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

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Abstract

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

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

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

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

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

Introduction

Type 2 diabetes is a leading cause of premature morbidity and mortality, much of which can be explained by the increased risk of cardiovascular disease (CVD). Previous studies have shown that adhering to specific dietary patterns such as the Dietary Approaches to Stop Hypertension diet (DASH-diet) or the Mediterranean Diet can lower the risk of developing CVD, even among those at initially high risk i.e. individuals with diabetes. A common theme underpinning these diets is an emphasis on consuming more fruits and vegetables (F&V). A large number of studies have demonstrated independent health benefits of a diet rich in F&V[1–3], findings which are supported by several studies which examined associations using plasma vitamin C as an objective biomarker of F&V intake[4,5]. More recently, variety of F&V intake, independent of quantity, has been considered in relation to risk of diabetes and CVD[6–8]. To our knowledge, no studies have examined the relationship between repeat measures of quantity and variety of F&V intake and CVD risk factors among individuals with diabetes over five-years of follow-up, corroborated using plasma vitamin C levels.

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

Methods

Study Design

The design and rationale for the ADDITION-Cambridge study have been reported in detail elsewhere[9]. In brief, individuals were recruited from 49 general practice (GP) clinics in the

East of England, UK, for a stepwise diabetes screening programme. Diabetes was diagnosed according to WHO criteria[10]. Eligible individuals were aged 40-69 years with no known diabetes and were within the top 25% of a diabetes risk score[9,11]. Exclusion criteria included being pregnant or lactating, having an illness with a prognosis of death within one year or a psychiatric illness which was likely to preclude involvement or informed consent. Of the 33,539 individuals who were invited to attend screening, 867 were identified to have diabetes and agreed to participate in the randomised control trial. The aim of the trial was to compare intensive treatment of multiple risk factors with routine care in individuals with screen-detected diabetes. Participants were cluster randomised by GP clinic. In the intensive treatment group practitioners were encouraged to follow a stepwise target-led treatment regimen to reduce and control CVD risk factors including blood glucose and lipid levels. This group additionally received theory-based health promotion materials including encouragement to consume at least five portions of F&V per day. The routine care group received care which followed UK national guidelines for diabetes management[12]. Participants attended for follow-up health assessments after one- and five-years. As there was no interaction by trial group, we pooled both trial groups and conducted a cohort analysis.

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

Fruit and vegetable intake and plasma vitamin C levels

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

frequency of food consumption on a nine-point scale ranging from "never or less than once per month" to "more than six times per day". Variety of F&V intake was derived by summing the total number of unique fruit and vegetable items consumed at least once per week. Possible variety ranged from 0 to 37 items. Quantity of F&V intake was derived by summing the total quantity (in grams/day) of different F&V consumed over the period of one week, divided by seven to quantify daily intake. We did not include potatoes in our analyses as they differ from vegetables in terms of energy and carbohydrate content and are commonly substituted for cereals rather than vegetables[15]. We also did not include fruit juice as it is not considered to be equivalent to whole fruit regarding fibre content and satiety value[16].

Measurement of cardiovascular risk factors

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

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

Covariates

Self-report questionnaires were used to obtain information on age, sex, occupation and ethnicity. Occupational social class was defined according to the Registrar General's occupation-based classification and comprised three categories: "professional, managerial and technical", "skilled – manual and non-manual" and "partly skilled or unskilled". Total energy and alcohol intake were assessed using an FFQ[14]. Time spent in moderate-to-vigorous physical activity (MVPA) was assessed by self-report using the previously validated European Prospective Investigation into Cancer-Norfolk physical activity questionnaire (EPAQ-2)[17]. Medication use was assessed using a self-report questionnaire adapted from the Aberdeen Health Service Research Unit questionnaire[9]. Very few people reported taking multivitamin supplements therefore this was not included in the analysis.

Statistical analysis

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

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

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

Sensitivity analyses

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

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

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

Results

In total, 603 individuals attended for all three clinic visits, of whom 401 had complete data for F&V intake, cardiometabolic risk factor levels and covariates (Supplementary Figure 1). 177 individuals were in the intensive treatment trial group and 224 in the routine care trial group. The mean age of the study participants was 61.4 (SD 6.6) years at baseline. Men comprised 57% of the cohort (**Table 1**). Participants with missing follow-up data tended to have a larger waist circumference and higher HbA_{1c} levels at baseline compared with those with complete data. Those with missing data also reported consuming a lower quantity and variety of F&V and had lower plasma vitamin C levels at baseline and at one- and five-years of follow-up.

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

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

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

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

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

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

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

Discussion

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

Although previous studies have examined the associations between quantity of F&V intake and CVD[18], few have done so in a population of people with diabetes with an extended duration of follow-up[19,20], and none have examined the association using plasma vitamin C as a biomarker of F&V intake. Among 10,000 individuals with diabetes who were followed-up for nine years in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, each 80g increase in self-reported F&V intake (including legumes) was associated with a hazard ratio (HR) of 0.88 (95% CI: 0.81, 0.95) for CVD mortality whereas each 80g increase in fruit was associated with a HR of 0.90 (0.81, 0.91)[19]. In contrast, among 1,400 Japanese adults with diabetes who were followed-up for eight years, Tanaka and colleagues did not find an association between self-reported F&V intake and incident coronary heart disease (CHD), although a protective effect on incident stroke was reported[20]. However, because F&V intake was assessed only at baseline in both studies, the potential benefits of increases in intake could not be examined.

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

Consistent with our finding that increased fruit intake was associated with a smaller waist circumference over one year, Bertoia and colleagues show that each increase in daily serving of fruit and vegetable is associated with a 240g and 110g reduction in weight, respectively, over four-years of follow-up[21]. To put only this finding into clinical context, if everybody with newly developed diabetes were to increase their quantity of F&V intake by 250g per day

(1 SD in our study), they would experience an approximate reduction in waist circumference of 1 cm – the benefit of which would be a 2% reduction in the risk of cardiovascular event[22].

Although the associations between plasma vitamin C levels and cardiovascular risk factor levels were not discrepant with those for quantity of F&V intake, there are several reasons which might explain why the associations were weaker for plasma vitamin C when the opposite might have been expected[4]. Firstly, because an increasing number of foods are enriched with vitamin C, F&V can no longer be assumed to be the main source of intake of this vitamin. Secondly, plasma levels of vitamin C plateau at the upper end of the normal range[23], meaning that any additional increase in intake will not be correctly reflected in plasma levels. Finally, a number of factors such as physical activity, BMI and the efficiency with which the body metabolises vitamin C, which is partially genetically determined, have all been associated with plasma vitamin C levels[24]. Thus, to gain a better understanding of the association between F&V intake and cardiovascular risk factor levels, future studies should use a complementary approach in which several F&V biomarkers are used in combination, as has been done previously[25].

Whilst the mechanisms by which F&V might improve cardiovascular risk factor levels are not yet fully understood, there are several plausible hypotheses. F&V provide an abundant source of vitamins, minerals and phytochemicals which could help reduce cardiovascular risk factor levels, by acting both alone and in synergy, by counteracting the potentially harmful effects of oxidative stress[26]. A second explanation could be that that because F&V generally have a low energy content any increase in intake could displace energy dense foods from the diet[27], thereby aiding weight-loss.

Strengths and Limitations

Our study has a number of important strengths, including the population-based study design, use of repeat measures of all exposures and outcomes over five-years of follow-up and complementary analyses using plasma vitamin C as an objective biomarker of F&V intake. While it is known that F&V intake reported using an FFQ generally leads to an over-estimation of intake in comparison to a seven-day dietary recall questionnaire[28], an additional major strength of our study is that we used the FFQ across time points to estimate change in intake, for which it has been shown to be equally as valid as multiple 24-hour recalls[29]. However, as with any self-reported measure, FFQs may be vulnerable to recall and social desirability biases. We were also able to adjust for a wide range of potential confounders, reducing the likelihood that our findings are explained by confounding.

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

Conclusions

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

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The authors' responsibilities were as follows—MJEL and AJMC: designed and conducted the analysis, interpreted the data, and wrote the manuscript; SJG: assisted in interpreting the data and edited the manuscript; and SJS: provided statistical expertise and gave critical comments on the manuscript. None of the authors had a conflict of interest.

References

1 Cooper AJ, Forouhi NG, Ye Z, Buijsse B, Arriola L, Balkau B *et al.* Fruit and vegetable intake and type 2 diabetes: EPIC-InterAct prospective study and meta-analysis. *Eur J Clin Nutr* 2012; **66**: 1082–92.

2 Oyebode O, Gordon-Dseagu V, Walker A, Mindell JS. Fruit and vegetable consumption and all-cause, cancer and CVD mortality: analysis of Health Survey for England data. *J Epidemiol Community Heal* 2014; **68**: 856–862.

3 Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W *et al.* Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* 2014; **349**: g4490–g4490.

4 Harding A-H, Wareham NJ, Bingham SA, Khaw K, Luben R, Welch A *et al.* Plasma vitamin C level, fruit and vegetable consumption, and the risk of new-onset type 2 diabetes mellitus: the European prospective investigation of cancer--Norfolk prospective study. *Arch Intern Med* 2008; **168**: 1493–9.

5 Pfister R, Sharp SJ, Luben R, Wareham NJ, Khaw K-T. Plasma vitamin C predicts incident heart failure in men and women in European Prospective Investigation into Cancer and Nutrition-Norfolk prospective study. *Am Heart J* 2011; **162**: 246–53.

6 Cooper AJ, Sharp SJ, Lentjes M a H, Luben RN, Khaw K-T, Wareham NJ *et al.* A prospective study of the association between quantity and variety of fruit and vegetable intake and incident type 2 diabetes. *Diabetes Care* 2012; **35**: 1293–300.

7 Oude Griep LM, Verschuren WMM, Kromhout D, Ocké MC, Geleijnse JM. Variety in fruit and vegetable consumption and 10-year incidence of CHD and stroke. *Public Health Nutr* 2012; **15**: 2280–6.

8 Bhupathiraju SN, Wedick NM, Pan A, Manson JE, Rexrode KM, Willett WC *et al.* Quantity and variety in fruit and vegetable intake and risk of coronary heart disease. *Am J Clin Nutr* 2013; **98**: 1514–23.

9 Echouffo-Tcheugui JB, Simmons RK, Williams KM, Barling RS, Prevost AT, Kinmonth AL *et al.* The ADDITION-Cambridge trial protocol: a cluster -- randomised controlled trial of screening for type 2 diabetes and intensive treatment for screen-detected patients. *BMC Public Health* 2009; **9**: 136.

10 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539–53.

11 Griffin SJ, Little PS, Hales CN, Kinmonth AL, Wareham NJ. Diabetes risk score: towards earlier detection of type 2 diabetes in general practice. *Diabetes Metab Res Rev* 2000; **16**: 164–171.

12 McIntosh A, Hutchinson A, Home P, Brown F, Bruce A, Damerell A *et al.* Clinical guidelines and evidence review for Type 2 diabetes: Blood glucose management. 2001; : Sheffield: ScHARR, University of Sheffield.

13 Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K. Which Plasma Antioxidants Are Most Related to Fruit and Vegetable Consumption? *Am J Epidemiol* 2001; **154**: 1113–1118.

14 Bingham S, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S *et al.* Validation of dietary

assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. *Int J Epidemiol* 1997; **26**: 137S–151.

15 Agudo A, Slimani N, Ocké MC, Naska A, Miller AB, Kroke A *et al.* Consumption of vegetables, fruit and other plant foods in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts from 10 European countries. *Public Health Nutr* 2002; **5**: 1179–96.

16 Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA *et al.* Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006; **114**: 82–96.

17 Wareham NJ, Jakes RW, Rennie KL, Mitchell J, Hennings S, Day NE. Validity and repeatability of the EPIC-Norfolk Physical Activity Questionnaire. *Int J Epidemiol* 2002; **31**: 168–74.

18 Gan Y, Tong X, Li L, Cao S, Yin X, Gao C *et al.* Consumption of fruit and vegetable and risk of coronary heart disease: a meta-analysis of prospective cohort studies. *Int J Cardiol* 2015; **183**: 129–37.

19 Nöthlings U, Schulze MB, Weikert C, Boeing H, van der Schouw YT, Bamia C *et al.* Intake of vegetables, legumes, and fruit, and risk for all-cause, cardiovascular, and cancer mortality in a European diabetic population. *J Nutr* 2008; **138**: 775–781.

20 Tanaka S, Yoshimura Y, Kamada C, Tanaka S, Horikawa C, Okumura R *et al.* Intakes of dietary fiber, vegetables, and fruits and incidence of cardiovascular disease in Japanese patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 3916–22.

21 Bertoia ML, Mukamal KJ, Cahill LE, Hou T, Ludwig DS, Mozaffarian D *et al.* Changes in Intake of Fruits and Vegetables and Weight Change in United States Men and Women Followed for Up to 24 Years: Analysis from Three Prospective Cohort Studies. *PLoS Med* 2015; **12**: e1001878.

22 de Koning L, Merchant AT, Pogue J, Anand SS. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *Eur Heart J* 2007; **28**: 850–6.

23 Baldrick FR, Woodside J V, Elborn JS, Young IS, McKinley MC. Biomarkers of fruit and vegetable intake in human intervention studies: a systematic review. *Crit Rev Food Sci Nutr* 2011; **51**: 795–815.

24 Johnston CS, Beezhold BL, Mostow B, Swan PD. Plasma vitamin C is inversely related to body mass index and waist circumference but not to plasma adiponectin in nonsmoking adults. *J Nutr* 2007; **137**: 1757–1762.

25 Cooper AJM, Sharp SJ, Luben RN, Khaw K-T, Wareham NJ, Forouhi NG. The association between a biomarker score for fruit and vegetable intake and incident type 2 diabetes: the EPIC-Norfolk study. *Eur J Clin Nutr* 2015; **69**: 449–54.

26 Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 2003; **78**: 517S–520S.

27 Rolls BJ, Ello-Martin JA, Tohill BC. What Can Intervention Studies Tell Us about the Relationship between Fruit and Vegetable Consumption and Weight Management? *Nutr Rev* 2004; **62**: 1–17.

28 Michels KB, Welch AA, Luben R, Bingham SA, Day NE. Measurement of fruit and vegetable consumption with diet questionnaires and implications for analyses and interpretation. *Am J Epidemiol* 2005; **161**: 987–94.

29 Kristal R, Kristal AR, Beresford SA, Lazovich D. Assessing change in diet-intervention research. *Am J Clin Nutr* 1994; **59**: 185S–189S.