

Fruit and vegetable intake and cardiovascular risk factors in people with newly diagnosed type 2 diabetes

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1 **Abstract**

2

3 **Background/objectives:** The cardiovascular benefit of increasing fruit and vegetable (F&V)
4 intake following diagnosis of diabetes remains unknown. We aimed to describe how quantity
5 and variety of F&V intake, and plasma vitamin C, change after diagnosis of diabetes and
6 examine if these changes are associated with improvements in cardiovascular risk factors.

7

8 **Methods:** 401 individuals with screen-detected diabetes from the *ADDITION-Cambridge*
9 study were followed-up over five-years. F&V intake was assessed by food frequency
10 questionnaire and plasma vitamin C at baseline, one- and five-years. Linear mixed models
11 were used to estimate associations of changes in quantity and variety of F&V intake, and
12 plasma vitamin C, with cardiovascular risk factors and a clustered cardiometabolic risk score
13 (CCMR), where a higher score indicates higher risk.

14

15 **Results:** F&V intake increased in year one but decreased by year five whereas variety
16 remained unchanged. Plasma vitamin C increased at one- and five-years. Each SD increase
17 (250g between baseline and one-year and 270g between one- and five-years) in F&V intake
18 was associated with lower waist circumference (-0.92 (95% CI:-1.57, -0.27) cm), HbA_{1c} (-
19 0.11 (-0.20, -0.03) %) and CCMR (-0.04 (-0.08, -0.01)) at one-year and higher HDL-
20 cholesterol (0.04 (0.01, 0.06) mmol/l) at five-years. Increased plasma vitamin C (per SD,
21 22.5µmol/l) was associated with higher HDL-cholesterol (0.04 (0.01, 0.06) mmol/l) and
22 lower CCMR (-0.07 (-0.12, -0.03)) between one- and five-years.

23

24 **Conclusions:** Increases in F&V quantity following diagnosis of diabetes are associated with
25 lower cardiovascular risk factors. Health promotion interventions might highlight the

26 importance of increasing, and maintaining increases in, F&V intake for improved
27 cardiometabolic health in patients with diabetes.

28

29 **Introduction**

30 Type 2 diabetes is a leading cause of premature morbidity and mortality, much of which can
31 be explained by the increased risk of cardiovascular disease (CVD). Previous studies have
32 shown that adhering to specific dietary patterns such as the Dietary Approaches to Stop
33 Hypertension diet (DASH-diet) or the Mediterranean Diet can lower the risk of developing
34 CVD, even among those at initially high risk i.e. individuals with diabetes. A common theme
35 underpinning these diets is an emphasis on consuming more fruits and vegetables (F&V). A
36 large number of studies have demonstrated independent health benefits of a diet rich in
37 F&V¹⁻³, findings which are supported by several studies which examined associations using
38 plasma vitamin C as an objective biomarker of F&V intake^{4,5}. More recently, variety of F&V
39 intake, independent of quantity, has been considered in relation to risk of diabetes and CVD⁶⁻
40 ⁸. To our knowledge, no studies have examined the relationship between repeat measures of
41 quantity and variety of F&V intake and CVD risk factors among individuals with diabetes
42 over five-years of follow-up, corroborated using plasma vitamin C levels.

43

44 Using data from the Anglo-Danish-Dutch Study of Intensive Treatment In People with
45 Screen Detected Diabetes in Primary Care (ADDITION)-Cambridge study, we examined the
46 longitudinal relationship between quantity and variety of F&V intake and plasma vitamin C
47 levels with CVD risk factors in participants with diabetes who were followed-up for five-
48 years.

49

50 **Methods**

51 *Study Design*

52 The design and rationale for the ADDITION-Cambridge study have been reported in detail
53 elsewhere⁹. In brief, individuals were recruited from 49 general practice (GP) clinics in the

54 East of England, UK, for a stepwise diabetes screening programme. Diabetes was diagnosed
55 according to WHO criteria¹⁰. Eligible individuals were aged 40-69 years with no known
56 diabetes and were within the top 25% of a diabetes risk score^{9,11}. Exclusion criteria included
57 being pregnant or lactating, having an illness with a prognosis of death within one year or a
58 psychiatric illness which was likely to preclude involvement or informed consent. Of the
59 33,539 individuals who were invited to attend screening, 867 were identified to have diabetes
60 and agreed to participate in the randomised control trial. The aim of the trial was to compare
61 intensive treatment of multiple risk factors with routine care in individuals with screen-
62 detected diabetes. Participants were cluster randomised by GP clinic. In the intensive
63 treatment group practitioners were encouraged to follow a stepwise target-led treatment
64 regimen to reduce and control CVD risk factors including blood glucose and lipid levels. This
65 group additionally received theory-based health promotion materials including
66 encouragement to consume at least five portions of F&V per day. The routine care group
67 received care which followed UK national guidelines for diabetes management¹². Participants
68 attended for follow-up health assessments after one- and five-years. As there was no
69 interaction by trial group, we pooled both trial groups and conducted a cohort analysis.

70

71 Ethical approval was obtained from the Eastern Multi-Centre Research Ethics Committee
72 (reference number 02/5/54) and all participants gave written informed consent. The
73 ADDITION-Cambridge trial is registered as ISRCTN86769081.

74

75 *Fruit and vegetable intake and plasma vitamin C levels*

76 Plasma vitamin C, an objective biomarker of F&V intake^{4,13}, was measured using a
77 Fluoroskan Ascent FL fluorometer. Self-reported F&V intake was assessed using a validated
78 130-item food frequency questionnaire (FFQ)¹⁴. Participants were asked to report the

79 frequency of food consumption on a nine-point scale ranging from “never or less than once
80 per month” to “more than six times per day”. Variety of F&V intake was derived by
81 summing the total number of unique fruit and vegetable items consumed at least once per
82 week. Possible variety ranged from 0 to 37 items. Quantity of F&V intake was derived by
83 summing the total quantity (in grams/day) of different F&V consumed over the period of one
84 week, divided by seven to quantify daily intake. We did not include potatoes in our analyses
85 as they differ from vegetables in terms of energy and carbohydrate content and are commonly
86 substituted for cereals rather than vegetables¹⁵. We also did not include fruit juice as it is not
87 considered to be equivalent to whole fruit regarding fibre content and satiety value¹⁶.

88

89 *Measurement of cardiovascular risk factors*

90 Baseline, one- and five-year health assessment visits to the study clinic included clinical and
91 anthropometric measures. HbA_{1c} was measured in venous samples using an ion-exchange
92 high-performance liquid chromatography method (Tosoh Bioscience, Redditch, UK). Serum
93 total cholesterol, high density lipoprotein (HDL)-cholesterol and triglycerides were measured
94 in non-fasted samples using enzymatic techniques (Dade Behring Dimension analyser,
95 Newark). Blood pressure was determined based on the mean of three measurements
96 performed after 10 minutes of rest, while participants were seated with a cuff placed on their
97 predominant arm at the level of the heart, using an automated sphygmomanometer (Omron
98 M4, UK). Height and weight were measured in light clothing, without shoes, using a fixed
99 rigid stadiometer and scale (SECA, UK). Waist circumference was derived based on the
100 mean of two measurements taken with a tape measure halfway between the lowest point of
101 the rib cage and the anterior superior iliac crest whilst standing.

102

103 Clustered cardiometabolic risk scores (CCMR) were derived for all clinic visits by averaging
104 standardised values for waist circumference, systolic blood pressure, HbA_{1c}, the natural log
105 of triglycerides and the inverse of HDL-cholesterol. Variables were standardised by
106 subtracting from them sex-specific population means and dividing by sex-specific SDs.
107 Means and SDs at baseline were used to standardise all follow-up CCMR scores. A lower
108 score therefore indicates lower risk.

109

110 *Covariates*

111 Self-report questionnaires were used to obtain information on age, sex, occupation and
112 ethnicity. Occupational social class was defined according to the Registrar General's
113 occupation-based classification and comprised three categories: "professional, managerial
114 and technical", "skilled – manual and non-manual" and "partly skilled or unskilled". Total
115 energy and alcohol intake were assessed using an FFQ¹⁴. Time spent in moderate-to-vigorous
116 physical activity (MVPA) was assessed by self-report using the previously validated
117 European Prospective Investigation into Cancer-Norfolk physical activity questionnaire
118 (EPAQ-2)¹⁷. Medication use was assessed using a self-report questionnaire adapted from the
119 Aberdeen Health Service Research Unit questionnaire⁹. Very few people reported taking
120 multivitamin supplements therefore this was not included in the analysis.

121

122 *Statistical analysis*

123 Descriptive characteristics at baseline and at one- and five-years of follow-up were
124 summarised using means and SDs or frequencies and percentages. t-tests or chi-squared tests
125 were used to examine differences in participant characteristics between those included for
126 these analyses and those excluded due to missing data.

127

128 Linear mixed models, with participant specific random intercepts, were used to estimate the
129 associations between each SD increase in change in quantity of F&V intake from baseline to
130 one-year, and from one-year to five-years, with CVD risk factor levels and CCMR at one-
131 and five-years, respectively. Models were adjusted for age and sex (Model 1), exposure and
132 outcome at baseline or one-year (where applicable), intervention group, occupational social
133 class, smoking status, alcohol intake, total energy intake and self-reported MVPA at each
134 baseline and follow-up (Model 2). We additionally adjusted for use of antihypertensive, lipid-
135 lowering and glucose-lowering medications at each baseline and follow-up in Model 2, as
136 appropriate. The same approach was used to examine the associations for variety of F&V
137 intake as well as for plasma vitamin C levels. Associations of quantity of intake were
138 adjusted for variety and *vice versa*.

139

140 We assessed for interaction of each exposure with sex in Model 2. To examine whether
141 associations were mediated by changes in waist circumference (when waist circumference
142 was not the outcome), we additionally adjusted for changes in waist circumference in Model
143 2. We also examined whether associations with CCMR were primarily driven by an
144 association with waist circumference by generating a CCMR score excluding waist
145 circumference (CCMR_{excluding•waist}) and with additional adjustment for waist circumference.

146

147 *Sensitivity analyses*

148 As the main analyses were limited to individuals who had data for all variables included in
149 the CCMR score, we also repeated all analyses for each of the cardiometabolic risk factors
150 independently by including the largest number of participants with data for that risk factor.
151 To explore the impact of missing covariate data on our results, we also used multiple
152 imputation by chained equations. For each exposure-outcome relationship, 10 imputed

153 datasets were created, and parameter estimates were combined using Rubin's rules. Each
154 imputation model included both the outcome of interest and all covariates in the analysis
155 models.

156

157 All statistical analyses were performed using Stata/SE 13.1 (Stata-Corp, College Station,
158 TX).

159

160

161 **Results**

162 In total, 603 individuals attended for all three clinic visits, of whom 401 had complete data
163 for F&V intake, cardiometabolic risk factor levels and covariates (Supplementary Figure 1).

164 177 individuals were in the intensive treatment trial group and 224 in the routine care trial
165 group. The mean age of the study participants was 61.4 (SD 6.6) years at baseline. Men
166 comprised 57% of the cohort (**Table 1**). Participants with missing follow-up data tended to
167 have a larger waist circumference and higher HbA_{1c} levels at baseline compared with those
168 with complete data. Those with missing data also reported consuming a lower quantity and
169 variety of F&V and had lower plasma vitamin C levels at baseline and at one- and five-years
170 of follow-up.

171

172 As shown in **Table 1**, intake of F&V increased in both men and women over the first year of
173 follow-up but decreased between one- and five-years. The most commonly eaten fruits were
174 apples, oranges and bananas and the most commonly eaten vegetables were carrots, peas,
175 tomatoes and green salad. Variety of F&V intake remained unchanged over one- and five-
176 years of follow-up. Plasma vitamin C levels increased across follow-ups. Quantity and
177 variety of F&V intake were moderately correlated at each time point ($r=0.54, 0.45$ and 0.40 at

178 baseline, one-year and five-years, respectively). There was a weak correlation between
179 quantity of vegetable intake and plasma vitamin C ($r=0.10-0.19$, at all time points) and a
180 slightly stronger correlation between fruit and combined fruit and vegetable intake and
181 plasma vitamin C ($r=0.24-0.30$, at all time points).

182

183 There was no suggestion of interaction by sex ($p>0.05$) so all results are presented for men
184 and women combined. Each SD change in quantity of F&V intake between baseline and one-
185 year (250g) was independently associated with a 0.92 (95% CI: 0.27, 1.57) cm lower waist
186 circumference, a 0.11 (0.03, 0.20) % lower HbA_{1c} level and a 0.04 (0.01, 0.08) lower CCMR
187 score at one-year (**Table 2**). Except for HDL-c, F&V intake was not associated with any
188 other CVD risk factor between one- and five-years. Change in the quantity of fruit intake (per
189 SD, 192g) was associated with a lower waist circumference (-0.89 (-1.56, -0.23) cm), HbA_{1c}
190 level (-0.12 (-0.20, -0.03) %) and CCMR (0.04 (0.01, 0.08)) at one-year as well as lower
191 triglyceride levels (-0.10 (-0.19, -0.01) mmol/l) and higher HDL-c (0.03 (0.01, 0.05) mmol/l)
192 at five-years (per SD, 197g). In contrast, change in quantity of vegetable intake (per SD,
193 151g) was associated with a 1.89 (0.29, 3.48) mmHg lower systolic blood pressure at one-
194 year. Increases in plasma vitamin C were not associated with any of the CVD risk factors or
195 with CCMR between baseline and one-year (Table 2). Between one- and five-years however,
196 each SD increase in plasma vitamin C (22.5 μ mol/l) was associated with lower triglyceride
197 levels (-0.11 (-0.21, -0.01), CCMR (-0.07 (-0.12, -0.03)) and with higher HDL-c levels (0.04
198 (0.01, 0.06) mmol/l).

199

200 Additional adjustment for waist circumference had no effect on the observed associations.
201 Associations between change in combined F&V intake and fruit intake separately with
202 CCMR_{excluding•waist} were not statistically significant between baseline and one-year but were

203 significant between one-year and five-years. The results were similar after adjustment for
204 waist circumference in the CCMR_{excluding•waist} model. The associations between change in
205 plasma vitamin C and CCMR_{excluding•waist} did not differ from the CCMR score including waist
206 circumference (data not shown).

207

208 As shown in **Table 3**, changes in variety of F&V intake combined and separately were not
209 associated with any of the individual cardiometabolic risk factors or CCMR at one- or five-
210 years.

211

212 Our findings remained unchanged when we included all participants who had complete data
213 for the cardiometabolic risk factor being analysed (data not shown) and when we performed
214 the analyses following multiple imputation of covariate data (data not shown).

215

216 **Discussion**

217 We demonstrate that while patients with screen-detected diabetes tend to increase the
218 quantity of F&V they consume in the first year following diagnosis of diabetes, this increase
219 is not the result of a change in the variety of F&V consumption, and is not maintained long-
220 term. Nevertheless, we show, for the first time, that even modest increases in F&V intake are
221 associated with clinically meaningful improvements in a number of important CVD risk
222 factor levels, namely waist circumference, HbA_{1c} and HDL-cholesterol. Increased vegetable
223 intake is associated with improved systolic blood pressure whereas increased fruit intake is
224 associated with improved triglyceride levels. These findings are corroborated by the inverse
225 association between change in plasma vitamin C and overall clustered cardiometabolic risk.

226

227 Although previous studies have examined the associations between quantity of F&V intake
228 and CVD¹⁸, few have done so in a population of people with diabetes with an extended
229 duration of follow-up^{19,20}, and none have examined the association using plasma vitamin C as
230 a biomarker of F&V intake. Among 10,000 individuals with diabetes who were followed-up
231 for nine years in the European Prospective Investigation into Cancer and Nutrition (EPIC)
232 study, each 80g increase in self-reported F&V intake (including legumes) was associated
233 with a hazard ratio (HR) of 0.88 (95% CI: 0.81, 0.95) for CVD mortality whereas each 80g
234 increase in fruit was associated with a HR of 0.90 (0.81, 0.91)¹⁹. In contrast, among 1,400
235 Japanese adults with diabetes who were followed-up for eight years, Tanaka and colleagues
236 did not find an association between self-reported F&V intake and incident coronary heart
237 disease (CHD), although a protective effect on incident stroke was reported²⁰. However,
238 because F&V intake was assessed only at baseline in both studies, the potential benefits of
239 increases in intake could not be examined.

240

241 To our knowledge, only two other studies have investigated associations between variety of
242 F&V intake and CVD risk, and while they were both in non-diabetes specific populations,
243 neither were able to find an association with incident CHD, despite sample sizes of 20,000
244 and 143,000 with follow-up durations of 10- and 20-years, respectively^{8,7}.

245

246 Consistent with our finding that increased fruit intake was associated with a smaller waist
247 circumference over one year, Bertoia and colleagues show that each increase in daily serving
248 of fruit and vegetable is associated with a 240g and 110g reduction in weight, respectively,
249 over four-years of follow-up²¹. To put only this finding into clinical context, if everybody
250 with newly developed diabetes were to increase their quantity of F&V intake by 250g per day

251 (1 SD in our study), they would experience an approximate reduction in waist circumference
252 of 1 cm – the benefit of which would be a 2% reduction in the risk of cardiovascular event²².

253

254 Although the associations between plasma vitamin C levels and cardiovascular risk factor
255 levels were not discrepant with those for quantity of F&V intake, there are several reasons
256 which might explain why the associations were weaker for plasma vitamin C when the
257 opposite might have been expected⁴. Firstly, because an increasing number of foods are
258 enriched with vitamin C, F&V can no longer be assumed to be the main source of intake of
259 this vitamin. Secondly, plasma levels of vitamin C plateau at the upper end of the normal
260 range²³, meaning that any additional increase in intake will not be correctly reflected in
261 plasma levels. Finally, a number of factors such as physical activity, BMI and the efficiency
262 with which the body metabolises vitamin C, which is partially genetically determined, have
263 all been associated with plasma vitamin C levels²⁴. Thus, to gain a better understanding of the
264 association between F&V intake and cardiovascular risk factor levels, future studies should
265 use a complementary approach in which several F&V biomarkers are used in combination, as
266 has been done previously²⁵.

267

268 Whilst the mechanisms by which F&V might improve cardiovascular risk factor levels are
269 not yet fully understood, there are several plausible hypotheses. F&V provide an abundant
270 source of vitamins, minerals and phytochemicals which could help reduce cardiovascular risk
271 factor levels, by acting both alone and in synergy, by counteracting the potentially harmful
272 effects of oxidative stress²⁶. A second explanation could be that that because F&V generally
273 have a low energy content any increase in intake could displace energy dense foods from the
274 diet²⁷, thereby aiding weight-loss.

275

276 *Strengths and Limitations*

277 Our study has a number of important strengths, including the population-based study design,
278 use of repeat measures of all exposures and outcomes over five-years of follow-up and
279 complementary analyses using plasma vitamin C as an objective biomarker of F&V intake.
280 While it is known that F&V intake reported using an FFQ generally leads to an over-
281 estimation of intake in comparison to a seven-day dietary recall questionnaire²⁸, an additional
282 major strength of our study is that we used the FFQ across time points to estimate change in
283 intake, for which it has been shown to be equally as valid as multiple 24-hour recalls²⁹.
284 However, as with any self-reported measure, FFQs may be vulnerable to recall and social
285 desirability biases. We were also able to adjust for a wide range of potential confounders,
286 reducing the likelihood that our findings are explained by confounding.

287

288 The limitations of our study also warrant discussion. Our analyses were limited to only 46%
289 of the original *ADDITION-Cambridge* study population due to missing data at one or more of
290 the follow-up clinic visits. Excluded participants reported having a lower intake of F&V, a
291 larger waist circumference and higher HbA_{1c} levels at baseline. However, when we performed
292 multiple imputation analyses, the results were similar suggesting bias due to missing data is
293 unlikely. In addition, due to the number of hypothesis tests conducted, statistically significant
294 results may have occurred due solely to the play of chance. As the majority of our population
295 is white and middle-aged, the generalisability of our findings to other ethnicities and age
296 groups requires caution. Furthermore, due to the lack of heterogeneity in variety of F&V
297 intake observed in our study cohort, we cannot rule out variety in intake as playing an
298 important role in CVD – we therefore suggest that this be studied in future cohorts.

299

300 **Conclusions**

301 Increased intake of F&V early in the course of diabetes is associated with improvements in a
302 number of important cardiovascular risk factors. It will be beneficial to investigate why the
303 early increases in F&V intake are not maintained in the longer term and future research
304 should focus on identifying strategies to help patients maintain improvements in diet.

305

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314

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332

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334 the analysis, interpreted the data, and wrote the manuscript; SJG: assisted in interpreting the
335 data and edited the manuscript; and SJS: provided statistical expertise and gave critical
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