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Title: Derivation and validation of a total fruit and vegetable intake prediction model to identify targets for biomarker discovery using the UK National Diet and Nutrition Survey.

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Running title: Total fruit and vegetable intake prediction model.

List of abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion; DINO, Diet In Nutrients Out; FV, fruit and vegetable; LR, likelihood ratio; MBPs, multi-metabolite biomarker panels; MG, modelling group; TFVpred, Total fruit and vegetable prediction; VG, validation group; VIF, variance inflation factor.

1 **Abstract (297 words)**

2 **Background:**

3 Dietary assessment in research and clinical settings is largely reliant on self-reported
4 questionnaires. It is acknowledged that these are subject to measurement error and biases
5 and that objective approaches would be beneficial. Dietary biomarkers have been purported
6 as a complimentary approach to improve accuracy of dietary assessment. Tentative
7 biomarkers have been identified for many individual fruit and vegetables (FV) but an objective
8 total FV intake assessment tool has not been established.

9 **Objective:**

10 We aimed to derive and validate a prediction model of total FV intake (TFVpred) to inform
11 future biomarker studies.

12 **Methods:**

13 Data from the National Diet and Nutrition Survey (NDNS) were used for this analysis. A
14 modelling group (MG) consisting of participants aged >11 years from the NDNS years 5-6 was
15 created (n=1746). Intake data for 96 FVs were analysed by stepwise regression to derive a
16 model that satisfied three selection criteria: standard error of the estimate (SEE) ≤ 80 , $R^2 > 0.7$,
17 and ≤ 10 predictors. The TFVpred model was validated using comparative data from a
18 validation group (VG) created from the NDNS years 7-8 (n=1865). Pearson's correlation
19 coefficients were assessed between observed and predicted values in the MG and VG. Bland-
20 Altman plots were used to assess agreement between TFVpred estimates and total FV intake.

21 **Results:**

22 A TFVpred model, comprised of tomatoes, apples, carrots, bananas, pears, strawberries and
23 onions, satisfied selection criteria ($R^2=0.761$, $SEE=78.81$). Observed and predicted total FV

24 intake values were positively correlated in the MG ($r=0.872$, $P<0.001$, $R^2=0.761$) and the VG
25 ($r=0.838$, $P<0.001$, $R^2=0.702$). In the MG and VG, 95.0% and 94.9% of TFVpred model residuals
26 were within the limits of agreement, respectively.

27 ***Conclusions:***

28 Intakes of a concise FV list can be used to predict total FV intakes in a UK population. The
29 individual FVs included in the TFVpred model present targets for biomarker discovery aimed
30 at objectively assessing total FV intake.

31 ***Keywords:*** fruit and vegetables, prediction model, dietary assessment, biomarkers, dietary
32 questionnaires.

33 **Background**

34 Non-communicable diseases (NCDs) accounted for 71.3% of worldwide mortality in 2016 (1).
35 The objective measurement of modifiable risk factors is vital in informing strategies to reduce
36 the public health burden incurred by NCDs. Fruit and vegetable (FV) intake has been
37 associated with a lower risk of cardiovascular disease (2–5), type 2 diabetes (6,7), and some
38 forms of cancer (2,8). These NCDs accounted for approximately 28.6 million deaths in 2016,
39 equating to half of global mortality (1), thus increasing FV consumption presents a potential
40 opportunity to reduce the burden of disease.

41 Recent meta-analyses assessing the relationship between the quantity of FV intake and
42 relative risk of all-cause mortality have produced equivocal results. Findings consistently
43 indicate that relative risk of all-cause mortality is proportionately lower with increased
44 consumption of FVs, yet the reported plateau in risk reduction ranges from 5 servings (5), to
45 10 servings of FV per day (2). This two-fold variation in the threshold of daily FV consumption
46 at which there is the lowest relative risk of all-cause mortality is congruent with disparities in
47 public health recommendations. The World Health Organization and Public Health England
48 currently recommend the consumption of at least five servings (400 g) of FV per day (9,10),
49 whereas the Danish Ministry of Food recommend the equivalent of 7.5 servings (600 g) per
50 day (11). Findings from Aune *et al.* (2) infer that current recommendations, such as the UKs
51 presented in the Eatwell Guide (9), may not sufficiently encourage higher levels of FV
52 consumption that pertain to a lower risk of all-cause mortality. The evidence regarding
53 optimal daily intake of FVs remains inconclusive, thus presenting a barrier toward informing
54 public health recommendations, emphasising the necessity for further elucidation of the
55 relationship between FV intake and NCDs.

56 Epidemiological studies aiming to determine diet-disease relationships assess dietary intake
57 using self-report methods such as food diaries, 24-hour recalls and food frequency
58 questionnaires (12–14). While necessary for obtaining data representative of habitual dietary
59 intake, such methods are inherently subject to measurement error and biases and can be
60 burdensome on participants (12,15–17). A more succinct method of intake data collection,
61 i.e. reporting a single food group of interest could alleviate the burden on participants, while
62 conversely reducing the utility of the data when the exploration of whole diet-disease
63 associations is required. Appropriate study designs and methodologies can mitigate the
64 measurement error and biases inherent to self-report methods (18). A combined approach,
65 comprised of the simultaneous measurement of dietary biomarkers and self-report methods
66 has been purported to improve the accuracy of dietary exposure measurements, thus
67 facilitating the elucidation of diet-disease relations (18,19).

68 Candidate dietary exposure biomarkers for the objective measurement of total FV intake,
69 including carotenoids and polyphenols (20,21), have been explored and shown to have limited
70 utility. The establishment of an objective tool to assess total FV intake, rather than individual
71 FV intake, has not yet proved efficacious or been validated (22). Untargeted metabolomic
72 techniques are increasingly prevalent within the literature, making significant progress in the
73 identification and quantification of specific dietary exposure biomarkers (23,24). The
74 predominant focus of this research has been identifying single biomarkers for specific
75 foods/food groups. Further to the identification of novel biomarkers, the use of a panel of
76 biomarkers, by measuring a number of metabolites pertaining to a food/food group for a
77 more accurate representation of dietary exposure, has been proposed (25). Multi-metabolite
78 biomarker panels (MBPs) have been identified for the quantification of walnuts (26), bread

79 (27), cocoa (28), orange juice (29), wine (30) and whole dietary patterns (31,32), however a
80 panel for total FV intake is yet to be established.

81 The National Diet and Nutrition Survey (NDNS) is a continuous, cross-sectional survey,
82 designed to collect detailed quantitative information on the food consumption, nutrient
83 intake and nutritional status of the UKs general population (33). Analysis of these data can
84 provide novel insight into total FV eating habits. The aim of this research was to identify a
85 concise number of FVs that are predictive of total FV intake. Identifying such FVs stands to
86 direct future metabolomic biomarker studies that pursue the objective measurement of FV
87 intake.

88 **Methods**

89 **Study Design**

90 This study analysed cross-sectional intake data of individuals from years 5-6 (2012/13 –
91 2013/14) and years 7-8 (2014-15 – 2015/16) of the NDNS rolling programme (33,34). The
92 modelling dataset (years 5-6) and validation dataset (years 7-8) were retrieved from the UK
93 data archive in September 2017 and January 2019, respectively.

94 **Data Source**

95 Full methodological details of the NDNS have been described elsewhere (35). In short, the full
96 NDNS years 5 - 6 dataset was comprised of 2,546 participants (age 30 ± 24 years, mean \pm SD)
97 recruited from 323 postal sector random sampling units across the UK. Data were collected
98 over 12 months to account for seasonal variation. Samples were stratified by country,
99 ensuring proportional representation from England, Scotland, Wales and Northern Ireland.
100 Following initial interviews to obtain background information and familiarise participants with
101 the intake data collection method, 4-day food diaries were completed and participants over

102 the age of 4 years who consented to a nurse visit had anthropometric measurements (height,
103 weight, waist and hip circumference, demi-span, blood pressure), and blood and urine
104 samples taken. The modelling group (MG) dataset was obtained from this sample and
105 included all participants > 11 years old (n = 1746).

106 ***Data Processing***

107 The fraction of NDNS data used in the current analysis consisted of food and drink
108 consumption data collected using 4-day un-weighed food diaries (portions were quantified
109 by household measures). Participants recorded the contents of all eating and drinking
110 occasions over four consecutive days, including one weekend day. Food diaries were
111 processed and coded using an adapted version of Health Nutrition Research's dietary
112 assessment system DINO (Diet In Nutrients Out) (36). DINO disaggregates composite items
113 and items that differ by preparation into individual foods with a unique code. The current
114 analysis aggregated data of the same fruit/vegetable with differing codes, to form a daily
115 intake value for individual FVs (g/day). Fruit juices, potatoes, and pulses (except for green
116 beans, runner beans, and broad beans) were excluded from the analysis due to differences in
117 nutrient composition from FV as included in the UK Eatwell Guide (9). We multiplied dried
118 fruit intake by three, based on the respective water and micronutrient content, to standardize
119 dried and non-dried FV intake (34). **Supplementary Table 1** outlines the details of individual
120 FV intake data aggregation, FV consumption prevalence and mean daily intake in consumers
121 only. Daily intake of 96 FVs were calculated and used as potential predictor variables.
122 Individual FV intakes were summed to calculate total FV intake (g/day).

123 **Statistical Analysis**

124 All data were obtained and processed using IBM SPSS Statistics 24 (SPSS, Inc., Chicago, IL,
125 USA) and analysed using Stata version 15 (College Station, TX: StataCorp LLC). The
126 assumptions of multiple linear regression analysis were satisfied prior to analysis. Normality
127 of residuals and homoscedasticity of the data were confirmed, and no transformations were
128 applied to any variables. All potential predictors had a linear relationship with total FV intake.
129 We conducted automated forward stepwise regression analyses. Models began with an
130 intercept and were iteratively constructed by selecting the predictor variable (individual FV
131 intake) that accounts for the most unique variance in total FV intake. Subsequent models
132 incorporated the individual fruit or vegetable that accounted for the most unique variance in
133 total FV intake among the remaining predictor variables. Predictor variables were added with
134 each model iteration until there was no longer an improvement in total FV intake variance
135 accounted for by the model. Regression significance ($P < 0.05$) was taken to indicate that the
136 independent variable predicts total FV intake. The variance inflation factor (VIF) was used to
137 quantify correlation of predictors in a model, to detect any collinearity. Regression
138 coefficients represent the mean change in outcome for one unit of change in the predictor
139 variable and were used to compile regression the equation. The standard error of the
140 estimates (SEE) was calculated and R^2 used to denote the proportion of variance in total FV
141 intake explained by each model.

142 **Model Selection Criteria**

143 The rationale underpinning model selection criteria was to produce a regression equation
144 that could be used to facilitate the discovery of FV biomarkers. The future utility of the model
145 is dependent upon having few predictors to moderate the extent of biomarker measurement

146 required, while explaining a large proportion of the variance in predicted total FV intake. We
147 established iterative models that satisfied three pragmatically determined selection criteria;
148 a SEE \leq an 80 g FV serving, variance in total FV intake (R^2) > 0.7 , and the number of predictors
149 in the model was capped at 10 to produce a concise assessment tool. Comparative
150 assessment of regression models was facilitated by calculating adjusted R^2 , Akaike
151 information criterion (AIC), Bayesian information criterion (BIC) and penalised likelihood ratio
152 (LR) testing. The aim of all comparative assessments was to ensure that all subsequent models
153 were an improvement on the previous model.

154 ***Model Validation***

155 Validation of the final total FV prediction model iteration (TFVpred) was conducted using a
156 novel dataset from the NDNS years 7-8, with participants aged > 11 years. NDNS data
157 collection methodologies were consistent with the years 5-6 used as the MG. The current
158 analysis applied the same data processing procedure described above to the validation group
159 (VG) dataset to obtain comparable FV intake data. The TFVpred equation was applied to the
160 VG dataset to predict total FV intake (g/day). Pearson's r correlation coefficient was measured
161 to determine linearity between observed and predicted total FV values. Correlational
162 coefficient of determination (R^2) was calculated to measure the amount of variance in
163 TFVpred estimated total FV intake explained by the observed total FV intake. Correlational
164 analysis was conducted with observed and predicted FV intake in vegetarian and vegan
165 subsets of the MG and VG to assess the validity of the prediction model in a subset of the
166 population with known differences in FV consumption patterns. Bland-Altman plots were
167 generated to assess the agreement between TFVpred estimates and observed total FV intake
168 in modelling and validation groups. Limits of agreement were plotted at ± 1.96 SDs of the
169 mean difference between the observed and predicted values of total FV intake.

170 **Results**

171 **Multiple Linear Regression Models for prediction of total FV intake**

172 In total, 4-day food diaries were analysed from 1746 participants in the MG, and 1865
 173 participants in the VG. Forward stepwise regression model summaries are displayed in **Table**
 174 **1**. Total FV prediction model 7 (TFVpred) was the first model iterated that met all model
 175 selection criteria, with an $R^2 > 0.7$, a SEE < 80 and contained ≤ 10 predictor variables. All seven
 176 models predicted total FV intake ($P < 0.05$). The proportion of variance explained by
 177 regression models (R^2) increased from 0.277 to 0.761 between models 1 and 7. Incremental
 178 reductions in SEE were observed with each regression model including a novel predictor.
 179 TFVpred, comprised of seven predictor FV coefficients and constant, is displayed in **Eq. 1**:

$$180 \text{TFVpred} = 1.773(\text{tomatoes}) + 1.428(\text{apples}) + 2.439(\text{carrots}) + 1.211(\text{bananas}) +$$

$$181 \quad 1.422(\text{pears}) + 1.714(\text{strawberries}) + 1.519(\text{onions}) + 29.88(\text{constant}).$$

182 The TFVpred equation highlights the seven predictor FVs accounting for the most variance in
 183 total FV intake, namely tomatoes, apples, carrots, bananas, pears, strawberries and onions,
 184 thus presenting targets for intake biomarker discovery. Five FVs included in the TFVpred
 185 model (tomatoes, onions, carrots, bananas and apples) were within the top six most
 186 commonly consumed FVs (as per number of consumers), while strawberries and pears were
 187 within the top 15 and 24, respectively (**Supplementary Table 1**). All predictor variable FVs
 188 were within the top 40 FVs for mean daily intakes in consumers only.

189 **Model Comparison**

190 Comparison of regression models is shown in **Table 2**. The variance in total FV intake
 191 explained by models, when corrected for the number of predictors, incrementally increased
 192 with additional model iteration. The size of incremental augmentation in adjusted R^2

193 diminished as regression models progressed, with the maximum change being an increase of
194 0.174 from model 1 to model 2, and the smallest change was 0.028, observed between
195 models 6 and 7. Penalised-LR criteria, AIC and BIC, are presented for each model in Table 2.
196 AIC and BIC values were incrementally smaller as more predictors were added to the
197 regression models. LR tests for nested models were significant with all subsequent iterations,
198 indicating successive improvements in goodness of fit.

199 ***Model Validation***

200 In the MG, observed and predicted values of total FV intake were positively correlated ($r =$
201 $0.872, P < 0.001$) with an $R^2 = 0.761$ (**Figure 1A**). Observed and predicted total FV intake values
202 in the VG were also positively correlated ($r = 0.838, P < 0.001$) with an $R^2 = 0.702$ (**Figure 1B**).
203 Bland-Altman plots determined there was good agreement between observed and predicted
204 total FV intake values, with the MG (**Figure 2A**) and VG (**Figure 2B**) demonstrating 95.0% and
205 94.9% of residuals were within the limits of agreement, respectively. Observed and predicted
206 total FV intake values within vegetarian and vegan subsets were positively correlated in the
207 MG ($r = 0.882, P < 0.001, R^2 = 0.777$, **Supplementary Figure 1A**) and VG ($r = 0.839, P < 0.001,$
208 $R^2 = 0.704$, **Supplementary Figure 1B**).

209 ***Discussion***

210 To our knowledge, this is the first study to elucidate a concise group of individual FVs that are
211 predictive of total FV intake, accounting for 76.1% of total variance. The 7th model iteration,
212 TFPred, was the first to satisfy predetermined selection criteria and was subsequently used
213 to predict total FV intake in the VG, using individual intake values of tomatoes, apples, carrots,
214 bananas, pears, strawberries and onions. Correlational analysis and Bland-Altman plots were
215 used to assess the efficacy of the TFPred model when applied to the VG and demonstrated

216 strong agreement between observed and predicted values. TFVpred thus provides a potential
217 assessment tool in estimating total FV intake, where valid measurements of seven individual
218 FV intakes (tomatoes, apples, carrots, bananas, pears, strawberries and onions) are available.
219 A multitude of comparisons between models were conducted to determine that TFVpred
220 outperforms other models by AIC, BIC and LR test statistics, thereby the most appropriate
221 model for estimating total FV intake (37). This research has the potential to consolidate the
222 applicability of existing individual FV measurements obtained using dietary questionnaires.
223 Furthermore, the identified FVs signify clear targets for novel biomarker discovery.
224 Subsequent integration of validated biomarkers within the TFVpred equation provide
225 additional utility as a potential tool for total FV intake estimation.

226 ***Dietary Questionnaires***

227 Self-report methods of dietary intake assessment, such as food diaries, 24-hour recalls and
228 food frequency questionnaires, have been a longstanding topic of debate in nutritional
229 research (17,38), while remaining the most prevalent techniques to assess diet-disease
230 relationships (4,39). Critics state that the reliance on memory and the influence of
231 researcher/social-approval biases can incur random and systematic measurement errors,
232 such as the over-reporting of FV intake (12–14,17). Furthermore, the accuracy of self-
233 reported data may be influenced by the ability of individuals, or the sensitivity of the
234 assessment method, to quantify the size and contents of a FV serving (40,41). Proponents of
235 self-report methods acknowledge that while limitations exist, study design considerations
236 and corrections for measurement error can be applied to gather insightful intake data,
237 currently unobtainable using other means (42,43). The NDNS dataset used in the current
238 study aimed to collect data accurately pertaining to the UK population by mitigating the effect
239 of some of these limitations through appropriate study design. Daily food diaries were

240 completed over four consecutive days to minimise reliance on memory (42). Upon completion
241 of food diaries, trained interviewers met with participants to aid the quantification of the food
242 diary constituents, where original visual aids were insufficient (35). The NDNS dataset
243 presents a useful source when compiling inferential statistical models, as in the present
244 analysis. Given the robustness of the NDNS methodology, validation with an updated NDNS
245 dataset was necessary and demonstrated the efficacy of the TFVpred model as a practical tool
246 for total FV intake estimation.

247 Novel assessment of total FV intake using the TFVpred model could utilise existing methods
248 of individual FV intake from dietary questionnaires. Measurements could be obtained via
249 amended food frequency questionnaires, i.e. condensed to include only FV assessment,
250 providing sufficient validation is conducted (39,44,45). Kristjansdottir *et al.* (44) reported that
251 FV intake estimated using a combined 24-hour recall and food frequency questionnaire was
252 associated with 7-day food diary reported intake, with a spearman's coefficient of 0.73 ($P <$
253 0.001). Furthermore, Block *et al.*(46) correlated FV intake obtained using 100-item food
254 frequency questionnaires (47), and a single page screener questionnaire, reporting a
255 spearman's coefficient of 0.71 ($P < 0.001$). Using a screener to assess FV intake could provide
256 a time-effective alternative to a lengthy questionnaire and provide specific FV intake data. A
257 practical application of the predictive FVs identified in the present analysis would be to
258 incorporate these FVs in screener questionnaires or as prompts in multiple pass dietary
259 assessment methods. Adopting such changes may increase the accuracy of dietary intake
260 data, though amendments to validated dietary assessment tools would require subsequent
261 validation. Incorporating measurements of the FVs identified in the TFVpred model within

262 existing dietary questionnaires presents an inexpensive tool for internal validation to improve
263 the precision of dietary intake assessment.

264 ***Combining Dietary Questionnaires and Biomarkers***

265 The prevailing recommendations from prominent research groups within the field of nutrition
266 and dietary assessment include the combined assessment of diet using dietary questionnaires
267 and biomarker quantification (18,19,25). A prospective application of the TFVpred model
268 validated in the present analysis would be to integrate biomarker assessments for the seven
269 FVs, providing an objective assessment tool that can be obtained from biological samples and
270 be used to assess FV exposure alongside appropriately conducted questionnaires. The NDNS
271 represents an example of how this may be achieved, due to the concurrent collection of self-
272 report data and urine samples, however the assessment of a validated FV biomarker
273 assessment panel is yet to be established (35). Systematic reviews exploring the efficacy of
274 objective assessments of FV intake by dose-dependent concentration biomarkers have
275 ascertained that no single candidate biomarker can accurately measure total FV intake
276 (20,48). However, putative dose-dependent urinary biomarkers have been identified for
277 some FVs including grapes (49), peas, apples, onions (50), red cabbage, strawberries and
278 beetroot (31). Prevalent techniques aiming to identify a panel of biomarkers pertaining to
279 individual foods/food groups include targeted and untargeted tandem high-performance
280 liquid-chromatography mass-spectrometry, as well as proton nuclear magnetic resonance
281 spectroscopy, with subsequent multivariate modelling (Principal Component-Discriminant
282 Analysis, Partial Least Squares, and Random Forest Classification) (27,32,51). This has led to
283 the identification of numerous metabolites purported as biomarkers of dietary exposure,
284 although validation as dose-dependent biomarkers of intake, necessary prior to TFVpred

285 model integration, is less pervasive (49,52,53). The specificity of putative biomarkers ranges
286 from individual foods (including FVs) to broad dietary pattern identification (32,54,55).

287 Potential confounding factors for biomarker identification include inherent genetic variance
288 between individuals, physiological and lifestyle factors that may influence metabolism,
289 biological sample handling and the analytical methodology (22). Future research should aim
290 to negate some of these factors. For example, Garcia-Aloy *et al.* (25) propose the use of MBPs
291 to provide an insight into dietary exposure. MBPs enable the simultaneous measurement of
292 numerous metabolites that pertain to a specific food/food group, capturing a broader fraction
293 of dietary exposure. Once validated, prospective MBPs of individual FV intake could be
294 integrated with the regression equation modelled in the present study as a method of
295 estimating total FV intake. Dragsted *et al.* (56) identified a stringent set of post-discovery
296 validity criteria for biomarkers, including assessments of: 1) biochemical plausibility and
297 stability, 2) dose-dependency with low abundance when intake is zero and saturation kinetics,
298 3) time-responsiveness to inform when biological samples can be collected, 4) robustness
299 after co-ingestion with other foods, 5) reliability to ensure biomarkers are comparable to
300 assessments from other questionnaire or biomarker measurements, 6) a reproducible
301 analytical methodology. Meeting these standards is imperative if biomarkers are to improve
302 the precision and accuracy of dietary assessment. Considerable work is necessary to elucidate
303 in particular time-responsiveness and dose-dependency of putative FV biomarkers (25). At
304 present, the limitations associated with both facets of dietary assessment cannot be fully
305 alleviated by adopting sole usage of the alternate technique, thus combinations of dietary
306 questionnaires and biomarker assessments should be explored (16,25).

307 ***Strengths & Limitations***

308 FV servings of 80 g were used in the present analysis to compute regression models, thus FVs
309 that deviated from the standard 80 g serving sizes, such as dried fruits, required numerical
310 transformation prior to be considered a FV portion. This was conducted to prevent the
311 potential exclusion of a subset of FVs that contribute to total FV intake, but do not constitute
312 a regular FV serving. Some semi-dried fruits were not included in the current analysis due to
313 the unknown composition of portion sizes. Consistent with other nutritional epidemiology
314 research (57,58), children aged < 12 years (MG, n = 763; VG, n = 822) were excluded from the
315 current analysis to mitigate the systematic error incurred by having dissimilar eating trends
316 and serving sizes to adolescents and adults. As the current analysis was conducted using
317 intake data from UK based participants ≥ 12 years, prospectively the TFVpred model should
318 not be used to estimate total FV intake in children < 12 years. Deriving the TFVpred model
319 using stepwise linear regression modelling and pragmatic predetermined selection criteria
320 facilitated the formation of a model that included a combination of influential FVs that were
321 predictive of total FV intake and frequently consumed in the population. TFVpred predictor
322 FVs were among the most pervasively consumed in the MG and VG, indicating good suitability
323 within a UK population. Future research should investigate the efficacy of the TFVpred model
324 in other developed countries and further validation is required prior to use in non-UK based
325 populations, as FV intake is variable between countries (59,60). A prominent challenge within
326 the present study was producing a model with a small number of predictors that captured a
327 substantial proportion of the variance in total FV intake, without including relevant cofactors
328 such as socioeconomic status(61,62), food availability(63) and vegetarianism(64). The
329 TFVpred model predictions were accurate for subsets of the population known to have
330 different FV consumption patterns, as demonstrated by the correlation between observed

331 and predicted total FV intake in vegetarians and vegans. The TFVpred model also performed
332 well across a broad variety of FV intakes, the small proportion of individuals that fall outside
333 the upper LOA. Bland-Altman plots (Fig. 2) indicate that 4.70 % and 4.86 % of individuals in
334 the MG and VG, respectively, fall outside the upper LOA, thus consuming a variety of FVs that
335 are not accounted for by the model. The simultaneous assessment of cofactors of total FV
336 intake and additional FVs would increase the accuracy of prediction models, however the aim
337 of the present study was to identify a concise number of predictor FVs that can be integrated
338 into dietary questionnaires to reliably estimate total FV intake in a UK population and identify
339 targets for biomarker discovery, rather than establish a multifaceted prediction model of total
340 FV intake.

341 ***Conclusions***

342 The TFVpred model (Eq. 1) established in the current study provides a valuable tool for
343 estimating total FV intake. Future utility of the TFVpred model would be improved with the
344 integration of dose-dependent biomarkers/MBPs for the FVs that predict total FV intake
345 (tomatoes, apples, carrots, bananas, pears, strawberries and onions). The identification of
346 these FVs, through the establishment and validation of the TFVpred model provides a clear
347 pathway for future research by identifying dose-dependent biomarker targets. Advances in
348 biomarker identification and validation provide a valuable opportunity to obtain objective
349 assessments of total FV intake that, in parallel with appropriate self-report techniques, could
350 denote notable improvements in the accuracy of dietary assessment.

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352 ***Statement of authors' contributions to manuscript***

353 EJO, TPD and LMO'C developed the research question and planned the analysis; EJO
354 performed the analysis with support from SP and LMO'C; EJO drafted the manuscript with
355 editorial support from OF, TPD, and LMO'C; and all authors read and approved the final
356 manuscript.

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Tables

Table 1 – Multiple linear regression models using individual fruit and vegetable (FV) intake data from the National Diet and Nutrition Survey Rolling Programme years 5-6 to predict total FV intake (n = 1746).

Model	Predictor Variables	Regression <i>P value</i>	Regression			Variance Inflation Factor	
			Constant	Coefficient (β)	Standard Error of the Estimate		R^2
1	Tomatoes	< 0.001	134.089	2.672	0.277	136.81	1.00
2	Tomatoes	< 0.001	104.069	2.352	0.451	119.24	1.02
	Apples	< 0.001		2.030			1.02
3	Tomatoes	< 0.001	69.595	2.277	0.567	105.92	1.02
	Apples	< 0.001		1.823			1.04
	Carrots	< 0.001		2.982			1.02
4	Tomatoes	< 0.001	46.973	2.091	0.664	93.26	1.04
	Apples	< 0.001		1.546			1.07
	Carrots	< 0.001		2.849			1.02
	Bananas	< 0.001		1.406			1.06
5	Tomatoes	< 0.001	45.125	2.060	0.702	87.91	1.04
	Apples	< 0.001		1.452			1.08
	Carrots	< 0.001		2.720			1.03
	Bananas	< 0.001		1.292			1.08
	Pears	< 0.001		1.362			1.05
6	Tomatoes	< 0.001	39.892	1.995	0.732	83.33	1.04
	Apples	< 0.001		1.453			1.08
	Carrots	< 0.001		2.673			1.03
	Bananas	< 0.001		1.250			1.08
	Pears	< 0.001		1.391			1.05
	Strawberries	< 0.001		1.762			1.01
7	Tomatoes	< 0.001	29.877	1.773	0.761	78.81	1.11
	Apples	< 0.001		1.428			1.08
	Carrots	< 0.001		2.439			1.05
	Bananas	< 0.001		1.211			1.08
	Pears	< 0.001		1.422			1.05
	Strawberries	< 0.001		1.714			1.01
	Onions	< 0.001		1.519			1.11

Table 2 – Comparison of multiple linear regression models using individual fruit and vegetable (FV) intake data from the National Diet and Nutrition Survey Rolling Programme years 5-6 to predict total FV intake (n = 1746).

Model	Cumulative Predictor Variables	Adjusted R ²	Change in adjusted R ²	Akaike information criterion	Bayesian information criterion	Likelihood		
						Ratio Models Tested	Likelihood Ratio Test statistic	Likelihood Ratio Test P
1	Tomatoes	0.276	-	22133	22144	-	-	
2	Apples	0.450	0.174	21654	21670	1 and 2	481.13	< 0.001
3	Carrots	0.566	0.116	21241	21263	2 and 3	414.65	< 0.001
4	Bananas	0.664	0.098	20798	20825	3 and 4	445.38	< 0.001
5	Pears	0.701	0.037	20592	20625	4 and 5	207.35	< 0.001
6	Strawberries	0.732	0.031	20406	20445	5 and 6	187.86	< 0.001
7	Onions	0.760	0.028	20213	20256	6 and 7	195.84	< 0.001

Figures

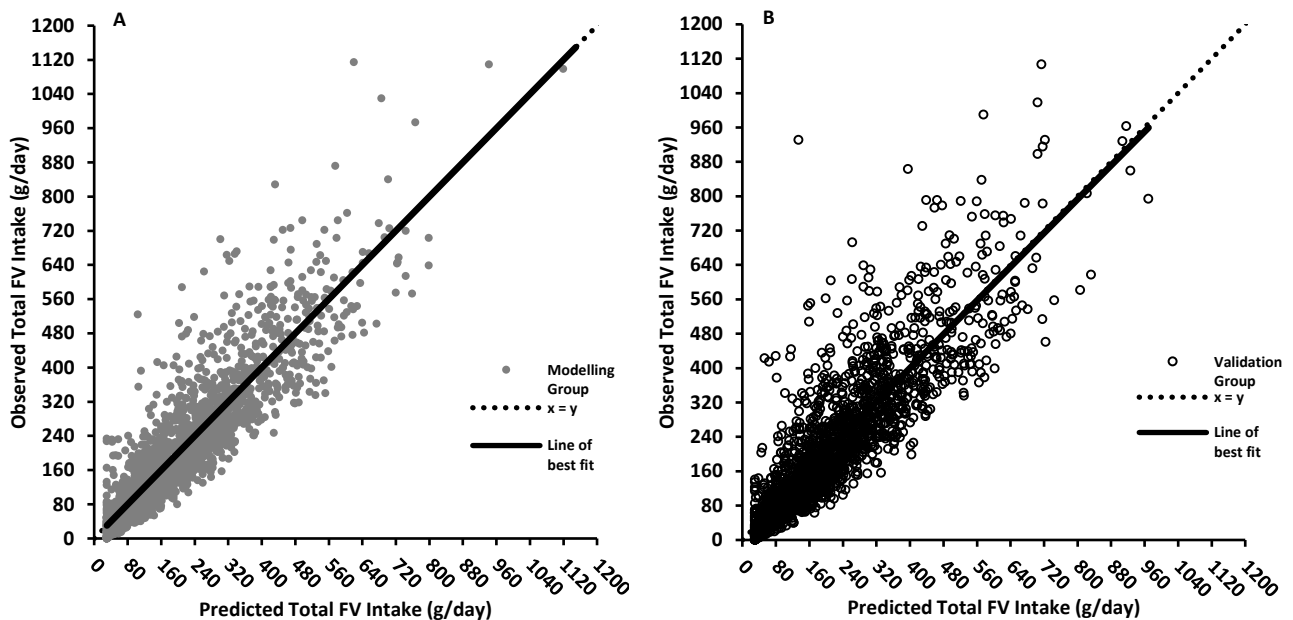


Figure 1 - Correlation between observed and predicted total FV intake using the TFVpred equation for the (A) modelling group (NDNS years 5-6, n = 1746) and (B) validation group (NDNS years 7-8, n = 1865). FV, fruit and vegetable.

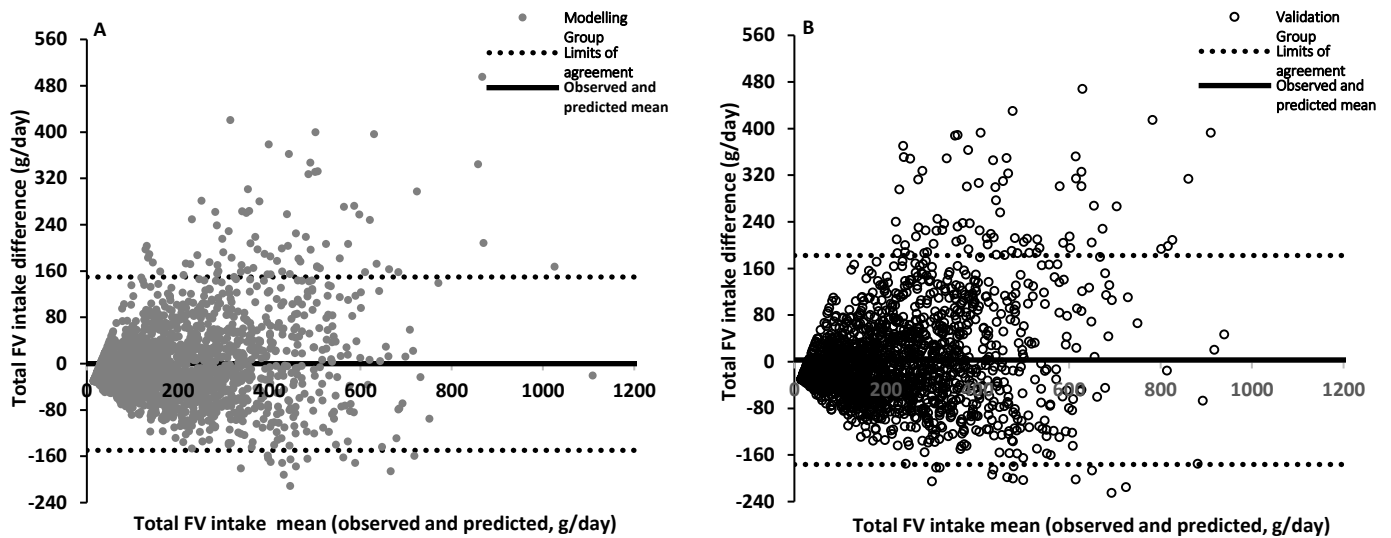


Figure 2 - Bland-Altman plots of total FV intake predictions in the modelling group (A, n = 1746) and validation group (B, n = 1865). Plots display the difference between total FV intake measured by the NDNS and total FV intake predicted by TFVpred model vs. the observed and predicted mean. Limits of agreement (dotted lines) are displayed at ± 1.96 SDs of the mean difference between the observed and predicted values of total FV intake. FV, fruit and vegetable.