic scale, we applied the 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 concentration on the original scale, X is the fruit and vegetable intake, and σ^2 is the sum of the variance components estimated in the mixed model.

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

Prediction models of fruit and vegetable intake

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

predictors was established in all 10 imputed data sets separately and the final model was established by majority voting[27].

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

RESULTS

Search and data retrieval

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

Table 2. The data of four studies were unfortunately unavailable, and three authors did not respond to our request. Information from these studies is available in Supplemental Table A. For four studies specific 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]. For the study of Miller II *et al.*[44], intake of fruit and vegetables in servings was converted to grams per day by multiplying the number of servings by 80 g. For the study of Itsiopoulos et al.[40] intake of fruit and vegetables was known for 15 subjects. For the remaining 12 subjects the vegetable intake was imputed as the mean of the intake as reported in the paper (i.e., 466 g/d vegetables and 162 g/d fruit). Where needed α-carotene, β-carotene and lycopene were converted from µg/mL to µmol/L.

Dose-response analysis

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

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

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

205

206 **Prediction model**

207 The regression coefficients of the final prediction model are presented in Table 3 and the
208 performance measures are presented in Table 4. The power and variable selection process of the
209 MFP and the reduced MFP model is shown in Supplemental Tables D and E. For fruit, vegetable
210 and juice intake, the reduced MFP model showed the lowest RMSE (i.e., 258.0 g) and the
211 highest correlation between observed and predicted (i.e., 0.78) compared to the linear model and
212 the full pre-specified MFP model. The mean difference of the reduced MFP model (-1.7 g) was a
213 little higher than of the other two models (linear model: -1.6 g; MFP model: -1.5 g), but the
214 limits of agreement were markedly smaller than those of the other two models. Bland-Altman
215 plots are presented in Supplemental Figure B.
216 For fruit and vegetable intake (excluding juices) the MFP model was the best model. It showed
217 the lowest RMSE (201.1 g), the highest correlation (0.65) and the lowest mean bias (2.4 g) with
218 the smallest limits of agreement (-368.2; 373.0 g).
219 The prediction model for fruit and vegetable intake (excluding juices) showed a somewhat lower
220 correlation and higher absolute mean difference than the model of fruit and vegetable intake
221 which included juices. Therefore, we investigated whether a model including a predictor variable
222 that represented the juice intake (in g/d) would improve the prediction for fruit and vegetable
223 intake when juices were excluded. However, this did not markedly change the results. The MFP
224 model including juice as a predictor variable had a RMSE of 202.8 g, a correlation of 0.64, mean
225 bias of 0.2 g (limits of agreement: -374.1; 374.6 g). Therefore, the more simple model without
226 juice as a predictor variable is preferred as a prediction model for fruit and vegetable intake
227 (excluding juices).
228 To be able to compare the performance of the prediction model with the current practice of using
229 the sum of carotenoids or any of the single biomarkers, we calculated the correlation coefficients
230 between the observed intakes and the sum of carotenoids and between observed intakes and the
231 single biomarkers (Table 5). For fruit, vegetable and juice intake the correlations range between
232 0.04 and 0.32, which is much lower than the 0.65 of the prediction model. Also for fruit and
233 vegetable intake (excluding juices) the correlations (between 0.15 and 0.38) are lower than that
234 of the prediction model (0.64).

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

ACKNOWLEDGEMENTS

FINANCIAL SUPPORT

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

CONFLICT OF INTEREST

None

AUTHORSHIP

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

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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).

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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 *et al.*[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 *et al.*[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 *et al.*[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 *et al.*[38]; Moller *et al.*[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 *et al.*[39]; Misikangas *et al.*[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 *et al.*[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	Mediterrean diet (R)	768	216	768	216
Karlsen *et al.*[41]; Bohn *et al.*[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 *et al.*[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 *et al.*[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 *et al.*[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 *et al.*[51]; Briviba *et al.*[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 *et al.*[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	Smoking	Plasma folate (nmol/l)		Vitamin C (µmol/l)	
		mean	SD	mean	SD	% male	% smoking	mean	SD	mean	SD
Broekmans *et al.*[33]	47	*49.3**	*5.1**	25.7	3.1	51.1	25.5	13.7	7.1	49.4	18.6
Castenmiller *et al.*[35, 36]	58	22.8	7.7	22.1	2.1	39.7	0	15.3	4.2	-	-
Chopra *et al.*[37]	34	37.2	8.7	-	-	0	-	-	-	-	-
Dragsted *et al.*[38, 46]	31	27.3	7.3	23.1	2.3	48.4	0	10.8	4.0	-	-
Freese *et al.*[39, 45]	77	25.1	6.6	22.6	3.2	26.0	5.2	10.0	4.1	51.9	16.5
Itsiopoulos *et al.*[40]	27	59.1	7.1	30.2	3.7	59.3	-	-	-	-	-
Karlsen *et al.*[29, 41]	33	56.7	6.4	24.8	2.7	100	100	-	-	46.7	17.0
Miller III *et al.*[44]	60	*52.0**	*10.0**	*29.6**	*4.4**	*44**	*14**	-	-	-	-
Van het Hof *et al.*[49]	43	22.4	6.4	22.4	2.1	27.9	0	-	-	66.6	17.4
Van Loo – Bouwman *et al.*[50]	24	22.0	4.0	21.8	2.2	41.7	0	-	-	-	-
Watzl *et al.*[32, 51]	63	31.2	9.0	23.7	2.7	100	0	-	-	83.7	16.6
Winkels *et al.*[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 (µmol/l)		β-carotene (µmol/l)		β-cryptoxanthin (µmol/l)		Lycopene (µmol/l)		Lutein (µmol/l)		Zeaxanthin (µmol/l)		Lutein+zeaxanthin (µmol/l)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Broekmans *et al.*	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 *et al.*	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 *et al.*	0.10	0.07	0.38	0.29	0.13	0.09	0.34	0.16	0.23	0.10	-	-	-	-
Dragsted *et al.*	-	-	0.36	0.23	-	-	-	-	0.26	0.12	-	-	-	-
Freese *et al.*	0.20	0.10	0.60	0.30	0.10	0.05	0.62	0.19	0.26	0.10	-	-	-	-
Itsiopoulos *et al.*	0.08	0.05	0.31	0.20	0.16	0.14	0.43	0.20	-	-	-	-	0.35	0.13
Karlsen *et al.*	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 *et al.*	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 *et al.*	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 *et al.*	0.10	0.06	0.75	0.36	0.34	0.14	-	-	-	-	-	-	-	-
Watzl *et al.*	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 *et al.*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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

		Linear model		MFP model			Reduced MFP model		
Predictors		β	SE	β	SE	Power	β	SE	Power
FVJ									
Constant		-172.8	158.9	-1691.4	526.9	-	1043.2	180.0	-
α-carotene	µmol/l	479.8	142.2	607.8	133.4	0.5	674.1	90.1	0.5
β-carotene	µmol/l	123.1	53.1	101.5	50.9	1	-	-	-
Lutein + zeaxanthin	µmol/l	193.2	68.8	154.6	70.6	1	-153.7	36.8	-0.5
β-cryptoxanthin	µmol/l	162.1	138.5	141.2	138.3	1	-	-	-
Lycopene	µmol/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	µmol/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	µmol/l	939.2	205.0	830.9	219.9	1	-	-	-
β-carotene	µmol/l	104.1	45.9	95.4	45.1	1	300.2	65.2	1
Lutein + zeaxanthin	µmol/l	276.8	69.5	414.4	90.2	1	-158.3	29.3	-0.5
				-562.4	140.2	1			
β-cryptoxanthin	µmol/l	146.1	105.7	74.4	100.7	1	-	-	-
Lycopene	µmol/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	µmol/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 (μmol/l)	0.29	0.26
β-carotene at follow-up (μmol/l)	0.27	0.24
Cryptoxanthin at follow-up (μmol/l)	0.08	0.16
Lycopene at follow-up (μmol/l)	0.19	0.24
Combined lutein and zeaxanthin at follow-up (μmol/l)	0.08	0.15
Sum of carotenoids (μmol/l)	0.23	0.33
Serum/plasma folate at follow-up (nmol/l)	0.32	0.26
Serum/plasma vitamin C at follow-up (μmol/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 *et al.*[33]	340.9	-127.9	-743.2; 487.5	209.8	-88.3	-457.4; 280.8
Castenmiller *et al.*[35, 36]	188.2	10.1	-358.4; 378.6	126.8	17.0	-224.7; 258.8
Dragsted *et al.*[38, 46]	303.4	-198.9	-631.7; 233.9	191.9	-80.1	-407.9; 247.6
Freese *et al.*[39, 45]	274.7	94.7	-410.3; 599.7	304.0	150.2	-368.1; 668.5
Itsiopoulos *et al.*[40]	271.0	4.8	-492.4; 502.0	253.6	129.6	-289.5; 548.8
Karlsen *et al.*[29, 41]	673.8	555.8	-159.4; 1271.0	228.7	33.0	-408.2; 474.2
Miller III *et al.*[44]	242.0	46.7	-326.1; 419.6	236.4	50.4	-370.8; 471.7
Van het Hof *et al.*[49]	125.5	27.0	-170.3; 224.2	88.9	16.0	-146.1; 178.0
Van Loo – Bouwman *et al.*[50]	181.4	0.48	-305.9; 306.9	195.1	-156.1	-331.4; 19.2
Watzl *et al.*[32, 51]	270.1	-141.1	-576.3; 294.1	210.6	-64.8	-441.2; 311.7
Winkels *et al.*[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

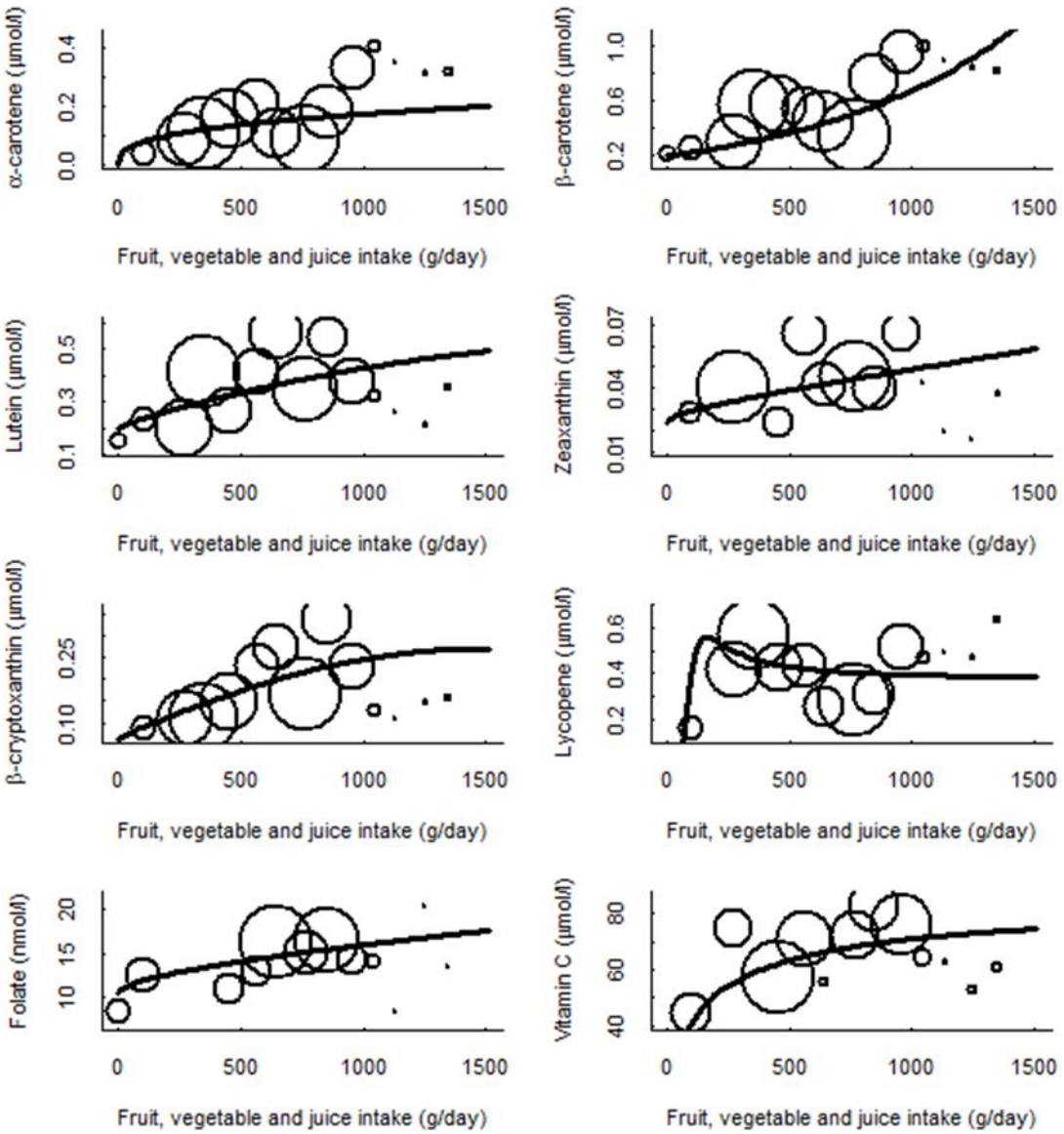
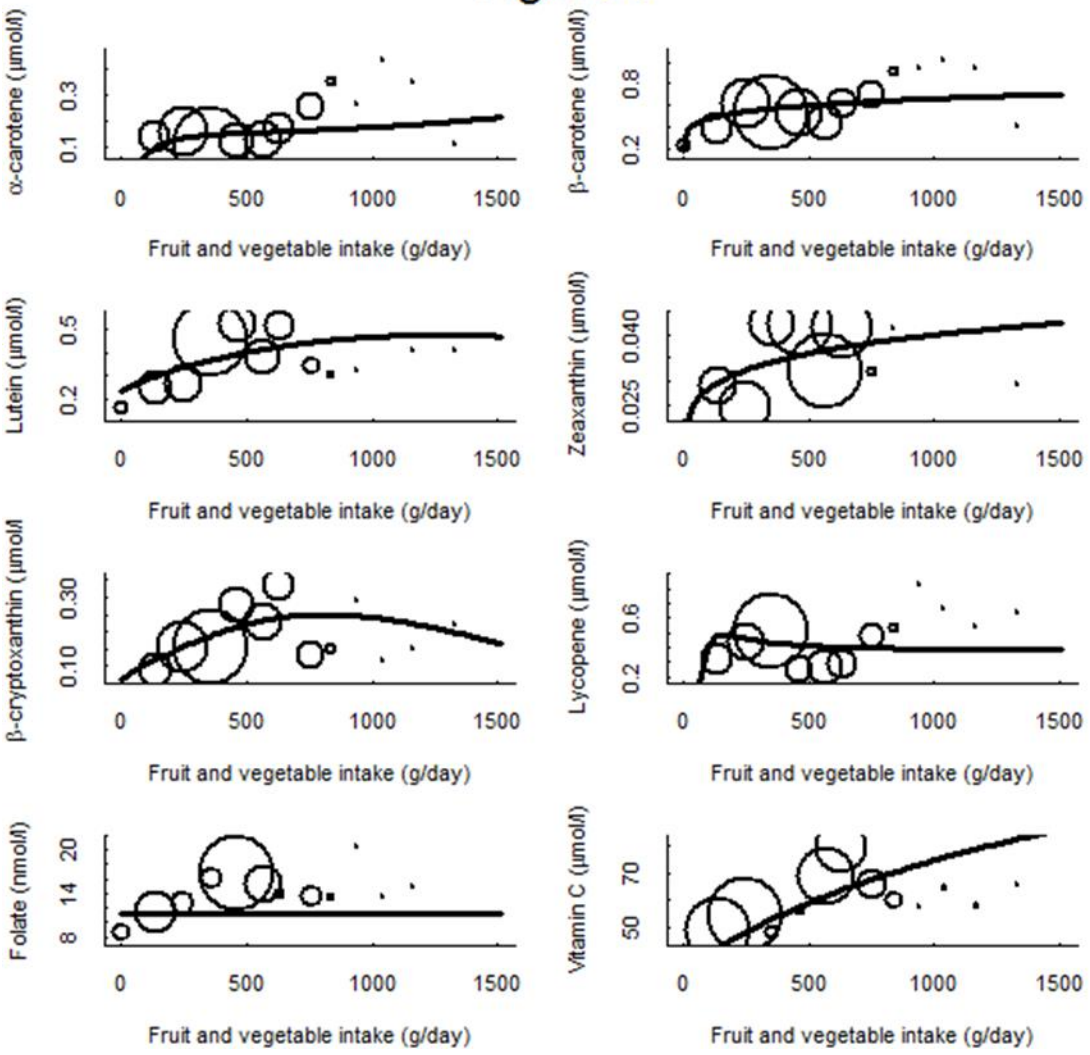

α-carotene (µmol/l)
0.0 0.2 0.4
β-carotene (µmol/l)
0.2 0.6 1.0
Lutein (µmol/l)
0.1 0.3 0.5
Zeaxanthin (µmol/l)
0.01 0.04 0.07
β-cryptoxanthin (µmol/l)
0.10 0.25
Lycopene (µmol/l)
0.2 0.4 0.6
Folate (nmol/l)
10 15 20
Vitamin C (µmol/l)
40 60 80
0 500 1000 1500
Fruit, vegetable and juice intake (g/day)

Figure 3


α-carotene (µmol/l)
0.1
0.3
β-carotene (µmol/l)
0.2
0.8
Lutein (µmol/l)
0.2
0.5
Zeaxanthin (µmol/l)
0.025
0.040
β-cryptoxanthin (µmol/l
0.10
0.30
Lycopene (µmol/l)
0.2
0.6
Folate (nmol/l)
8
14
20
Vitamin C (µmol/l)
50
70
0
500
1000
1500
Fruit and vegetable intake (g/day)