



## Conference on 'Translating nutrition: integrating research, practice and policy' Nutrition Society Silver Medal Lecture

# Fruits and vegetables: measuring intake and encouraging increased consumption

Jayne V. Woodside\*, Ian S. Young and Michelle C. McKinley

Centre for Public Health, Queen's University Belfast, Grosvenor Road, Belfast BT12 6BJ, UK

A high intake of fruit and vegetables (FV) is associated with reduced risk of chronic disease, although the evidence base is mostly observational. Blood biomarkers offer an objective indicator of FV intake, potentially improving estimates of intakes based on traditional methods. A valid biomarker of overall FV intake would be able to confirm population intakes, more precisely evaluate the association between intakes and health outcomes and confirm compliance in FV interventions. Several substances have been proposed as biomarkers of FV intake: vitamin C, the carotenoids and polyphenols. Certain biomarkers are strong predictors of single FV; however, the proposed single biomarkers of FV consumption are only modestly predictive of overall FV consumption. This is likely to be due to the complexity of the FV food group. While accurately measuring FV intake is important in nutrition research, another critical question is: how best can an increase in FV intake be achieved? Increased FV intake has been achieved in efficacy studies using intensive dietary advice. Alternative, less intensive methods for encouraging FV consumption need to be developed and tested for population level intervention. Systematic reviews suggest peer support to be an effective strategy to promote dietary change. This review will describe the evidence for a link between increased FV intake and good health, outline possible novel biomarkers of FV consumption, present the most recently available data on population intake of FV and examine the usefulness of different approaches to encourage increased consumption of FV.

### Fruit: Vegetables: Biomarkers: Dietary change

#### Fruit and vegetables and disease risk

Diets rich in fruit and vegetables (FV) are associated with a reduced risk of chronic disease<sup>(1)</sup>, and therefore increased consumption of FV is recommended as part of a healthy diet<sup>(2,3)</sup>. In this review, the evidence supporting the association of FV with chronic disease risk will be discussed alongside approaches to measure FV intake and strategies to encourage increased FV consumption at the population level.

#### *Fruit and vegetables and CVD risk*

Meta-analyses of observational studies have consistently shown an association between increased FV intake and reduced risk of CHD<sup>(4,5)</sup> and stroke<sup>(6,7)</sup>, with some evidence of a dose–response effect<sup>(4,6)</sup>, although there was

considerable between-study heterogeneity<sup>(8)</sup>. Individual prospective cohort studies published since these meta-analyses have tended to confirm this association<sup>(9–15)</sup>, although these studies are heterogeneous in terms of dietary assessment and statistical analysis methodology. There has been some inconsistency regarding the questions of whether it is total FV intake that is associated with reduced CVD risk, or fruit alone, vegetables alone, or specific classes of FV<sup>(13)</sup> and whether the association is demonstrated in both sexes<sup>(9)</sup> or is stronger in a particular subset of the population studied, e.g. smokers<sup>(14)</sup>.

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study investigators published the largest prospective analysis of FV intake and CVD risk to date<sup>(16)</sup>. A total of 313 074 men and women without a

**Abbreviations:** EPIC, European Prospective Investigation into Cancer and Nutrition; FV, fruits and vegetables.

**\*Corresponding author:** Dr Jayne V. Woodside, fax +44 2890 235900, email j.woodside@qub.ac.uk



previous history of myocardial infarction or stroke from eight European countries were followed for an average of 8.4 years. Participants consuming at least eight portions of FV daily had a 22% lower risk of fatal IHD than those consuming less than three portions per d (relative risk 0.78, 95% CI 0.56, 0.95). The authors were able to calibrate the FV intake estimates to account for the different dietary assessment techniques used in participating centres and, after this adjustment, also found that a one portion increment in FV was associated with a 4% lower risk of fatal IHD (95% CI 0.92, 1.00;  $P = 0.033$  for trend). Such an observation is in agreement with the previous meta-analysis that demonstrated a similar estimate of difference in risk per portion of daily FV intake increase<sup>(5)</sup>.

#### *Fruit and vegetables and cancer risk*

The World Cancer Research Fund report<sup>(17)</sup> rated the evidence that increased FV intake was associated with reduced cancer risk as being either 'probable' or 'limited-suggestive', depending on the cancer site.

Several further analyses of prospective epidemiological studies have been published since the World Cancer Research Fund report, including an analysis of the EPIC cohort by Boffetta *et al.*<sup>(18)</sup>, which included almost 400 000 individuals, who developed approximately 30 000 cancers at all sites over nearly 9 years of follow-up. Only a weak, although statistically significant, inverse association was seen, with a 4% reduction in risk of cancer for every 200 g increase in FV (hazard ratio 0.96; 95% CI 0.94, 0.98). Such a weak association makes it difficult to exclude the possibility that the association has occurred because of residual confounding, although the authors and an accompanying editorial<sup>(19)</sup> note that there is still the possibility that one type of FV, or a group of FV, or a specific compound within a certain FV could be associated with reduced risk or that FV, in general, could be protective at certain cancer sites.

A more recent review carried out by Key<sup>(20)</sup> summarised the evidence for a potential link between various main cancer sites, including stomach, colorectal, oesophageal, oral cavity and pharynx, lung, breast, prostate and FV intake, while the overall cancer risk was also examined. Including the EPIC analysis described earlier, Key also concludes that the evidence is relatively weak, but suggests that those eating very low amounts of FV may still benefit from increasing intake, while, as earlier, Key also suggests that particular FV or certain nutrients/compounds could still have beneficial effects.

#### *Fruit and vegetables and diabetes*

A recent systematic review and meta-analysis was carried out by Carter *et al.*<sup>(21)</sup>, examining the relationship between FV intake and type 2 diabetes incidence. Summary estimates illustrated that comparing the highest intake of green leafy vegetables (1.35 portions per d) with the lowest intake (0.2 portions per d) was associated with a statistically significant 14% reduction in the risk of type 2 diabetes (hazard ratio 0.86; 95% CI 0.77, 0.97;  $P = 0.01$ ). No significant associations were found between fruit

only (hazard ratio 0.93; 95% CI 0.83, 1.01), vegetables only (hazard ratio 0.91; 95% CI 0.76, 1.09) or FV combined (hazard ratio 1.00; 95% CI 0.92, 1.09) and incidence of diabetes.

Cooper *et al.*<sup>(22)</sup> have published a similar meta-analysis to Carter *et al.*<sup>(21)</sup> but included their own data from a large case-cohort study, the EPIC-InterAct study. They found similar associations as Carter<sup>(21)</sup>, in relation to green leafy vegetables (relative risk 0.84; 95% CI 0.74, 0.94), and also observed a weak association between total FV intake and diabetes risk when comparing the lowest and highest quartiles of FV intake (relative risk 0.93; 95% CI 0.87, 1.00). Furthermore, the EPIC-InterAct data indicated an inverse association between root vegetables and diabetes risk (relative risk 0.87; 95% CI 0.77, 0.99); however, this was not evident in two other studies that examined this vegetable subgroup<sup>(23,24)</sup>.

Therefore, the results of both meta-analyses suggest that an increased consumption of green leafy vegetables may reduce the incidence of diabetes, with no association or weak associations demonstrated for total FV intake; however, the former observation regarding green leafy vegetables is, at present, based on a limited number of studies. Other FV groups, such as root vegetables, may also play a role, but this requires further study. FV consumption separately showed no association with the risk of diabetes.

#### *Fruit and vegetables and cognitive decline/risk of Alzheimer's disease*

As the populations of developed countries age, maintaining cognitive function will be of increasing importance so that the ageing population maintain independent living for as long as possible. Regular consumption of FV has for long been considered to be associated with a reduced risk of cognitive decline and Alzheimer's disease, but the evidence has only recently been systematically reviewed. Loeff and Walach<sup>(25)</sup> found that nine prospective studies with a follow-up period longer than 6 months, incorporating just over 44 000 participants, examined FV intake and either cognitive decline or Alzheimer's disease endpoints. Out of the six studies examining FV separately, five found that a higher consumption of vegetables, but not fruit, was associated with reduced risk of dementia or cognitive decline. A similar association was found for the three further studies that examined FV intake combined.

#### *Fruit and vegetable intervention studies*

Studies examining health benefits of FV have mostly been observational to date, and therefore only associations, rather than cause and effect, can be demonstrated. Lifestyle behaviours tend to cluster, meaning that a higher intake of FV is also associated with a better overall diet, with increased levels of physical activity, with not smoking and with being of a higher social class. Statistical analysis can attempt to control for all of these other lifestyle behaviours, yet it is likely that this is not completely successful, and therefore that residual confounding will account for at least part of the demonstrated associations in prospective cohort studies.

FV intervention studies conducted to date, which allow the demonstration of cause and effect, have been limited mostly to studies examining cardiovascular risk factors, although many studies with hard clinical endpoints, where increased FV intake has been combined with other dietary and lifestyle changes, have also been conducted<sup>(26–31)</sup>. For example, the Dietary Approaches to Stop Hypertension study demonstrated a beneficial effect of increased FV intake on blood pressure over 8 weeks, with systolic and diastolic blood pressure decreasing by 2.8 and 1.1 mmHg, respectively<sup>(32)</sup>. Participants with hypertension had greater reductions in blood pressure than those without hypertension. A similar blood pressure lowering effect was demonstrated by John *et al.*<sup>(33)</sup> in a study of brief negotiation to encourage FV intake in the primary care setting, while a beneficial effect of increased FV consumption on microvascular function has recently been demonstrated<sup>(34)</sup>. Evidence for the effects of increased FV intake on other cardiovascular risk factors, including hypercholesterolaemia and obesity, is limited at present, and further appropriately designed studies are required to confirm or refute the few studies that have been performed<sup>(35–39)</sup>. A beneficial effect of increased FV intake on inflammatory markers has been demonstrated in some<sup>(40)</sup>, but not all<sup>(34)</sup> studies. In terms of intervention studies with endpoints related to other disease states, no effect of increased FV consumption has been demonstrated on oxidative stress and inflammation in chronic obstructive pulmonary disease<sup>(41)</sup>, while an effect of increased FV intake on response to vaccination in older people has recently been shown<sup>(42)</sup>.

*If fruit and vegetables have beneficial effects, what is causing those beneficial effects?*

If it is assumed that FV are beneficial to health and that accumulating evidence from intervention studies demonstrate this and confirm the already-existing data from observational epidemiological studies, then it will be of scientific interest to know whether or how specific components of FV influence these health-promoting effects and also to investigate ways in which FV might indirectly benefit health, for example, by displacing less desirable food components from the diet and thus improving the overall dietary profile. Research has tended to focus on the health benefits of the micronutrients and polyphenols present in FV; however, FV also have a high water content, low energy content (and therefore low energy density) and high fibre content; it is likely, therefore, that increasing FV intake results in a number of changes to the overall diet profile. In a systematic review and meta-analysis of FV intervention studies, we have recently assessed whether increasing FV consumption has an impact on diet profile. In the systematic review, all twelve studies demonstrated increases in micronutrient intakes, while a meta-analysis showed no significant difference between the intervention and control groups in energy (kJ) in seven studies (mean difference = 4.184 kJ (1 kcal; 95% CI –115, 117);  $P = 0.98$ ), significant decreases in total fat (% energy) in five studies (mean difference = –4%; 95% CI –5, –3;  $P < 0.00001$ ) and significant increases in fibre in six studies (mean difference 5.36 g; 95% CI 4, 7;  $P < 0.00001$ ) and

total carbohydrate (% energy) in four studies (mean 4%; 95% CI 2, 5;  $P < 0.00001$ ). Results indicate that increased FV consumption increases micronutrient, carbohydrate and fibre intakes, and possibly reduces fat intake, with no overall effect on energy intake. Therefore, health benefits of FV are likely to act through an improvement in overall diet profile alongside direct effects of increased intakes of micronutrients and other phytochemicals<sup>(43)</sup>.

*Fruit and vegetables quantity v. variety*

The focus to date has been on the quantity of FV consumed, with most countries setting a five portions per d target<sup>(3)</sup>. However, attention in the research setting and within dietary guidelines has focused more recently on the concept of food synergy and dietary variety<sup>(44)</sup>, with both American and UK dietary guidelines advocating FV variety<sup>(2,45,46)</sup>. In fact, little is known about how a focus on variety rather than quantity is associated with FV health benefits. Bhupathiraju and Tucker<sup>(47)</sup> showed that increased FV variety, but not quantity, was associated with reduced C-reactive protein concentrations in fully adjusted models in a cross-sectional study of about 1200 Puerto Rican adults. Oude Griep *et al.*<sup>(48)</sup> found quantity and variety to be highly correlated ( $r 0.81$ ), and reported that greater variety was associated with higher intakes of micronutrients, particularly vitamin C, but did not demonstrate an association of fruit or vegetable variety with CHD or stroke in a prospective study of 20 000 men and women in the Netherlands. In the only study to examine variety of FV intake and diabetes risk, the EPIC-Norfolk investigators showed an association between increased vegetable, but not fruit, intake and type 2 diabetes risk, but, in terms of variety, greater variety of fruit, vegetables and combined FV was significantly associated with reduced risk of type 2 diabetes<sup>(49)</sup>. In a similar analysis of the full EPIC cohort, variety was associated with risk of oesophageal squamous cell carcinoma, but not gastric or oesophageal adenocarcinoma<sup>(50)</sup>. These limited studies do not allow firm conclusions to be drawn. Further studies are required to demonstrate whether an increased emphasis on FV variety is likely to enhance disease prevention efforts.

*Methods of classifying fruit and vegetables: colour and the preparation method*

While most studies have examined total FV intake in portions per d, FV can be further classified in a number of ways<sup>(51)</sup>, and more recent studies have attempted to classify FV, either by colour or by whether FV was consumed in the raw or processed state. Oude Griep *et al.*<sup>(52)</sup> showed an association between white FV (mostly apples and pears) and stroke risk, and an association between deep orange FV (mostly carrots) and risk of CHD<sup>(53)</sup>. For every 25 g increase in white FV, stroke risk was reduced by 9% (hazard ratio 0.91; 95% CI 0.85, 0.97)<sup>(52)</sup>, while for every 25 g increase in deep orange FV, CHD risk was reduced by 26% (95% CI 0.55, 1.00), although a borderline significant result was also demonstrated for total FV intake (hazard ratio 0.98, 95% CI 0.97, 1.01)<sup>(53)</sup>. The same investigators also examined their data by dividing FV into



whether it was consumed raw or had been processed in some way, including cooking<sup>(54,55)</sup>. Processed FV were not related to stroke incidence, but total stroke incidence tended to be 30% lower for participants with a high intake of raw FV (upper v. lowest quartile; hazard ratio 0.70; 95% CI 0.47, 1.03;  $P = 0.07$  for trend)<sup>(54)</sup>. Raw vegetable intake was significantly associated with ischaemic stroke, while raw fruit intake was borderline significantly associated with haemorrhagic stroke<sup>(54)</sup>. In a similar analysis of CHD endpoints, both raw and processed FV intake were significantly associated with reduced CHD risk (highest v. lowest quartile; raw hazard ratio 0.70; 95% CI 0.47, 1.04; processed hazard ratio 0.79; 95% CI 0.54, 1.16)<sup>(55)</sup>. These studies provide some evidence of a differential association of FV with CVD risk, both by the method of classification of FV, and depending on CVD sub-type.

### Measuring fruit and vegetable intake

Methods for measuring FV consumption through traditional dietary assessment methods, such as food diaries, FFQ or 24-h recalls, can be flawed<sup>(56–58)</sup>. For example, it is well recognised that self-reported intake can often be inaccurate<sup>(59)</sup> due, for example, to reliance on a participant's memory, the inability of some methods to account for day-to-day variation in intake, or the fact that respondents tend to change their usual eating patterns in order to simplify record keeping and/or to impress the investigator<sup>(60,61)</sup>. These problems can be further compounded by coding and data entry errors<sup>(60,62)</sup>. Different dietary intake methodologies also tend to agree poorly with each other<sup>(63)</sup>, and this has also been shown to lead to variation in the demonstrated associations with disease risk<sup>(64)</sup>.

Given the difficulties associated with the dietary intake methodology outlined earlier, more objective indices of FV intake, such as nutritional biomarkers in biological samples, are therefore of interest. Accurate measurement of the concentrations of compounds found in FV in biological samples would allow the confirmation of low intakes of FV in populations, the evaluation of the association between intake and disease risk, and could also be used as biomarkers of compliance in FV intervention studies. Such biomarkers of intake need to be able to discriminate between differences in intakes<sup>(65,66)</sup>, should be non-invasive or minimally invasive<sup>(65,67)</sup>, reproducible, easily measured<sup>(68)</sup> and highly responsive to the intervention being carried out<sup>(65)</sup>. Plasma, serum and urinary biomarkers have been explored as potentially useful indicators of FV intake. For instance, FV are the primary source of carotenoids in the diet and, as carotenoids cannot be synthesised by human subjects, they are considered to be good candidate biomarkers of intake<sup>(69)</sup>. Six carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin, lycopene and lutein) are found in appreciable amounts in human serum<sup>(65,70)</sup>. Other candidate biomarkers of FV intake include antioxidant vitamins such as vitamin C<sup>(71,72)</sup> and flavonoids<sup>(73,74)</sup>, including quercetin<sup>(75)</sup>. Some of these compounds have been reliably associated with a particular fruit or vegetable, or a class of FV in observational studies, but less successfully with total FV consumption<sup>(69,70,72,76–80)</sup>. This is almost certainly due to the complexity of FV

and the large number of bioactive compounds present, but also potentially because of other dietary sources of these compounds.

We have recently conducted a systematic review to examine the usefulness of the main biomarkers of FV intake to act as objective indicators of compliance in dietary intervention studies<sup>(81)</sup>. A comprehensive search of the literature was conducted using six databases, and suitable papers were selected and relevant data extracted ( $n = 96$ ). The papers were categorised into three sub-groups: whole diet interventions; mixed FV interventions; and studies involving individual fruits or vegetables. Overall, the most commonly measured, and most consistently responsive, biomarkers were the carotenoids and vitamin C. Single biomarkers were good predictors of single classes of FV, e.g. quercetin was a good biomarker of onion consumption, and lycopene was a good biomarker of tomato consumption. However, single biomarkers cannot accurately and reliably predict total FV consumption. It therefore remains prudent to measure a panel of biomarkers in FV trials (notably  $\alpha$ - and  $\beta$ -carotene, lutein, zeaxanthin,  $\beta$ -cryptoxanthin, lycopene and vitamin C).

It is likely that further research will develop some more novel biomarker approaches to assess FV intake. The association between dietary intake and biomarker status may depend on certain population characteristics, for example BMI<sup>(82)</sup>. It must also be remembered that biomarkers will never solely reflect dietary intake of FV, as status will also reflect a number of physiological processes, providing a measure of nutrient availability at the tissue level, and therefore there may always be a role for the more traditional dietary assessment methodologies, perhaps in partnership with biomarker assessment<sup>(83)</sup>. Recently, for carotenoids and potentially a broader range of markers, combining biomarkers and self-reported measures using regression calibration equations has been suggested to allow more powerful tests of association with disease risk<sup>(84,85)</sup>. Urinary pH has been suggested as an indicator of FV intake<sup>(86)</sup>, while urinary K can act as a biomarker, both of FV intake<sup>(87)</sup> and diet quality in general<sup>(88)</sup>. The use of Resonance Raman Spectroscopy has also been suggested as a feasible method to measure dermal carotenoids<sup>(89)</sup>.

A number of studies have suggested that summing individual flavonoids (sum of quercetin, kaempferol, isorhamnetin, tamarixetin, naringenin, hesperetin and phloracetin) excreted in 24 h urine samples correlates better with FV intake than individual flavonoid excretion<sup>(90,91)</sup>. Mennen *et al.*<sup>(73)</sup> found that the same sum correlated with fruit and fruit juice intake, but not total FV intake, but these authors suggested that adding more polyphenols from fruits to the panel might have further improved the accuracy of such a biomarker to take into account the diversity of the fruit sources. It may therefore be possible to combine one or more potential biomarkers of FV intake (including vitamin C and carotenoids) and better predict intake with a combined biomarker approach rather than using single markers or a panel of single markers.

Such a combined biomarker approach outlined earlier automatically leads to the consideration of a much broader range of biomarkers to monitor FV intake, for example,

using a metabolite fingerprinting approach. Although there are considerable methodological challenges<sup>(92)</sup>, a number of studies have suggested possible biomarkers of individual FV or classes of FV: proline betaine for citrus fruit<sup>(93,94)</sup>, novel ascorbate derivatives for broccoli or raspberries<sup>(95)</sup>, sulfonated caffeic acid and sulfonated methyl-epicatechins after raspberry consumption<sup>(95)</sup> and phenyl-acetylglutamine for vegetable intakes<sup>(96)</sup>.

### Do we need to increase fruit and vegetable consumption? Current population intakes

Before considering how to most effectively increase FV consumption in the general population, it must first be demonstrated that such an increase is necessary. In the National Diet and Nutrition Survey in adults aged 19–64 years in 2004, the mean daily FV intake was 2.7 portions per d for men and 2.9 portions per d for women<sup>(97)</sup>. In the new National Diet and Nutrition Survey adult survey for years 1, 2 and 3 combined (2008–9 to 2010–11), adults (19–64 years) consumed on average 4.1 portions per d and older adults (>65 years) consumed 4.4 portions per d<sup>(98)</sup>. A total of 31% of adults and 37% of older adults met the ‘5-a-day’ recommendation<sup>(98)</sup>. The methodologies differed between these two surveys (moving from a 7 d weighed food diary in 2004 to a 4 d estimated diary and with composite foods now being disaggregated), and therefore direct comparisons between the surveys and conclusions about changes over time are not possible, but it can be concluded with some certainty that the majority of the adult population is still not meeting the 5-a-day recommendation. Similar data exist from Northern Ireland (which was not included in the original National Diet and Nutrition Survey, but is now part of the rolling programme), where in a representative sample of 426 adults >65 years old, the mean intake, measured using a telephone-based recall method, was about four portions per d<sup>(99)</sup>.

The new National Diet and Nutrition Survey results from the rolling programme are not yet comprehensive enough, with large enough sample sizes, to draw conclusions on the geographical variation in FV intake and how this might impact on disease risk. However, macrosimulation modelling carried out by researchers in Oxford, using data from the Family Food Survey, has suggested that if the average diet in Wales, Scotland and Northern Ireland was improved to that achieved in England, then the mortality gap in these countries could be considerably reduced (reduction in Wales 81% (95% credible interval 62%, 108%); Scotland 40% (33%, 51%); Northern Ireland 81% (67%, 99%)), suggesting considerable variation in diet quality between countries in the UK<sup>(100)</sup>. The same research group has used similar modelling techniques to focus on FV intake and concluded that 33 000 deaths per year could be avoided if UK dietary recommendations were met, with more than 15 000 of these avoided deaths being due to increased FV intake<sup>(101)</sup>.

Lock *et al.*<sup>(102)</sup> have similarly attempted to estimate the global burden of disease attributable to low consumption of FV. The associations, stratified by fourteen geographical regions, sex and age, were estimated using information on

FV consumption in the population and six health outcomes (IHD, stroke, stomach, oesophageal, colorectal and lung cancer). The analysis suggested that 2.6 million deaths worldwide and 31% of CVD (and between 2 and 19% of cancers) may be attributed to inadequate consumption of FV. About 1.8% of the total burden of disease worldwide was estimated to be attributable to inadequate FV consumption, compared with 1.3% for physical activity, 2.3% for overweight and obesity, 2.8% for high cholesterol and 4.1% for tobacco<sup>(102)</sup>.

A more recent study by Hall *et al.*<sup>(103)</sup> confirmed a low intake of FV worldwide. The authors found that, of 196 373 respondents who resided in fifty-two countries of mostly low and middle income, approximately 78% of both males and females consumed less than five portions of FV daily. FV intakes were assessed by responses to survey questions asked as part of the World Health Survey<sup>(103)</sup>.

### Encouraging increased fruit and vegetable consumption

Evidence available to date indicates that FV are, overall, beneficial to health and that current intakes in Western populations remain low. Public health specialists are, therefore, faced with the challenge of finding methods for encouraging more people to eat more FV and for sustaining this behaviour in the long term. It is generally agreed that interventions to promote increased FV intake should have a theoretical basis<sup>(104)</sup> and frameworks exist to guide the development of such interventions<sup>(105)</sup>. Common behaviour theories and constructs used to effect changes in FV intake have recently been summarised<sup>(106)</sup>.

Two systematic reviews have examined interventions to increase FV intake in adults<sup>(106,107)</sup>, with a further two carrying out a similar analysis in children (one focused on interventions within primary schools)<sup>(108,109)</sup>.

Pomerleau *et al.*<sup>(107)</sup> published their systematic review in 2005. They included a total of forty-four studies (only excluding acutely ill adults), and showed that small increases in FV intake were possible in healthy adults (of the order of 0.1–1.4 servings per d), and that this could be achieved by a variety of approaches. Positive effects were seen consistently in studies which used face-to-face education or counselling strategies, although interventions delivering information by telephone or via computer were also effective. These successful modes of intervention could collectively be described as peer support, which encompasses a variety of methods including face-to-face or group self-management programmes, peer coaching, the use of community health workers, telephone-based peer support and web- and email-based peer support. In addition, community-based multi-component interventions also produced increases in FV intake. In general, studies were able to demonstrate larger increases in those with pre-existing health disorders.

In a more recent review of behavioural interventions to promote intake of FV in both adults and children, Thomson and Ravia<sup>(106)</sup> only included US-based studies, which resulted in the inclusion of only thirty-four studies. A total of thirty-eight non-US studies were excluded, as were studies that included those with pre-existing disease (*n* 40).



A final condition for inclusion was those studies which specifically reported the theory basis or constructs used when the intervention was designed. Such strict inclusion and exclusion criteria will have resulted in a different profile of studies included compared with the earlier review, but a similar result was demonstrated. An increase in FV intake (+1.13 servings per d) was shown for adults, and +0.39 servings per d for children, and in most studies these increases were statistically significant. However, as the authors note, in most cases these observed increases would not have been big enough to allow the populations included to achieve the recommended intake levels. The studies included in the review employed most commonly the transtheoretical model (with stages of change construct) and social cognitive theory as a basis for the interventions.

The systematic reviews of interventions to promote increased FV intake in adults made a number of recommendations for further research. Pomerleau *et al.*<sup>(107)</sup> considered that the effectiveness of all new interventions had to be tested, particularly in developing countries, where several new programmes had been developed, but without formal evaluation. Any new intervention shown to be effective in a particular setting would have to be retested in a new setting, as factors particular to the local setting may affect the effectiveness of the intervention. The authors suggested that the reports should provide a better description of the methods used in order to allow exact repetition of the interventions, and the reports should include estimates of variability for the selected outcomes in order to allow power calculations. The specific components of intervention should be examined in more depth and how these individual effects differ in different populations. Pomerleau *et al.*<sup>(107)</sup> also considered that there are further gaps in the literature, suggesting a need to better understand factors influencing FV consumption, including economic, social and environmental factors that affect both food availability and the ability of an individual to make healthy choices, and the barriers to making changes towards increased FV intake.

The recent systematic review by Thomson and Ravia<sup>(106)</sup> was rigorous in terms of selecting only theory-based trials in healthy volunteers, and it applied a number of other exclusions when selecting studies. However, within the studies included, the authors still considered that there are a number of design limitations. Self-reported dietary instruments tended to be used, rather than objective biomarkers; cost, which is a potentially important barrier to increased consumption, was frequently not assessed; and there was a lack of clear linkage between the behaviour theories and constructs and the intervention developed. The authors recommend combining behavioural interventions with social marketing, behavioural economics approaches and technology-based behaviour change models in future studies, and always including both process and outcome evaluation.

Two further systematic reviews have focused on increasing FV intake in children. In 2006, Knai *et al.*<sup>(108)</sup> were able to find fifteen studies that met their inclusion criteria, and ten of these demonstrated significant effects on intake, from +0.3 to +0.99 servings per d. All but one

of the fifteen studies were school-based. Multi-component interventions seemed to be the most effective, and the following seemed to be important factors for a successful intervention: an intervention duration of at least 6 months, the complete involvement of the whole school community, for teacher training and curriculum integration to be included, the interventions to include peer leadership and encouragement and involve canteen staff, and finally, to include parents, both at home and in the school.

A second systematic review focusing on children, but including only primary school interventions, was published in 2011<sup>(109)</sup>. Nineteen strong and moderate quality studies were included, with a further eight being excluded as being of low quality. Studies were categorised as being game- or computer-based, multi-component, free or subsidised programme, or other studies, and meta-analyses were then conducted. The 'other studies' classification included only two studies that could not be meta-analysed; although both of these produced significant increases in FV intake, the free/subsidised category showed no effect of these types of interventions, while computer-based interventions seemed to be effective (standardised mean difference 0.33; 95% CI 0.16, 0.50). Finally, there were eleven multi-component intervention studies, but only seven of these were randomised controlled trials and could be combined in a meta-analysis. Six of the seven individual studies produced an increased intake of FV, but the combined analysis showed no effect of these interventions on intake (standardised mean difference 0.08; 95% CI -0.00, 0.17), although this combined effect did approach significance ( $P = 0.06$ ). The authors also made suggestions to improve the design of future studies, including larger samples, longer follow-up and clarification of the method of randomisation, while further studies in developing countries are also required.

## Conclusion

In conclusion, the majority of observational studies have shown an association between increased FV intake and a reduced risk of CVD. The evidence, to date, is weaker for cancer than for CVD, and prospective studies indicate that certain sub-groups of FV intake may be associated with reduced risk of diabetes. A high intake of FV may also be associated with a slower rate of cognitive decline and risk of mild cognitive impairment and Alzheimer's disease. The randomised controlled trial evidence elucidating the precise health benefits of increasing FV intake is currently limited but is developing. Alongside the assessment of FV intake, assessment of a panel of blood biomarkers, including vitamin C and the carotenoids can provide a useful and more objective indicator of FV intakes. Despite public health recommendations to increase FV intake, intakes of FV worldwide are lower than recommended levels. Achieving a sustained increase in FV intake is challenging and will require the development and testing of further intervention approaches.

## Acknowledgements

The authors declare no conflicts of interest. This research received no specific grant from any funding agency in the

public, commercial or not-for-profit sectors. J. V. W. drafted the manuscript. M. C. M. and I. S. Y. critically reviewed the manuscript and suggested appropriate revisions.

### References

- Boeing H, Bechthold A, Bub A *et al.* (2012) Critical review: vegetables and fruit in the prevention of chronic diseases. *Eur J Nutr* **51**, 637–663.
- Department of Health (2011) <http://www.nhs.uk/LiveWell/5ADAY/Pages/5ADAYhome.aspx> (accessed November 2012).
- World Health Organisation. (2003) [http://www.who.int/dietphysicalactivity/publications/f&v\\_promotion\\_initiative\\_report.pdf](http://www.who.int/dietphysicalactivity/publications/f&v_promotion_initiative_report.pdf) (accessed November 2012).
- He FJ, Nowson CA, Lucas M *et al.* (2007) Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. *J Hum Hypertens* **21**, 717–728.
- Dauchet L, Amouyel P, Hercberg S *et al.* (2006) Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *J Nutr* **136**, 2588–2593.
- He FJ, Nowson CA & MacGregor GA (2006) Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet* **367**, 320–326.
- Dauchet L, Amouyel P & Dallongeville J (2005) Fruit and vegetable consumption and risk of stroke: a meta-analysis of cohort studies. *Neurology* **65**, 1193–1197.
- Dauchet L, Amouyel P & Dallongeville J (2009) Fruits, vegetables and coronary heart disease. *Nat Rev Cardiol* **6**, 599–608.
- Nakamura K, Nagata C, Oba S *et al.* (2008) Fruit and vegetable intake and mortality from cardiovascular disease are inversely associated in Japanese women but not in men. *J Nutr* **138**, 1129–1134.
- Takachi R, Inoue M, Ishihara J *et al.* (2008) Fruit and vegetable intake and risk of total cancer and cardiovascular disease: Japan Public Health Center-Based Prospective Study. *Am J Epidemiol* **167**, 59–70.
- Nagura J, Iso H, Watanabe Y *et al.* (2009) Fruit, vegetable and bean intake and mortality from cardiovascular disease among Japanese men and women: the JACC Study. *Br J Nutr* **102**, 285–292.
- Holmberg S, Thelin A & Stiernström EL (2009) Food choices and coronary heart disease: a population based cohort study of rural Swedish men with 12 years of follow-up. *Int J Environ Res Public Health* **6**, 2626–2638.
- Bendinelli B, Masala G, Saieva C *et al.* (2011) Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study. *Am J Clin Nutr* **93**, 275–283.
- Dauchet L, Montaye M, Ruidavets JB *et al.* (2010) Association between the frequency of fruit and vegetable consumption and cardiovascular disease in male smokers and non-smokers. *Eur J Clin Nutr* **64**, 578–586.
- Oude Griep LM, Geleijnse JM *et al.* (2010) Raw and processed fruit and vegetable consumption and 10-year coronary heart disease incidence in a population-based cohort study in the Netherlands. *PLoS One* **5**, e13609.
- Crowe FL, Roddam AW, Key TJ *et al.* (2011) Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study. *Eur Heart J* **32**, 1235–1243.
- World Cancer Research Fund. (2007) Diet and Cancer Report. <http://www.dietandcancerreport.org/> (accessed November 2012).
- Boffetta P, Couto E, Wichmann J *et al.* (2010) Fruit and vegetable intake and overall cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *J Natl Cancer Inst* **102**, 529–537.
- Willett WC (2010) Fruits, vegetables, and cancer prevention: turmoil in the produce section. *J Natl Cancer Inst* **102**, 510–511.
- Key TJ (2011) Fruit and vegetables and cancer risk. *Br J Cancer* **104**, 6–11.
- Carter P, Gray LJ, Troughton J *et al.* (2010) Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *BMJ* **341**, e4229.
- Cooper AJ, Forouhi NG, Ye Z *et al.* (2012) Fruit and vegetable intake and type 2 diabetes: EPIC-InterAct prospective study and meta-analysis. *Eur J Clin Nutr* **66**, 1082–1092.
- Liu S, Serdula M, Janket SJ *et al.* (2004) A prospective study of fruit and vegetable intake and the risk of type 2 diabetes in women. *Diabetes Care* **27**, 2993–2996.
- Villegas R, Shu XO, Gao YT *et al.* (2008) Vegetable but not fruit consumption reduces the risk of type 2 diabetes in Chinese women. *J Nutr* **138**, 574–580.
- Loef M & Walach H (2012) Fruit, vegetables and prevention of cognitive decline or dementia: a systematic review of cohort studies. *J Nutr Health Aging* **16**, 626–630.
- Burr ML (2007) Secondary prevention of CHD in UK men: the Diet and Reinfarction Trial and its sequel. *Proc Nutr Soc* **66**, 9–15.
- Burr ML, Fehily AM, Gilbert JF *et al.* (1989) Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* **2**, 757–761.
- de Lorgeril M, Salen P, Martin JL *et al.* (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* **99**, 779–785.
- Pierce JP, Natarajan L, Cnaan BJ *et al.* (2007) Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. *JAMA* **298**, 289–298.
- Appel LJ, Champagne CM, Harsha DW *et al.* (2003) Effects of comprehensive lifestyle modification on blood pressure control: main results of the PREMIER clinical trial. *JAMA* **289**, 2083–2093.
- Howard BV, Van Horn L, Hsia J *et al.* (2006) Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA* **295**, 655–666.
- Appel LJ, Moore TJ, Obarzanek E *et al.* (1997) A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* **336**, 1117–1124.
- John JH, Ziebland S, Yudkin P *et al.* (2002) Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomized controlled trial. *Lancet* **359**, 1969–1974.
- McCall DO, McGartland CP, McKinley MC *et al.* (2009) Dietary intake of fruits and vegetables improves microvascular function in hypertensive subjects in a dose-dependent manner. *Circulation* **119**, 2153–2160.
- Broekmans WMR, Klopping-Ketelaars WAA, Kluit C *et al.* (2001) Fruit and vegetables and cardiovascular risk



- profile: a diet controlled intervention study. *Eur J Clin Nutr* **55**, 636–642.
36. Whybrow S, Harrison CL, Mayer C *et al.* (2006) Effects of added fruits and vegetables on dietary intakes and body weight in Scottish adults. *Br J Nutr* **95**, 496–503.
  37. Buijsse B, Feskens EJ, Schulze MB *et al.* (2009) Fruit and vegetable intakes and subsequent changes in body weight in European populations: results from the project on Diet, Obesity, and Genes (DiOGenes). *Am J Clin Nutr* **90**, 202–209.
  38. Smith-Warner SA, Elmer PJ, Tharp TM *et al.* (2000) Increasing vegetable and fruit intake: randomized intervention and monitoring in an at-risk population. *Cancer Epidemiol Biomarkers Prev* **9**, 307–317.
  39. Zino S, Skeaff M, Williams S *et al.* (1997) Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. *BMJ* **314**, 1787–1791.
  40. Watzl B, Kulling SE, Moseneder J *et al.* (2005) A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am J Clin Nutr* **82**, 1052–1058.
  41. Baldrick FR, Elborn JS, Woodside JV *et al.* (2012) Effect of fruit and vegetable intake on oxidative stress and inflammation in COPD: a randomised controlled trial. *Eur Respir J* **39**, 1377–1384.
  42. Gibson A, Edgar JD, Neville CE *et al.* (2012) Effect of fruit and vegetable consumption on immune function in older people: a randomized controlled trial. *Am J Clin Nutr* **96**, 1429–1436.
  43. Fulton SL, McKinley MC, Young IS *et al.* (2011) The effect of increasing fruit and vegetable consumption on overall diet: a systematic review and meta-analysis. *Proc Nutr Soc* **70**(OCE3), E66.
  44. Jacobs DR Jr, Gross MD & Tapsell LC (2009) Food synergy: an operational concept for understanding nutrition. *Am J Clin Nutr* **89**, 1543S–1548S.
  45. Lichtenstein AH, Appel LJ, Brands M *et al.* (2006) Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* **114**, 82–96.
  46. US Department of Health and Human Services & US Department of Agriculture (2005) *Dietary Guidelines for Americans*. 6th ed. Washington, DC: USDHHS.
  47. Bhupathiraju SN & Tucker KL (2011) Greater variety in fruit and vegetable intake is associated with lower inflammation in Puerto Rican adults. *Am J Clin Nutr* **93**, 37–46.
  48. Oude Griep LM, Verschuren WM, Kromhout D *et al.* (2012) Variety in fruit and vegetable consumption and 10-year incidence of CHD and stroke. *Public Health Nutr* **15**, 2280–2286.
  49. Cooper AJ, Sharp SJ, Lentjes MA *et al.* (2012) A prospective study of the association between quantity and variety of fruit and vegetable intake and incident type 2 diabetes. *Diabetes Care* **35**, 1293–1300.
  50. Jeurnink SM, Büchner FL, Bueno-de-Mesquita HB *et al.* (2012) Variety in vegetable and fruit consumption and the risk of gastric and esophageal cancer in the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer* **131**, E963–973.
  51. Pennington JAT & Fisher RA (2009) Classification of fruits and vegetables. *J Food Compos Anal* **22S**, S23–S31.
  52. Oude Griep LM, Verschuren WM, Kromhout D *et al.* (2011) Colors of fruit and vegetables and 10-year incidence of stroke. *Stroke* **42**, 3190–3195.
  53. Oude Griep LM, Verschuren WM, Kromhout D *et al.* (2011) Colours of fruit and vegetables and 10-year incidence of CHD. *Br J Nutr* **106**, 1562–1569.
  54. Oude Griep LM, Verschuren WM, Kromhout D *et al.* (2011) Raw and processed fruit and vegetable consumption and 10-year stroke incidence in a population-based cohort study in the Netherlands. *Eur J Clin Nutr* **65**, 791–799.
  55. Oude Griep LM, Geleijnse JM, Kromhout D *et al.* (2010) Raw and processed fruit and vegetable consumption and 10-year coronary heart disease incidence in a population-based cohort study in the Netherlands. *PLoS ONE* **5**, e13609.
  56. Kirsh VA, Peters U, Mayne ST *et al.* (2007) Prospective study of fruit and vegetable intake and risk of prostate cancer. *J Natl Cancer Inst* **99**, 1200–1209.
  57. Mikkelsen TB, Olsen SF, Rasmussen SE *et al.* (2007) Relative validity of fruit and vegetable intake estimated by the food frequency questionnaire used in the Danish National Birth Cohort. *Scand J Public Health* **35**, 172–179.
  58. Tucker KL, Chen H, Vogel S *et al.* (1999) Carotenoid intakes, assessed by dietary questionnaire, are associated with plasma carotenoid concentrations in an elderly population. *J Nutr* **129**, 438–445.
  59. Horner NK, Patterson RE, Neuhauser ML *et al.* (2002) Participant characteristics associated with errors in self-reported energy intake from the Women's Health Initiative food-frequency questionnaire. *Am J Clin Nutr* **76**, 766–773.
  60. Lee RD & Nieman DC (2003) *Nutritional Assessments*, 3rd ed. New York: McGraw-Hill.
  61. Livingstone MBE & Black AE (2003) Markers of the validity of reported energy intake. *J Nutr* **133**, 895S–920S.
  62. Deharven G, Charrondiere UR, Slimani N *et al.* (1999) Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC. *Eur J Clin Nutr* **53**, 60–79.
  63. Michels KB, Welch AA, Luben R *et al.* (2005) Measurement of fruit and vegetable consumption with diet questionnaires and implications for analyses and interpretation. *Am J Epidemiol* **161**, 987–994.
  64. Bingham S, Luben R, Welch A *et al.* (2008) Associations between dietary methods and biomarkers, and between fruits and vegetables and risk of ischaemic heart disease, in the EPIC Norfolk Cohort Study. *Int J Epidemiol* **37**, 978–987.
  65. Crews H, Alink G, Anderson R *et al.* (2001) A critical assessment of some biomarker approaches linked with dietary intake. *Br J Nutr* **86**, S5–S35.
  66. Hunter D (1990) Biochemical indicators of dietary intake. In *Nutritional Epidemiology*, pp. 143–216 [W Willett, editor]. New York: Oxford University Press.
  67. Field JK, Brambilla C, Hirsch FR *et al.* (2001) Molecular biomarkers workshop: a European strategy for developing lung cancer molecular diagnostics in high risk populations. *Lung Cancer* **31**, 339–345.
  68. Stockley RA (2007) Biomarkers in COPD: time for a deep breath. *Thorax* **62**, 657–660.
  69. Jansen MCJF, van Kappel AL, Ocke MC *et al.* (2004) Plasma carotenoid levels in Dutch men and women, and the relation with vegetable and fruit consumption. *Eur J Clin Nutr* **58**, 1386–1395.
  70. Al-Delaimy WK, Ferrari P, Slimani N *et al.* (2005) Plasma carotenoids as biomarkers of intake of fruits and vegetables: individual-level correlations in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Clin Nutr* **59**, 1387–1396.
  71. Dehghan M, Akhtar-Danesh N, McMillan CR *et al.* (2007) Is plasma vitamin C an appropriate biomarker of vitamin C intake? A systematic review and meta-analysis. *Nutr J* **6**, 41–53.

72. Padayatty SJ & Levine M (2008) Fruit and vegetables: think variety, go ahead, eat! *Am J Clin Nutr* **87**, 5–7.
73. Mennen LI, Sapinho D, Ito H *et al.* (2006) Urinary flavonoids and phenolic acids as biomarkers of intake for polyphenol-rich foods. *Br J Nutr* **96**, 191–198.
74. Mennen LI, Sapinho D, Ito H *et al.* (2008) Urinary excretion of 13 dietary flavonoids and phenolic acids in free-living healthy subjects – variability and possible use as biomarkers of polyphenol intake. *Eur J Clin Nutr* **62**, 519–525.
75. McAnlis GT, McEneny J, Pearce J *et al.* (1999) Absorption and antioxidant effects of quercetin from onions, in man. *Eur J Clin Nutr* **53**, 92–96.
76. Al-Delaimy WK, Slimani N, Ferrari P *et al.* (2005) Plasma carotenoids as biomarkers of intake of fruits and vegetables: ecological-level correlations in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Clin Nutr* **59**, 1397–1408.
77. Ferrari P, Al-Delaimy WK, Slimani N *et al.* (2005) An approach to estimate between- and within-group correlation coefficients in multicenter studies: plasma carotenoids as biomarkers of intake of fruits and vegetables. *Am J Epidemiol* **162**, 591–598.
78. Drownowski A, Rock CL, Henderson SA *et al.* (1997) Serum beta-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. *Am J Clin Nutr* **65**, 1796–1802.
79. Bingham SA, Gill C, Welch A *et al.* (1997) Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and vitamin C and carotenoids as biomarkers. *Int J Epidemiol* **26**, S137–S151.
80. Campbell DR, Gross MD, Martini MC *et al.* (1994) Plasma carotenoids as biomarkers of vegetable and fruit intake. *Cancer Epidemiol Biomarkers Prev* **3**, 493–500.
81. Baldrick FR, Woodside JV, Elborn JS *et al.* (2011) Biomarkers of fruit and vegetable intake in human intervention studies: a systematic review. *Crit Rev Food Sci Nutr* **51**, 795–815.
82. Vioque J, Weinbrenner T, Asensio L *et al.* (2007) Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. *Br J Nutr* **97**, 977–986.
83. Pollard J, Wild CP, White KL *et al.* (2003) Comparison of plasma biomarkers with dietary assessment methods for fruit and vegetable intake. *Eur J Clin Nutr* **57**, 988–998.
84. Freedman LS, Midthune D, Carroll RJ *et al.* (2011) Using regression calibration equations that combine self-reported intake and biomarker measures to obtain unbiased estimates and more powerful tests of dietary associations. *Am J Epidemiol* **174**, 1238–1245.
85. Freedman LS, Kipnis V, Schatzkin A *et al.* (2010) Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? *Epidemiol Perspect Innov* **7**, 2.
86. Welch AA, Mulligan A, Bingham SA *et al.* (2008) Urine pH is an indicator of dietary acid-base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study. *Br J Nutr* **99**, 1335–1343.
87. Berry SE, Mulla UZ, Chowienczyk PJ *et al.* (2010) Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. *Br J Nutr* **104**, 1839–1847.
88. Mente A, Irvine EJ, Honey RJ *et al.* (2009) Urinary potassium is a clinically useful test to detect a poor quality diet. *J Nutr* **139**, 743–749.
89. Mayne ST, Cartmel B, Scarmo S *et al.* (2010) Noninvasive assessment of dermal carotenoids as a biomarker of fruit and vegetable intake. *Am J Clin Nutr* **92**, 794–800.
90. Nielsen SE, Freese R, Kleemola P *et al.* (2002) Flavonoids in human urine as biomarkers for intake of fruits and vegetables. *Cancer Epidemiol Biomarkers Prev* **11**, 459–466.
91. Krogholm KS, Haraldsdóttir J, Knuthsen P *et al.* (2004) Urinary total flavonoid excretion but not 4-pyridoxic acid or potassium can be used as a biomarker for the intake of fruits and vegetables. *J Nutr* **134**, 445–451.
92. Favé G, Beckmann M, Lloyd AJ *et al.* (2011) Development and validation of a standardized protocol to monitor human dietary exposure by metabolite fingerprinting of urine samples. *Metabolomics* **7**, 469–484.
93. Lloyd AJ, Beckmann M, Favé G *et al.* (2011) Proline betaine and its biotransformation products in fasting urine samples are potential biomarkers of habitual citrus fruit consumption. *Br J Nutr* **106**, 812–824.
94. Heinzmann SS, Brown IJ, Chan Q *et al.* (2010) Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption. *Am J Clin Nutr* **92**, 436–443.
95. Lloyd AJ, Favé G, Beckmann M *et al.* (2011) Use of mass spectrometry fingerprinting to identify urinary metabolites after consumption of specific foods. *Am J Clin Nutr* **94**, 981–991.
96. O’Sullivan A, Gibney MJ & Brennan L (2011) Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. *Am J Clin Nutr* **93**, 314–321.
97. NDNS (2004). The National Diet and Nutrition Survey: Adults aged 19 to 64 Years, Summary Report, Volume 5. London: The Stationery Office. <http://www.food.gov.uk/multimedia/pdfs/ndns5full.pdf> (accessed November 2012).
98. Department of Health, National Diet and Nutrition Survey (2012) <http://transparency.dh.gov.uk/2012/07/25/ndns-3-years-report/> (accessed November 2012).
99. Appleton KM, McGill R & Woodside JV (2009) Fruit and vegetable consumption in older individuals in Northern Ireland: levels and patterns. *Br J Nutr* **102**, 949–953.
100. Scarborough P, Morgan RD, Webster P *et al.* (2011) Differences in coronary heart disease, stroke and cancer mortality rates between England, Wales, Scotland and Northern Ireland: the role of diet and nutrition. *BMJ Open* **1**, e000263.
101. Scarborough P, Nnoaham KE, Clarke D *et al.* (2012) Modelling the impact of a healthy diet on cardiovascular disease and cancer mortality. *J Epidemiol Community Health* **66**, 420–426.
102. Lock K, Pomerleau J, Causser L *et al.* (2005) The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. *Bull WHO* **83**, 100–108.
103. Hall JN, Moore S, Harper SB *et al.* (2009) Global variability in fruit and vegetable consumption. *Am J Prev Med* **36**, 402–409.e5.
104. Craig P, Dieppe P, Macintyre S *et al.* (2008) Developing and evaluating complex interventions: the new Medical Research Council guidance. *BMJ* **337**, a1655.
105. Michie S, van Stralen MM & West R (2011) The behaviour change wheel: a new method for characterising and designing behaviour change interventions. *Implementation Sci* **6**, 42.



106. Thomson CA & Ravia J (2011) A systematic review of behavioral interventions to promote intake of fruit and vegetables. *J Am Diet Assoc* **111**, 1523–1535.
107. Pomerleau J, Lock K, Knai C *et al.* (2005) Interventions designed to increase adult fruit and vegetable intake can be effective: a systematic review of the literature. *J Nutr* **135**, 2486–2495.
108. Knai C, Pomerleau J, Lock K *et al.* (2006) Getting children to eat more fruit and vegetables: a systematic review. *Prev Med* **42**, 85–95.
109. Delgado-Noguera M, Tort S, Martínez-Zapata MJ *et al.* (2011) Primary school interventions to promote fruit and vegetable consumption: a systematic review and meta-analysis. *Prev Med* **53**, 3–9.