Food Standards Australia New Zealand
Diet-Disease Relationship Review

Dietary fruit and vegetable intake and
risk of coronary heart disease

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EXECUTIVE SUMMARY

In late 1999, a Canadian report presented a comprehensive analysis of studies on the relationship between dietary fibre and risk of coronary heart disease (CHD) that were published in the scientific literature. In addition to grains and grain products, fruits and vegetables comprised the major sources of dietary fibre. The overall conclusion was: "Diets low in saturated fat and cholesterol and rich in high-fibre, intact foods, such as whole grains, fresh fruits and vegetables, may reduce the risk of heart disease, a disease associated with many factors." The research reviewed and analysed by the authors clearly provided ample support for the conclusion; however, the level of evidence for the relationship between fruit and vegetable consumption and risk of CHD was less apparent in the report. An independent review of the summaries presented in the Appendices, focusing on research projects specifically addressing fruit and vegetable intake and CHD risk, revealed fairly consistent support for an inverse association between consumption of fruits and vegetables and risk of CHD in observational studies. This is confirmed by a pooled reanalysis of data from eight of these studies described further in this report (Part 2 and Appendix 4). Evidence at that time from randomised, controlled trials, primarily examining the effect of fruit intake on lipid levels, was less consistent, showing null or beneficial effects. The current report now critically reviews studies researching the relationship between fruit and vegetable intake and risk of CHD published since 2000.

A total of 13 research projects were identified that investigated outcomes related to CHD morbidity or mortality, including 9 prospective cohort studies and 4 retrospective case-control studies. Irrespective of study design and specific outcome, and similarly for study populations differing by age, gender, or nationality, an inverse association was generally reported for fruit and/or vegetable intake and CHD risk. The magnitude of the association varied across studies, and in some cases did not approach statistical significance. Nevertheless, as intake of fruits and/or vegetables increased, risk of CHD morbidity and mortality tended to decrease. Results remained consistent, although sometimes attenuated, following statistical adjustment for many CHD risk factors and/or factors considered potentially intermediary in the causal pathway between diet and CHD risk. The overall inverse relationship was evident throughout the range of moderate fruit and vegetable consumption characteristic of Australian and New Zealand residents, although there is some indication that there may be a threshold in intake beyond which additional improvements in disease risk are not observed.

Although the exact mechanism/s for the observed inverse relationship is/are not known, there are ample reasons to consider the association biologically plausible. Fruits and vegetables contain biologically active compounds including fibre, anti-oxidant vitamins, and anti-oxidant phytonutrients, among others. In addition to consumption of these possibly beneficial substances, eating fruits and vegetables may displace other foods rich in various fats, energy, cholesterol or sodium, which themselves are considered adverse risk factors for CHD. And there are a number of physiological processes potentially involved in atherosclerosis underlying CHD that may be altered as a consequence of fruit and vegetable consumption. Research focused on the role of fruit and/or vegetable consumption in relation to biomarkers of CHD risk provides additional evidence of this (18 studies reviewed).

Increased intake of fruits and/or vegetables generally has been associated with lower levels of systolic and diastolic blood pressures in randomised, controlled trials. Whereas inverse associations between blood lipid levels and fruit and/or vegetable consumption are usually observed in cross-sectional studies, results in intervention trials are sometimes, but less consistently, beneficial. Few studies have investigated homocysteine and c-reactive protein levels, but early results lend support to lower levels with increased intake of fruits and/or vegetables.

Dietary research is noteworthy for its challenges, particularly when assessing usual diet within free-living populations. In particular, difficulties in measurement of food intake related to types of fruits and vegetables, serving sizes, and frequency of consumption are well documented, but generally lead to underestimation of true effects. Proper measurement and control for potential confounding factors also are important to preclude attribution of results to diet when they are, in fact, a consequence of other characteristics that are correlated with diet. And rigorous design and implementation for the ascertainment and recruitment of study participants are necessary to ensure generalisability of findings to the population of interest. The methods used by the reviewed studies had varying strengths and limitations, but few were sufficiently flawed to undermine confidence in their results. ***Overall, the available research points to a convincing level of evidence for a relationship between a diet rich in vegetables and/or fruits and reduced risk of CHD, which is sufficiently consistent and substantial to underpin policy recommendations.***

Part 1: Critical appraisal of previous review of this diet-health relationship

1 (a) Appraisal of the selection and assessment of evidence in the review

The search methodology used in the Canadian report, entitled 'Short Literature Review for Fruits, Vegetables and Grain Products that Contain Fibre, Particularly Soluble Fibre, and Coronary Heart Disease,' was generally appropriate and would have covered the majority of the relevant literature available to the authors over the time period covered. One ramification of the choice to search for "cardiovascular diseases NOT neoplasms" is the exclusion of any studies that looked at overall mortality and also cause-specific mortality from major causes including cardiovascular disease (CVD) and neoplasms. Such studies are often large prospective studies with a high-quality level of evidence. However, it is unlikely that this would have resulted in a systematic bias towards inclusion of studies with a null, positive, or negative association.

Largely, we agree with the inclusion/exclusion criteria outlined in the previous report, with some minor hesitancies, specifically relating to appropriate study populations and appropriate study endpoints. For example, on p11 of the draft report, the authors claim:

> "Studies were excluded if the dietary intervention was conducted in subjects with diagnosed CHD or with a history of MI or coronary artery bypass surgery…Studies with healthy subjects or those with hyperlipidemia were included."

Findings need to be generalisable for primary prevention. We are assuming that the authors meant that valid population groups for studies were subjects at background risk for CHD, or with a major risk factor for coronary heart disease (CHD) (eg, hyperlipidemia, diabetes, hypertension), but not CHD itself. (The Canadian report described hypertension and hyperhomocysteinemia as risk factors for CHD along with dyslipidemia, therefore we assume that these at-risk groups were included rather than excluded.) We also are assuming that the authors did not exclude studies of general populations in which a minority of participants may have had prior CHD (which would be unnecessarily restrictive, especially since this issue can be addressed analytically).

Some studies included in our review cited studies that fit the time period of the Canadian review and were not found in the Canadian report. More careful review of these individual reports revealed that none were actually relevant to the question at hand, but rather addressed issues of tangential interest to the association between fruits, vegetables, or grain products that contain fibre and CHD risk. Overall their findings were supportive of an inverse association, but are not documented further in this report.

1 (b) Re-assessment of several pivotal studies cited in the review and consideration of the validity of the review's conclusions

Several of the studies cited in the Canadian report were independently reviewed, and in general, the authors of that report were accurate in summarising study findings, made reasonable comments on salient design and analytic issues, and presented an appropriate synthesis across studies. The report's conclusions are well substantiated by the papers available at that time. In brief, the overall conclusion was: "Diets low in saturated fat and cholesterol and rich in high-fibre, intact foods, such as whole grains, fresh fruits and vegetables, may reduce the risk of heart disease, a disease associated with many factors." Noteworthy in this conclusion are the two phrases (emphases added): '*fresh* fruits and vegetables' and '*may* reduce the risk of heart disease'. The current review may provide additional insights in relation to these statements.

Unfortunately, the focus of the Canadian report was on dietary fibre, including grain products as well as fruits and vegetables, and on soluble fibre in particular. Hence their emphasis was on the benefits of high-fibre, intact foods and fresh fruits and vegetables in their recommendation. In the context of the current report, the conclusions of the Canadian report and many of the included studies are of limited utility, because any beneficial effect of fruit and vegetable consumption in relation to CHD-related outcomes may be due to mechanisms unrelated to dietary fibre. In fact, most of the early studies included in the Canadian report, in which associations with intake of fruit and vegetables were addressed separately, showed inverse relationships with CHD morbidity or mortality and to a lesser extent lipid levels. This evidence is now followed up in the current report with a critical review of the scientific literature available from 2000-2005.

Part 2: Review of the evidence released since the time of the Canadian review

Identification of relevant studies

Search Strategy

Three major databases were searched: EBSCO Host, ProQuest, and Blackwell Synergy. Each search was conducted only of scholarly literature published from 2000 onwards. Searches were tailored to each database to minimise the chances of excluding relevant literature. The search terms for each database are provided in Appendix 1. (Hard-copy results from each search results are provided separately.) Additionally, reference lists contained in located articles and reviews were checked for any additional studies that appeared to relate to the topic.

Inclusion/ Exclusion of Studies

Articles and abstracts were scanned for relevance. Those not relevant because they were clearly unrelated to the topic of the health claim or reported research on non-human subjects were discarded. Articles that reported on studies published elsewhere were not included, but were used as an additional source to locate potentially relevant studies.

All relevant studies identified were summarised. Studies were included if they evaluated the relationship between CHD or its biomarkers and fruits and/or vegetables in whole or juice form. These studies may have tested sub-groups of fruits or vegetables, such as cruciferous vegetables or citrus fruits. Studies were excluded if they only tested the effects of a single fruit or vegetable (such as kiwifruit), extracts made from fruits and vegetables, or nutritional components of fruits and vegetables (such as vitamin C, antioxidants or fibre).

Intervention studies were included if they were conducted on healthy populations or those at higher risk of CHD (eg, with hyperlipidemia, hypertension). Interventions focusing on those with CHD were excluded.

Definition of Fruits and Vegetables

This review did not employ an *a priori* definition of fruits and vegetables, but rather used the definitions applied in the reviewed studies. These are described in the contexts of the reported findings and further addressed in Part 3 with respect to relevance of the findings for Australia and New Zealand. Claims cannot be substantiated on the basis of this review for types of fruits and vegetables that have not been considered by the authors of the original studies.

Some further caveats are worth noting. Fruits and vegetables are nutritionally heterogeneous. They each have multiple sub-group classifications and there are important nutritional differences both between and within these subgroups of fruits and vegetables. The studies in

this review generally were inclusive of vegetables. Notable exceptions are potatoes, which were often excluded from vegetable classifications, and legumes which were sometimes excluded or considered separately. The basis for these exclusions lies in the important nutritional dissimilarities between these and other vegetables. Potatoes were often excluded because they have a comparatively low content of substances expected to protect against CHD (such as vitamin C and other antioxidant vitamins) and a relatively high caloric content. Since potatoes contribute a substantial proportion to total vegetable intakes in many western populations, their exclusion is necessary to avoid distorting results. Legumes also warrant unique consideration, as their relatively high protein and caloric content, the amount and types of dietary fibres they contain, and their importance in vegetarian diets suggest they could have unique cardio-protective contributions that might be dissimilar to other vegetables. In addition, FSANZ specifically requested the exclusion of legumes from this review. Unlike potatoes, legumes contribute much smaller proportions to total vegetable intake in western populations and their inclusion in some studies is unlikely to have affected results. Researchers did not exclude any types of fruits in their studies.

The nutritional content (and therefore potential cardiovascular benefit) of fruits and vegetables are affected by many factors, such as growing conditions, harvesting, storage, processing, and preparation methods. The first three of these factors are unrealistic to capture in population research and their variation within and across populations may contribute to some heterogeneity in findings. Processing is potentially important as the nutritional content of fresh, frozen and canned fruits and vegetables varies for key nutrients which have physiological relevance for mechanisms of CHD aetiology (particularly vitamin C and folate). Some studies included only fresh vegetables, while others explicitly included frozen and canned vegetables, and others did not specify. Many studies employed food frequency questionnaires to measure dietary intake, which assessed the intake of mixed dishes but did not include these in the measures of fruit and vegetable intake. In addition, it should be noted that forms of processing used in developing appealing combined food products may have a different effect on nutritional quality and subsequent cardio-protection than freezing and canning, which are preservation techniques specifically designed to maximise product quality and minimise nutrient losses. Some studies included and others excluded fruits and vegetables consumed in juice form, although the contribution of juices to total intake was not clear. In juice form, fruits and vegetables lack the fibre they contain as whole foods, and therefore may not offer the same health benefits. Hence some heterogeneity in findings across studies may be due to the forms of the fruits and vegetables that ultimately were included in individual data analyses.

Measurement and Analysis of Fruit and Vegetable Intake

Accurate and valid measurement of the intake of fruits and vegetables is difficult [1]. The most commonly used and widely accepted techniques are weighed records, the diet history method (sometimes including a frequency cross-check), the 24-hour recall, and the food frequency questionnaire [2]. Direct observation is rare, and biomarkers (such as serum carotenoids or plasma vitamin C) are sometimes used, albeit typically in conjunction with other intake measures, as physiologically these biomarkers are not expected to correlate perfectly with intake [1, 3]. Each dietary assessment method has different strengths and weaknesses, and each may generate different estimates of the association between fruit and

vegetable intake and disease risk [4]. All the common methods used by studies in this review involve participants' self-report, which is subject to 'social desirability bias' and is problematic in that participants may under-report total food and energy intake, and intake of foods believed to be unfavourable to the researchers [5, 6], and perhaps over-report consumption of foods they perceive as healthy such as fruits and vegetables [7, 8].

Weighed records, diet histories and 24-hour recalls collect information on foods consumed, rather than food categories, and thus can obtain more detailed information than can food frequency questionnaires, for example about the types of fruits and vegetables people consume. In addition to the level of detail, intake assessment methods vary in their level of accuracy in quantifying total food intake. Weighed records are considered the most accurate dietary intake assessment techniques [9] and are the only measure that does not require participant recall. However, they can underestimate usual total intake, as respondents often temporarily change their food habits to accommodate having to weigh and report every item they consume [5]. Multiple 24-hour recall techniques are less accurate than weighed records, but considered superior to dietary histories or food frequency questionnaires [9]. Diet histories conducted by trained professionals can be accurate, especially if used in conjunction with food models or other visual tools to assist participants in judging portion sizes [2]. Food frequency questionnaires are the simplest and least costly to implement on a wide scale [7], however their quality varies widely. Some assess only the frequency of food intake, while semi-quantitative food frequency questionnaires include portion size information to measure quantities of daily intake. Their validity is usually established relative to one of the more accurate methods in a study sub-sample, however the quality of the 'validation' also depends on the quality of the reference assessment method [7].

Validation for nutrients of which fruits and vegetables are key sources (such as vitamin C) is more indicative of a valid fruit and vegetable assessment than validation for other micro- and macro- nutrients, such as fat intake or energy intake. Food frequency questionnaires that specify particular fruits and vegetables often yield higher intake estimates relative to other methods [10]. Food frequency questionnaires may show poor validity at the individual level, however they are often sufficient to classify individuals into quintiles of intake [11, 12]. Inaccuracy in all methods of dietary measurements means that some extent of misclassification bias is likely in all studies – however this bias is minimised in studies using the more accurate methods (multiple weighed records, multiple recall methods, dietary histories or high quality food frequency questionnaires) and is probably greater in studies using less accurate dietary intake methods (such as lower quality food frequency questionnaires, especially for those with low validity). Generally this misclassification results in under-estimations of true relationships with outcome variables [7, 12].

The time period of exposure also is a source of difficulty in meaningfully connecting dietary exposures to disease outcomes. Diets generally vary from day to day as well as over longer periods throughout the lifecourse. Further, the time period of importance may be different, depending on the mechanism of protection/damage, as some nutrients may be ingested intermittently but are cumulative, while others are not stored and therefore require regular intake to have an impact. Studies generally treat dietary effects as cumulative in terms of their effect on CHD, however no measure provides an accurate cumulative measurement of diet over a lifetime. Different methods vary in the time periods over which they assess diet, and

ususally measure current diet (eg. weighed records, multiple recalls) or diet over the last month, several months or year (eg. food frequency questionnaires). It is not known which dietary assessment method reflects the most appropriate temporal dietary exposure in terms of CHD prevention.

Where authors have used dietary assessment tools capable of quantifying dietary intake, by convention they generally describe findings in terms of metric quantities or 'servings'. In most papers reviewed, servings are typically USDA servings, which are approximately 150g and equivalent to a medium-sized piece of fruit, ½ cup of cooked vegetables or 1 cup of salad vegetables [13]. This is the definition of 'serving' employed by this review, which is roughly equivalent to sample serves in the Australian core foods approach to healthy eating [14]. However, dietary assessment methods that seek to gauge frequency rather than quantity of intake may allow participants to determine what constitutes a serving, and this may not equate to sample serves or USDA servings. For simplicity, this review uses the term portion when the term serving may not apply.

Finally, there are two problematic issues in relation to the statistical analysis of fruit and vegetable consumption and risk of CHD and related outcomes. First, there is the question of how the measure of intake is analysed. Some studies used dietary information as grams/day (ie, a continuous variable), while most categorised the dietary exposure, usually into quartiles or quintiles (ie, ordinal variables), but some simply indicated above or below some threshold (ie, dichotomous variable). Clearly the size of the risk reduction is influenced by the specific definition used, which is why we have not attempted to summarise the magnitude of the association between fruit and vegetable intake and risk of CHD. Instead, we have focused on the quality of the study in relation to its individual results and evaluating consistency in direction of the association across studies. More formal meta-analysis or pooled analysis is necessary to estimate the average effect size across the various studies.

The second analytic issue relates to statistical adjustment for other dietary characteristics, in particular total energy consumption. The dilemma is that energy intake may serve as a confounder (ie, independently related to the outcome and to the exposure of interest – fruit and vegetable intake), or it may lie on the causal pathway (either preceding fruit and vegetable intake or as an intermediary along the way to the CHD outcome). In the former case, adjustment is necessary to estimate the independent association with fruit and vegetable intake, whereas in the latter situation, controlling for energy would result in over-adjustment and an underestimation of the true RR or OR. Willet (1997) argues that studies of dietary intake and CHD should adjust for energy intake [15], as it is generally shown to reduce the risk of CHD. Also, people who consume more energy also generally consume more of most specific nutrients. Therefore, failure to adjust for energy intake can lead to a false conclusion that virtually all nutrients are associated with reduced risk of CHD. Adjustment for body mass index (BMI) and physical activity (which was common in the studies in this review) partially but not wholly removes this effect. Confounding is strongest for intake of energy carrying nutrients (such as fat), but also applies to other dietary intakes (such as intake of fruits and vegetables). Some of the studies in this review did not adjust for energy intake, while others did, using a variety of analytic methods. Furthermore, the adequacy of the adjustment may be limited by the use of categorical variables for dietary measures rather than the preferred continuous variable form. The similarity in the results across studies that

did and did not adjust for energy suggest that confounding by energy intake did not produce the observed risk reductions. However, the true size of the risk reductions in individual studies cannot be known with certainty.

Bases for Quality Ratings

The quality of the evidence provided by each study is multi-faceted and unable to be fully captured by a single score. Issues related to measurement and analysis of fruit and vegetable intake are discussed above. Furthermore, each study design has methodological strengths and weaknesses (eg, RCT, cohort, case-control, cross-sectional), and some studies manage to control the potential pitfalls of their chosen study design more effectively than others. Accordingly, this review separates the evidence by study design type and discusses the ways in which limitations of the study are likely to overestimate or underestimate the true relationship between fruit and/or vegetable intake and the coronary outcome measure. In addition, the quality of the studies is briefly summarised in the tables with a simple A, B, C rating – based on subjective appraisal of how well the study design was implemented and therefore able to substantiate the study's conclusions. It must be emphasised that this is not a validated quality measure but rather a visual tool to assist in reading the tables and should be used in conjunction with the other aspects of quality.

Quality Rating -

A- Well-conducted study, very minor problems only, unlikely to alter conclusions.

B- Generally well-conducted study, some limitations, may underestimate the true effect but unlikely to produce a type I error.

C- Study with serious limitations that are likely to substantially alter the conclusions, or which may have produced a type I error.

Summary of Findings for Risk of CHD

We now summarise the scientific literature on the possible relationships between fruit and vegetable consumption and CHD risk and mortality. The studies in this section are considered the most relevant, as CHD is the outcome under investigation in each of these studies, hence the findings are most directly relevant to the question posed. The statistics most commonly used to report the results are the relative risk (RR) for cohort studies and the odds ratio (OR) for case-control studies. The OR actually provides an estimate of the RR, so we focus our description here on that measure. A RR is simply the ratio of two absolute risks: the numerator is the absolute risk among those with the factor, while the denominator is the absolute risk among those without the factor. If exposure to a factor increases risk of disease or other outcome, then the RR will be great than 1.0; conversely, if the exposure reduces the risk of the outcome, the RR will be less than 1.0. The statements that 'the RR=0.85' or that 'the risk is reduced by 15%' are considered equivalent, keeping in mind that the comparison is between exposed and unexposed groups. The confidence intervals give the reader an idea of the uncertainty in the estimated RR or OR relative to the ***true*** RR or OR; it gives the ***range*** of estimates in which the ***true*** RR or OR lies.

At the end of Part 2, Table 1 summarises key features of each of the identified relevant studies. This is followed by some discussion of the possible underlying mechanisms to address the biological plausibility of the observed associations. In Part 3, we provide external information about diet and lifestyle among Australian and New Zealander populations. In Part 4, we review additional studies that focus on outcomes related to CHD risk factors, to provide further insight into the nature of the observed relationships. Tables in Appendices 2-4 then present details on each of the cited studies. Appendix 2 presents prospective observational studies (cohort), while Appendix 3 presents retrospective observational studies (case-control) and Appendix 4 presents meta-analyses (only one was identified). Additional Appendices appear for the studies reviewed in Part 4.

Observational Studies: Cohort Studies

Nine cohort studies [13, 16-22] were located, which evaluated whether intake of fruits, vegetables or both was related to subsequent development of CHD. They are discussed below in chronological order of publication. Most (n=7) address combined fruit and vegetable consumption, in addition, in some cases, to subgroups of fruit and/or vegetable intake. One (#4) addresses fruit intake, and another (#9) addresses vegetable consumption only. All of the associations tested between consumption of fruits, vegetables, or both and diagnoses related to CHD revealed inverse relationships (16 of 16). Of these, 14 were statistically significant in at least one stratum, or on a per serve basis. The eight studies that also tested the associations in population subgroups reported inverse relationships, and seven of these included statistically significant results (see Table 1 on page 19).

These studies provide the best quality evidence currently available for the hypothesis that fruit and/or vegetable intake reduces risk of CHD. Their prospective design ensures that the exposure (fruit and/or vegetable intake) precedes the outcome (CHD morbidity or mortality). However, because they are observational studies, potential confounding must be addressed in statistical analyses. When adjusted results are quite similar to unadjusted results, we report the latter to take advantage of the tighter confidence intervals; however, we acknowledge when statistical significance is lost as a consequence of further adjustment. Furthermore, the impact of incomplete participation, as a consequence of original ascertainment or recruitment difficulties or loss-to-follow-up, must be considered in relation to internal validity and generalisability of findings.

CHD Study 1: Liu and colleagues, 2000, studied almost 40,000 women health professionals in the U.S., with no prior history of CVD [13]. They explored the relationship of **combined fruit and/or vegetable intake** at baseline with two outcomes: myocardial infarction (MI) or CVD incidence over a 5-year follow-up period. They also tested associations with CVD in a subgroup that did not have either diabetes, history of hypertension, or history of high cholesterol at baseline on the premise that these conditions may affect participants' food intake. Comparing the highest with lowest quintiles of combined fruit and vegetable intake (which averaged 10.2 and 2.6 serves per day, respectively), risk of MI was substantially reduced (approximately 40%) but was not statistically significant (RR=0.62; 95%CI: 0.37-1.04). Estimates were statistically significant in quintiles two (average 4.1 serves/day; RR=0.49; 95% CI: 0.28-0.85) and four (average 7.1 serves/day; RR=0.50; 95% CI: 0.28-0.86). Risk of CVD in the general sample was reduced by approximately 15-25% for all quintiles beyond the first (none statistically significant), with or without adjustments for potential confounding and intermediary factors. However in the sub-sample without CVD co-morbidities, a significant and large risk reduction of 55-60% was observed for CVD, depending on the extent of adjustment conducted.

Similar results were observed when fruits and vegetables were analysed separately. When comparing the highest with lowest quintiles of **fruit intake** (which averaged 3.8-3.9 and 0.6 serves per day, respectively), the risk of MI among all participants and the risk of CVD among those without common CHD-related disorders at baseline were substantially reduced (approximately 40%; statistically significant for MI only). Risk of CVD for all participants was reduced minimally (4%). When comparing the highest with lowest quintiles of **vegetable**

intake (which averaged 6.8-6.9 and 1.5 serves per day, respectively), there were no significant associations with either CVD or MI among all participants. However among women without common CHD-related disorders at baseline, the risk of CVD was substantially and significantly reduced (approximately 55%) before and after statistical adjustment for potential confounders.

None of the associations described above reflected typical dose-response relationships; in most cases, reductions in risk for either endpoint became apparent with the second quintile of consumption of fruits, vegetables, or both. Most known potential confounding factors were considered, although some residual confounding is possible, as no adjustment was made for energy intake. Considering the magnitude of the observed inverse associations, this is unlikely to account for the reported findings. The dietary assessment method was appropriate, minimising the chance of misclassification bias. Overall, this study provides substantial support for an inverse relationship between consumption of fruits and vegetables and CVD.

CHD Study 2: Joshipura and colleagues, 2001, reported on the 8-year follow-up of over 84,000 women (aged 34-59 years at baseline) and over 42,000 men (aged 40-75 years at baseline), all of whom were health professionals and had no history of CVD, diabetes or cancer at baseline [17]. They found a significant reduction in CHD risk of approximately 4% with each additional daily serve of **fruits and vegetables** (RR=0.96; 95% CI: 0.94-0.99). This was reflected in a 20% risk reduction (RR=0.80; 95% CI: 0.69-0.93) among those consuming the highest compared with the lowest quintiles of fruit and vegetable intake, which had average daily intakes around 10 and 3 serves, respectively. Importantly, risk reduction with each additional daily serve of vitamin C-rich fruits and vegetables was slightly greater than the findings for any fruits and vegetables (6%; RR=0.94; 95% CI: 0.88-0.99).

They also found a significant, 6% reduction in risk of CHD with each additional daily serve of **fruit** (RR=0.94; 95% CI: 0.90-0.98). This was reflected in a significant 20% risk reduction in the top two highest quintiles compared with lowest quintiles of fruit intake, which had average daily consumptions around 4 servings and 1 serve, respectively. A non-significant 5% reduction in risk was observed with each additional daily serve of citrus fruits, but there was no association with citrus juice intake. Similarly, a significant, inverse association was observed for **vegetable** intake and CHD, with a 5% reduction in risk per daily serving of vegetables (RR=0.95; 95% CI: 0.92-0.99) and significant reductions in risk per daily serve of cruciferous vegetables (14%) and green leafy vegetables (23%). (Larger reductions were observed for the subgroups because total vegetable intake included potatoes and legumes, neither of which was inversely associated with CHD risk; hence the relative risk estimate for all vegetables in this study probably underestimates the true inverse relationship.) As for fruit, for each of these three vegetable categories, reductions in CHD risk were evident for both quintiles 4 and 5 relative to quintile 1.

The relationships in this study were independent of a large number of potential confounders. Also, further adjustments for fibre (and other dietary) intake did not substantially alter results, indicating fibre is unlikely to be the only component of fruits and vegetables responsible for the observed inverse associations. Subgroup analyses showed fruits and vegetables were similarly protective for persons with or without hypertension or diabetes, and for men and

women, but were slightly more protective for non-users than users of multivitamins and for current smokers than never or past smokers. The research used high-quality food frequency questionnaires, which still have some potential for misclassification of dietary intakes. However, this is more likely to lead to null findings than a significant inverse association and has less effect on comparisons between the upper and lower quintiles of intake. Generally the conclusions of this study are quite robust; however the analyses for some types of vegetables were limited by the very low intakes in the study population (eg. median intakes for highest versus lowest quintiles were 0-2 for citrus fruit, 0-1 for citrus juice, 0-1 for cruciferous vegetables, and 0-1.5 for green leafy vegetables).

CHD Study 3: Bazzano and colleagues, 2002, used 19-year follow-up data from the National Health and Nutrition Examination survey cohort of over 9,000 men and women aged 25-47 years and free of CHD at baseline [18] . They found significant, inverse relationships between frequency of **fruit and vegetable** consumption and mortality from ischemic heart disease (IHD) and CVD mortality, after considering age, race, gender and energy intake as possible confounders. Reductions of about 35% were observed for consuming fruits and vegetables 3+ times per day compared to <1 time per day (RR=0.66; 95% CI: 0.49-0.90 for IHD mortality; and RR=0.63; 95% CI: 0.51-0.79 for CVD mortality). Consideration of several other potential confounders attenuated the relationships, attenuating results to 25% reduction for both IHD and CVD mortality and causing the association with IHD mortality to lose statistical significance. Both mortality relationships showed significant dose-response trends. Results for IHD incidence showed a smaller magnitude of association (15% reduction when minimally adjusted), were not statistically significant and were generally considered inconclusive.

Some possibility for residual confounding exists as this study did not consider dietary confounders other than energy intake. Furthermore, the dietary assessment method had potential for misclassification, as it classified intake by frequency only (not quantity). One other methodological issue to consider is a small possibility of reverse causality. While the study excluded participants who used heart disease medications, or had a history of heart attack, heart failure or stroke at baseline, the study did not specify a minimum period between dietary assessment and outcome, and diet over "the last three months" may have been affected by illness. However, as this was a cohort study of long duration, it is unlikely that a substantial number of dietary assessments were taken immediately prior to the measured outcomes. This study also includes a more representative sample of participants than cohorts comprised of health professionals; however, their somewhat younger ages at baseline may have compromised the number of outcome events observed. Overall, the results of this study are considered supportive of an inverse relationship between fruit and vegetable consumption and IHD and CVD, but for mortality only.

CHD Study 4: Appelby and colleagues, 2002, reported on the 18-24-year follow-up of 10,741 'health conscious' British adults aged 16-89 years and enrolled in the Health Food Shoppers study [23]. No details on recruitment were provided. Participants were free from known cancer (except melanoma) at baseline, however participants with other health conditions, including CHD, were not excluded. Participants' dietary and other lifestyle habits and demographic characteristics were measured in a 'diet and lifestyle questionnaire'. Intake of at least one serve of **fresh fruit** daily was associated with significant reductions in

mortality from IHD (approximately 25%) and from all causes (approximately 20%). Risk reductions for IHD mortality were greater for women (48%) and non-smokers (33%), although this may reflect differences in distributions of intake rather than biologically different effects of fruit in these subgroups. Results were adjusted for many potential confounders, however several others were not considered (such as intake of energy, fat, saturated fat, physical activity). Dietary measurement was crude but likely sufficient to classify individuals above and below the one-serve threshold. The study deliberately included a 'health conscious' sample who may not reflect the general population, and thus findings are of limited generalisabilty. Furthermore, the study did not exclude participants with a baseline history of CHD, limiting the applicability of results for primary prevention. While this study was large and had a long follow-up period, its overall quality is considered low despite the fact that it produces similar findings to other studies.

CHD Study 5: Steffen and colleagues, 2003, reported on the 11-year follow-up results from the Atherosclerosis Risk in Communities (ARIC) Study, which included almost 12,000 men and women, aged 45-64 years at baseline and with no history of CVD, diabetes or cancer [20]. They found a linear, roughly dose-dependent relationship between **fruit and vegetable intake** and incidence of coronary artery disease (CAD) (when adjusted for energy intake, age, race and sex). This amounted to a significant 40% reduction in risk for the upper compared with lower quintiles of intake (RR=0.59; 95% CI: 0.42-0.81), who consumed averages of 7.5 and 1.5 serves per day, respectively. The reduction in risk was reduced substantially to around 15% and was no longer statistically significant following additional adjustment for other possible risk and intermediary factors. In race-specific sub-group analyses, this study found a significant ≈60% reduction in CAD risk for African Americans, comparing the upper and lower quintiles of intake after all adjustments were made (RR=0.37; 95% CI: 0.17-0.80), but no reduction in CAD risk for white Americans.

The apparent racial difference in association may represent metabolic differences or cultural differences in patterns of consumption, eg, the types and serving sizes of fruits and vegetables consumed; however, it should be noted that confidence intervals were relatively large and overlapped for the two groups. A food-frequency questionnaire was used for dietary assessment, similar to that used by most other cohort studies. The community-based nature of the study makes it one of the most potentially generalisable; however, this is compromised by the limited response rate of 60%. Overall, this study provides more limited support for the hypothesis under study.

CHD Study 6: Rissanen and colleagues, 2003, conducted a study in Finland (the Kuopio Ischaemic Heart Disease (KIHD) Risk Factor Study) comprised of just under 2000 men, aged 42-60 years and with no history of CVD at baseline. Using data from 12.8-years of follow-up, men in the upper quintile, who consumed at least 400g per day of **fruit and vegetables,** had a statistically significant ≈57% reduction in CVD-related death relative to those in the lowest quintile who consumed less than 133g daily (RR=0.43; 95% CI: 0.24-0.76, adjusted for age and education). This result was slightly attenuated to a relative risk of 0.56 (p=0.05) with additional adjustment for smoking and alcohol intake, and was still substantial (RR=0.61), but no longer statistically significant, after further adjustment for possible intermediary factors (lipids, diabetes, blood pressure, BMI and cardiovascular fitness). After adjustment for intakes of nutrients in fruits and vegetables (vitamin C, E, beta-carotene,

lycopene, folate, fibre), fruits and vegetables continued to carry a 34% reduction, leading the authors to comment constituents other than these are likely to contribute to the protective effect of fruits and vegetables. The authors reported that intakes of folate and vitamins C and E accounted for 36% of the protective effect of fruits and vegetables on CVD mortality. Four-day diet records were used as measures in this study, which minimises the possibility of misclassification bias. Fruits and vegetables were all included except potatoes. One methodological consideration in interpreting findings is that this study did not adjust for fat intakes. Baseline estimates showed saturated fat intake to be significantly lower (≈ 5%) in the upper compared with the lower quintiles of fruit and vegetable intake, but this small difference could not account for the entire observed association with CVD mortality. The representative nature of the sample and the excellent response rate (83%) make the results from this study highly generalisable to those with similar diets. Overall, this study is consistent with the other cohort study of mortality in providing evidence for an inverse association between fruit and vegetable consumption and death from CVD.

CHD Study 7: Genkinger and colleagues, 2004, combined data from two studies previously conducted on over 6000 adult residents in the U.S. state of Maryland [21]. They were 30-93 years at baseline in 1974 and did not exclude those with evidence of CVD or other diseases. Adults in the upper quintile of **fruit and vegetable** intake, consuming on average approximately 5 serves daily, had a significant 30% lower mortality from CVD compared with those in the lowest quintile consuming just under one serve daily (RR=0.71; 95% CI: 0.51-0.98 when adjusted for age and energy). Additional adjustment for other potential risk and intermediary factors attenuated this estimate only slightly (to ≈25%), but it lost its statistical significance. The reduced risk of death due to CVD was similar for both men and women, "ever" and "never" smokers, and across a range of body mass indices. A protective effect was also suggested (but not significant) when considering only intake of **cruciferous vegetables.** Those with the highest intakes (half a serve daily, on average) had a non-significant reduction in CVD mortality, 17%-11%, depending on the number of potential confounders included in the model. The small range of intakes for cruciferous vegetables undoubtedly limited the utility of these analyses. Although the results did not support a strict dose-response relationship within the narrow range of daily intakes, a rough extrapolation from these findings suggests that a 20-35% reduction in risk per serve would not be inconsistent with the results of this study, which would make this group of vegetables particularly cardioprotective.

While this study did not exclude participants with baseline histories of CVD, analysis showed results to be unaffected by baseline history of MI. Several potential dietary and lifestyle confounders were not considered, and these unmeasured factors may partially explain the findings of this study. The generalisability of the study's findings is uncertain, because the cohort was based on a non-random sample. Findings may have been biased towards the null as the food frequency assessment method did not have high validity for measurement of vitamin C and folate, suggesting that it may not have accurately captured intakes of fruits and vegetables. Nevertheless, the data support an inverse association between fruit and vegetable consumption and CVD mortality.

CHD Study 8: Tucker and colleagues, 2005, conducted a prospective study of 501 men aged 30-80 years at baseline and with no evidence of angina pectoris or MI (Baltimore Longitudinal Study of Ageing), also in the U.S. state of Maryland. After 18 years of follow-up, they observed a significant reduction in risk of CHD mortality of 21% for each daily serve of **fruits and vegetables** (RR=0.79; 95% CI: 0.69-0.92), when adjusted for a number of risk and intermediary factors. Further adjustment for additional dietary factors and secular trend further attenuated the risk reduction, and it became non-significant. **Fruit** consumption alone contributed minimally to the findings, revealing at most only a non-significant 14% reduced risk of CHD mortality. In contrast, each additional daily serve of **vegetables** was associated with a substantial and significant (27%) reduction in risk of death from CHD, after consideration of most possible confounding factors. Without adjustments, the risk reduction observed was larger (≈40%) (RR=0.60; 95% CI: 0.46-0.78).

Tucker and colleagues argue that statistical adjustment for saturated fat intake may understate the true contribution of fruits and vegetables to risk reduction for CHD mortality. They reported no significant interaction ($p>0.10$) between saturated fat and fruit and vegetable intakes, however they showed the effects of both were additive, and suggested that they probably reflect different mechanisms for reducing risk of CHD. Based on the most adjusted results, either a diet low in saturated fat (defined as <12%) with low fruit and vegetable intake (<5 serves/day), or a diet high in saturated fat (12+%) and high in fruit and vegetable intake (5+ serves/day), was associated with a 54-59% lower risk of CHD mortality. However, compared with consuming a diet low in vegetables and high in saturated fats, those consuming a diet low in saturated fat and high in fruits and vegetables experienced a 67% reduction in CHD mortality (RR=0.37; 95% CI: 0.16-0.81).

This study used a method that produces high-quality dietary measures (multiple food records), which would minimise misclassification. The study also employed an analytical strategy to ensure no temporal ambiguity, by excluding diet records within two years of a coronary outcome because diet may be affected by the disease process. Overall, this study was methodologically sound, and therefore the conclusions it presents are likely to be valid.

CHD Study 9: Liu and colleagues, 2001, also conducted analyses on data from the Physician's Health Survey, with involved 12-year follow-up of over 15,000 male physicians in the U.S. [16]. They found men with the highest quintile of **vegetable** intake (2.5 or more serves daily) had reductions in CHD risk and first MI of approximately 20% compared with men with the lowest intakes (<1 serve daily). The relationship was statistically significant for CHD incidence (RR=0.71; 95% CI: 0.57-0.89) but not for first MI (RR=0.79; 95% CI: 0.55-1.16). Interestingly, this study noted that the inverse association between CHD incidence and vegetable consumption among those who were overweight or obese ($BMI \geq 25 kg/m^2$) was similar to that observed for the whole sample, but a stronger, statistically significant 60% reduction was observed among smokers (RR=0.41; 95% CI: 0.18-0.97). Some residual confounding is possible, as this study did not adjust for intakes of energy or fatty acids. If anything, this study is likely to have underestimated the inverse association with vegetable intake, as the measurement tool did not demonstrate high validity, leading to possible misclassification of dietary exposures. The fact that complete dietary data were only

available for 69% of the cohort also introduces the possibility of some selection bias, which introduces further concern about the validity and generalisability of the results.

Observational Studies: Case-control Studies

Overview

Four case-control studies were located [24-28]. These measured the risk of CHD associated with fruits, vegetables, or combined fruits and vegetables. Five out of six associations tested showed an inverse relationship with CHD, and all were statistically significant. One study reported a significant, positive association between fruit intake and CHD (see Table 1).

Like cohort studies, case-control studies are observational and hence statistical adjustment for potential confounding is a necessary part of the analytic process. They also have some additional limitations, chiefly being subject to recall bias, since the onset of heart disease may result in cases differently recalling their intake of fruits and vegetables than controls. This bias would overestimate a putative protective effect of fruits and vegetables if cases recalled and reported lower than actual intake of fruits and vegetables than controls did, or it would underestimate the protective effect if cases tended to over-report their consumption compared to controls. Also, where studies have looked at intake immediately prior to CHD onset, there can be a reduced intake of all foods including fruits and vegetables, which would lead to an over-estimation of the inverse relationship between fruit and vegetable consumption and CHD.

Fruits and Vegetables

CHD Study 10: Yusuf and colleagues, 2004, reported results form a multi-centre case-control study conducted in 52 countries across Western, Eastern and Southern Europe, the Middle East, Africa, Asia, Australia & New Zealand, North, Central and South America [26]. Analyses included 15,152 cases ascertained from coronary care units in hospitals and 14,820 age- and sex-matched controls from either the same hospitals or the surrounding communities. Analyses addressed combined **fruit and vegetable** intake and revealed a significant, 30% reduction in CHD risk with daily consumption (OR=0.70; 95% CI: 0.64-0.77), compared with less frequent consumption. Findings were consistent across the regions studied and for those younger ($\leq$55 years for men or $\leq$65 years for women) and older (56+ years for men or 66+ years for women). The report gave few details regarding the method for assessing fruit and vegetable intake, and the actual amount of intake that was associated with a risk reduction is unknown. However, the stability of this finding across regions implies that the risk reduction applies to levels and types of fruit and vegetable intake that are achievable in many populations. Further, any error in quantifying fruit and vegetable intake by the measurement instrument is likely to reduce the chance of finding a significant protective effect of fruits and vegetables, and therefore should not discount the findings of this study.

CHD Study 11: Martínez-Gonazález and colleagues, 2002, conducted a hospital-based, case-control study in three hospitals located in Pamplona, Spain [24, 25]. Cases were 171 patients diagnosed with non-fatal acute MI and controls were 171 sex- and age-matched patients from the same hospital. In one set of fully-adjusted analyses [24, 25], they found a

significant 80% reduction in CHD risk (around 80%) for the top quintile (consuming an average of 750g/day) relative to the bottom quintile (consuming an average of 100g/day) of **fruit** (OR=0.20; 95% CI: 0.05-0.78). Importantly, a significant, inverse association was observed for all quintiles above baseline, giving an aggregate, statistically significant 75% reduction in risk of acute, non-fatal MI (OR=0.25; 95% CI: 0.08-0.74) for those consuming a median of 300g of fruit per day or more. Similar results were obtained in another set of analyses [24], showing a statistically significant 65% reduction in MI risk among those consuming at least 175g/day of fruit (OR= 0.35; 95% CI 0.14-0.89); however further adjustment for a list of other foods and food groups, which were highly correlated with fruit intake, attenuated the results substantially. This study used a measure of fruit and vegetable intake that included only fresh fruits, so findings cannot be generalised to other, processed fruits. Furthermore, a median daily intake of 300g in the middle quintile of this population is large, compared with typical intakes in Australia and New Zealand.

The results for **vegetables** in the Spanish case-control study provided more limited evidence for an inverse association with risk of non-fatal MI [24, 25]. In one analysis, the odds of MI were reduced by a non-significant 20% comparing the top quintile (median 950g/day) with the bottom quintile (median 278g/day) of vegetable intake (OR=0.80; 95%CI: 0.20-3.13, when fully adjusted) [24]. In the same paper, results differed only slightly across quintiles 2-5, but even the aggregated risk reduction for quintiles 3-5 failed to reach statistical significance. In the other report [25], odds of non-fatal MI were reduced by 55% when comparing quintiles 2-5 of daily vegetable consumption (347g/day or more) to the lowest quintile (OR=0.45; 95% CI: 0.21-0.98); however results were attenuated and became non-significant when further adjusted for other dietary characteristics. There is little explanation for the differences in results observed between the two sets of analyses published from this study, although adjustment for different sets of covariates remains one possibility. As with fruit intake, it should be noted that those in the lowest quintile were consuming an average of nearly 300g of vegetables daily, which is similar to the upper quintiles of intake in other studies and approaches recommended daily intakes for Australians. It is possible that the quantities of vegetables consumed in this population were too high to observe the putative protective effect on risk of CHD.

CHD Study 12: Rastogi and colleagues, 2004, conducted a hospital-based, case-control study in the urban areas of Delhi and Bangalore, India [27]. They included 350 cases diagnosed with acute MI and 700 non-cardiac patients as controls matched on age, sex, and hospital. An intake of more than 3 serves of **fruit** per day compared with one or fewer was associated with a 1.5-2.5 increased risk of acute MI; the positive association increased in magnitude and statistical significance with adjustment for additional covariates (OR=2.46; 95% CI: 1.15-5.25 from most adjusted model). One to three serves of fruit per day were positively associated with 45-30% increased odds of MI, but were not statistically significant. In contrast, a strong, statistically significant inverse association was demonstrated for **vegetable** intake. Consuming three or more compared with one or fewer serves of vegetables per day was associated with a 70% reduction in MI risk (OR=0.33; 95% CI: 0.13-0.82 from most adjusted model). A similar result was observed when green leafy vegetables were analysed separately (OR=0.34; 95% CI: 0.17-0.69 for >3 compared to <1 serve per day).

Due to the lack of an adequate dietary database for Indian foods, only limited adjustment for dietary covariates was possible, which did not include adjustment for total intakes of nutrients and energy. Use of mustard oil was the only fat variable associated with CHD in this study, and cereal intake was the major source of energy in this population. The magnitude of odds ratios in both sets of analyses suggests that these findings cannot be entirely explained by residual confounding, but the positive association indicating increased risk of MI with high fruit consumption is anomalous. The composition of diet also included many fruits and vegetables typical to India but not necessarily elsewhere. Hence the results of this study may not be generalisable to Australia and New Zealand, which both have population tendencies to over-nutrition.

CHD Study 13: Tavani and colleagues, 2004, combined data from three, hospital-based, case-control studies conducted in Northern Italy between 1983 and 1999 [28]. Analyses included a total of 1,713 cases comprised of patients diagnosed with non-fatal acute MI and 2317 controls comprised of patients with diagnoses unrelated to smoking or other MI risk factors and roughly matched on age, sex, and hospital. Only the association with **vegetable** intake was addressed. Among all participants, low consumption of vegetables (defined as <7.5 'portions' per week) was significantly associated with an increased risk of CHD (OR=1.26; 95% CI: 1.08-1.46); this is equivalent to a 20% reduced risk of MI with higher intakes of vegetables. Similar results (representing reductions of 22-26%) were observed among those reporting a family history of CHD (OR=1.35; 95% CI: 1.01-1.80) and those not (OR=1.29; 95% CI: 1.07-1.55). It is unclear whether the 'portions' used reflect a standardised, quantitative measure or are determined by respondents. Limited detail was provided on the measurement of vegetable intake, which leaves the possibility of misclassification of exposure and an underestimation of the protective effect of vegetable intake. Most potential confounders were considered by adjustment; however energy intake, total fat intake and intake of fatty acids were not. These factors cannot be ruled out as contributing to the findings of this study.

Meta-analysis

A meta-analysis [29] pooled results of seven studies of vegetable intake and CHD and eight studies of fruit intake and CHD, which were conducted between 1984 and 1999 in Europe and the US. Both incidence and mortality outcomes were included, as were all kinds of study designs (clinical trial, cohort, case-control, and cross-sectional), although the majority were prospective cohort studies. None of the results previously described in this section were included in this report due to the non-overlapping nature of the publication dates; however, earlier results from some of the studies likely contributed to the meta-analysis findings. The pooled results showed significant 20% and 15% reductions in risk of CHD, respectively, for those consuming the highest levels of vegetables (OR=0.77; 95% CI: 0.70-0.86) and fruits (OR=0.86; 95% CI: 0.77-0.96). Importantly the magnitude of these pooled relative risks were virtually unchanged whether they were adjusted for 'primary factors' (age, energy, smoking and 'dietary factors') or 'secondary factors' (use of supplements, family history, presence of many traditional coronary risks, physical activity, menopausal status, education, or use of aspirin). As the quantities consumed by the upper and lower quintiles are not presented in this analysis, the magnitude of the pooled relative risk can only be said to pertain to high compared with low intakes of fruits and vegetables in diets typical to Europe and the US.

Summary of the Evidence for an Association with CHD

The majority of the evidence supports an inverse association between fruit and/or vegetable consumption and CHD. The magnitude of the putative protective effect has not been quantified in this review, nevertheless, the overall consistency of results, irrespective of specific outcome studied, absolute quantities of fruits or vegetables consumed, or location of the research is noteworthy. The nature of the relationship does not appear to follow a strict dose-response pattern, but generally does show additional risk reduction with increases in fruit and vegetable intake. All of the studies addressing incidence or mortality from CHD were observational. These studies were generally high quality, although sometimes studies did not adjust for potential confounding factors (such as energy intake or physical activity) or adjusted for factors that may be intermediaries on a causal pathway between intake of fruits and vegetables and risk of CHD. For example, fruits and vegetables are generally low in energy, sodium, saturated fat and total fats. Therefore part of a protective mechanism of a high intake of fruits and vegetables may include reduction in obesity, BP and serum lipids as a result of replacing "less healthy" alternative foods in the diet. Consequently, adjustments for serum cholesterol, BP, and body mass index may be 'over-adjustments' removing part of the beneficial effect of fruit and vegetable consumption along with other confounding influences. Generally the biases in the studies were more likely to under-estimate than to over-estimate the inverse relationships between intake of fruits and vegetables and CHD. The only evidence of possible 'harm' was an observed positive relationship between fruit intake and CHD in a study from India, which may stem from differences in factors surrounding a high intake of fruits in this population. While none of the observational data could establish manipulation of the independent variable and reversibility (and therefore causality), the cohort studies were able to establish temporal sequence between intake of fruits and vegetables and outcomes related to CHD.

Table 1: Summary of findings related to association between consumption of fruits and vegetables and oucomes related to coronary heart disease

Study	#	Location	Design	Size	Duration	Incidence/ Mortality	Fruits & Vegetables	Fruit Only	Vegetables Only	Subgroup Analysis
WHS	1	US	cohort	39,876 women 84,251 women	5 years	I	inv +	inv *	inv, ns	inv *
NHS & HPS	2	US	cohort	42,158 men	8 years	I	inv *	inv*	inv *	inv*
NHANES/						I	inv +	N/A	N/A	
NHEFS	3	US	cohort	9,608 adults	16.6 years	M	inv *	N/A	N/A	
HFS	4	UK	cohort	10,741 adults	18-24 years	M	N/A	inv*	N/A	inv*
ARIC	5	US	cohort	11,940 adults	11 years	M	inv *	N/A	N/A	inv*
KIHD	6	Finland	cohort	2641 men	12.8 years	I	inv *	N/A	N/A	
Odyssey	7	US	cohort	6,151 adults	13 years	M	inv *	N/A	N/A	inv, ns
BLSA	8	US	cohort	501 men	18 years	M	inv *	inv, ns	inv *	inv *
PHS	9	US	cohort	15,220 men	12 years	I	N/A	N/A	inv *	inv *
INTERHEART	10	52 countries	case-control	15,152 cases 14,820 controls	N/A	I[a]	inv *	N/A	N/A	inv*
Pamplona Hospital Study	11	Spain	case-control	171 cases 171 controls	N/A	I[a]	N/A	inv *	inv +	n/a
India Multi-centre Study	12	India	case-control	350 cases 700 controls (women)	N/A	I[a]	N/A	pos *	inv *	
Three Italian Case-Control Studies	13	Italy	case-control	1,713 cases 2,317 controls	N/A	I[a]	N/A	N/A	inv *	inv *
TOTAL – cohort studies						5 incidence 4 mortality	8/8 inv 8/8 significant	4/4 inv 3/4 significant	4/4 inverse 3/4 significant	7/7 inverse 6/7 significant
TOTAL – case-control						4 incidence 0 mortality	1/1 inv 1/1 significant	1/2 inv 2/2 significant	3/3 inverse 3/3 significant	2/2 inverse 2/2 significant
TOTAL – all studies						9 incidence 4 mortality	9/9 inv 9/9 significant	5/6 inv 5/6 significant	7/7 inverse 6/7 significant	8/8 inverse 7/8 significant

WHS=Women's Health Study; NHS=Nurses' Health Study; HPS= Health Professionals' Study; HFS=Health Food Shoppers Study; ARIC=Atherosclerosis Risk in Communities Study; KIHD=Kuopio Ischemic Heart Disease Study; BLSA=Baltimore Longitudinal Study of Ageing; PHS=Physician's Health Study

I=Incidence; M=Mortality

+ = statistically significant in some strata but not highest; *= statistically significant in highest stratum or per serve; ns= not statistically significant; N/A= not assessed

[a] these case-control studies included incident cases of non-fatal MI only; deceased cases were not included

Mechanisms of Action

There is considerable overlap between the current models of CHD aetiology and the biological activity of compounds in fruits and vegetables, which would support a role for a diet high in fruits and vegetables in reducing the risk of CHD. Fruits and vegetables contain many compounds that are thought to be beneficial in preventing CHD and other degenerative diseases, through multiple mechanisms. Fruits and vegetables contain dietary fibre, antioxidant vitamins (vitamins C and E, beta carotene, selenium) and antioxidant phytonutrients, such as flavonoids, carotenoids, and polyphenols. In addition to cardiac benefit from ingesting beneficial substances, benefits of fruit and vegetable consumption may also stem from concurrent displacement from the diet of substances that may contribute to CHD, as fruits and vegetables do not contain large amounts of fats (particularly saturated fat), energy, cholesterol or sodium.

Aetiology of CHD

The main cause of CHD is atherosclerosis [30]. Activation of the atherosclerotic plaque and formation of a thrombus which occludes blood flow to the myocardium of the heart comprise the chief cause of heart attack (or MI) [31]. Prevention of the underlying atherosclerosis and plaque instability are therefore at the core of current efforts to prevent CHD. The mechanisms of plaque formation and rupture are still under research. The most widely accepted theory is the "response to injury" theory, which postulates that injury to the endothelium lining the arteries causes vascular inflammation, and a fibroproliferative response. Many factors are likely to injure the epithelium including oxidized LDL-cholesterol (referred to as LDL for brevity), some infectious agents, toxins (including those from cigarette smoking), hyperglycemia, and hyperhomocystinemia. Monocytes enter the blood vessel wall, take up LDL and form the foam cells seen in early atherosclerosis, which produce factors that injure the endothelium. Most people have a "fatty streak" by age 20 years which is an accumulation of serum lipoproteins, foam cells, T-lymphocytes and smooth muscle cells. Smooth muscle cells form a protective fibrous cap over the contents of the fatty streak, but this cap can be 'denuded' exposing the underlying contents to the circulating blood. The progression of atherosclerotic lesions to a MI usually involves these unstable plaques eroding or rupturing, and platelets adhering to the site, becoming activated, aggregating, and forming a thrombus [32].

Altered cholesterol metabolism

In addition to fruits providing a replacement in the diet for fattier, higher cholesterol foods, intake of dietary fibres from fruits and vegetables has been shown to lower cholesterol in humans [33]. Both total cholesterol and LDL may be reduced by dietary fibre [34]. Experimental studies show that high levels of insulin may promote dyslipidemia [35], and fibre decreases the glycemic and insulinemic response to foods by delaying carbohydrate absorption after meals [36, 37].

Lowering of blood lipids is important in preventing CHD. Choy et al. [38] make the claim

> "Results from both clinical and animal studies show that a high level of LDL in the blood may be the single most important risk factor in the production of atherosclerosis." (p214)

High serum lipid levels, especially high levels of LDL, relate strongly to the development of atherosclerosis [38]. Evidence that LDL in atherosclerotic plaques has a causal role, or is clinically significant, is provided by randomised clinical trials that show lowering lipids reduces coronary events [39].

Reduced oxidative modification of LDL

Antioxidants include manganese, selenium, zinc, vitamin C, vitamin E, beta-carotene and phytonutrients (lutein, lycopene, b-cryptoxanthin, flavonoids, isoflavones), most of which are contained in fruits and vegetables [33]. Antioxidants have been hypothesised to prevent CHD by reducing the oxidation of LDL [40]. Antioxidant intake has been shown to inhibit oxidation of LDL in animals (in vivo) and humans (ex vivo), but the reduced ex-vivo oxidation of LDL associated with antioxidants may have limited biological relevance [40]. The effect of antioxidants on LDL oxidation within the atherosclerotic plaque is somewhat unpredictable, as various oxidative processes occurring within the plaque are likely to be affected by different antioxidants [41]. Which antioxidants or combination of antioxidants, if any, can reduce the oxidation of LDL in the formation and progression of CHD is not definitively established and still under research.

An ability to prevent the oxidative modification of LDL may be relevant to preventing CHD, as the oxidative modification of LDL plays a substantial role in CHD development. In the formation of foam cells, LDL is modified before uptake by macrophages [38] in large amounts, since this process is not regulated by negative feedback mechanisms [42, 43]. LDL can be modified by various processes including oxidation [44]. Oxidatively-modified LDL is involved in other aspects of CHD development as it affects the regulation of vascular tone, activation of inflammatory responses and platelet aggregation [38]. However, there is question as to whether the oxidation of lipids and proteins in CHD is causal, as opposed to being a consequence of the disease process [41]

Effect on platelet activation and aggregation

Through a reduction in the insulineamic response, dietary fibre from fruits and vegetables may reduce the formation of atherosclerotic lesions, as high levels of insulin have also been shown to promote abnormalities in blood-clotting factors and atherothrombosis [35]. Substances in fruits and vegetables other than fibre have also shown a capacity to reduce the aggregation of platelets. Pharmacologic studies have shown garlic to inhibit the adhesion and aggregation of platelets [45] and these findings are also substantiated in research on humans [46, 47]. Garlic is believed to inhibit platelet aggregation by suppression of thromboxane B2 synthesis and alteration of platelet lipoxygenase and cyclooxygenase activities, and flavonoids present in some fruits and vegetables have also demonstrated a capacity to act in a similar manner [48].

Reduction in abnormal blood platelet aggregation may be important in preventing CHD. Platelet activation and aggregation play key roles in the process of arterial thrombosis [49], although the mechanisms by which these occur are not fully known. Patients with MI often have platelet 'hyperaggregability' [49] and prospective studies find risk of recurrent MI is increased by platelet hyperaggregabilty [50]. Further, the role of platelet aggregation appears causally important in the development of CHD, as inhibiting platelet aggregation successfully prevents arterial thrombotic events

including MI. For example, aspirin suppresses platelet aggregation (by blocking platelet thromboxane A2 synthesis) and prevents both CHD and stroke [51].

Reduced BP

Studies have shown intake of fruits and vegetables also reduce arterial BP [52, 53]. There is biological plausibility for this relationship, although there is no certainty as to which factors are most important, or whether all factors have been identified. Dietary factors have been shown to contribute to modulating endothelial dysfunction and subsequent elevated BP. Some of these factors are present in fruits and vegetables, including vitamins C and E, folic acid, coenzyme Q-10 (also found in meat and fish), which have been shown to have a beneficial effect on endothelial function and possibly in preventing CVD [54, 55]. Dietary fibre, magnesium, the high potassium/low sodium content of fruits and vegetables also appear to be important [56].

In a review, Houston [56] claims that antioxidant deficiency and excess free radical production have been linked to hypertension in numerous epidemiological, observational, and intervention studies. He further claims that the initiation, maintenance, pathogenesis, pathophysiology, and cardiovascular complications of hypertension may result from oxidative stress with an imbalance between reactive oxygen species and the antioxidant defense mechanisms [56]. People with hypertension have an impaired antioxidant defense mechanism, more oxidative stress with more reactive oxygen species produced and a greater-than-normal response to oxidative stress [56]. Thus antioxidants present in fruits and vegetables may be useful in preventing and treating hypertension and therefore reducing risk of CHD. Empirical support that antioxidant vitamins, particularly vitamin C, are potentially important components of fruits and vegetables for lowering BP can be found in studies that show hypotensive effects among people with hypertension [57], vitamin C supplements lowering BP [58] and improved vasodilation of coronary arteries in people with hypertension [59].

The high-potassium, low-sodium content of fruits and vegetables may be part of the mechanism by which fruits and vegetables lower BP. In both epidemiological and clinical trials, an increased sodium intake is associated with higher BP [60]. Reversibility is evident as restriction of sodium intake leads to a reduction in BP in people with hypertension, especially those who are ‘salt sensitive’ [61-64]. Increased dietary intake of potassium (abundant in fruits and vegetables) also has been shown to reduce BP in epidemiological studies and clinical trials [65, 66]. The magnesium content of fruits and vegetables may also provide a mechanism for lowering BP, as in many (but not all) studies, increased dietary magnesium intake is associated with a decrease in BP [56].

The evidence around fibre intake and reduced BP is not consistent (Houston, 2005). However, researchers have proposed many biological mechanisms by which dietary fibre might reduce BP: by improving insulin sensitivity, reducing endothelial dysfunction, reducing intravascular volume by increasing the excretion of sodium in the urine, decreasing sympathetic nervous system activity, reducing oxidised LDL, and by reducing the hypertriglyceridemia, hyperglycemia, endothelial dysfunction, and vasoconstriction that occurs after consuming high-fat meals [67-70].

The reduction in BP associated with fruit and vegetable intake may have a substantial capacity in preventing CHD. Trials show reduction in BP reduces strokes, heart failure and CHD, irrespective of the methods used to lower BP [71]. A target BP under 140/90 mmHg is considered appropriate for primary and secondary prevention in the general population, with a lower target for people with diabetes or renal parenchymal disease [72].

Homocysteine

Intake of fruits and vegetables, folate-rich vegetables, and folate have been associated with reduced circulating levels of homocysteine [73]. Manipulation of diet in interventions with healthy volunteers has shown that homocysteine can be reduced by supplemental folate intake [74] and intake of folate-rich fruits and vegetables [75].

The mechanisms by which reducing elevated levels of homocysteine may be important in preventing CHD are under investigation. There is some support to the possibilities that elevated homocysteine disrupts endothelial function [76, 77], proliferates smooth muscle cells [78, 79], increases cholesterol synthesis in the liver [80, 81] and is involved in LDL oxidation and thrombus formation [73]. In a review of folate and CAD prevention, Musiket [82] cautions that it is neither proven nor disproved whether mild elevation in homocysteine is a modifiable risk factor or a result of CAD, awaiting further intervention trials for confirmation. The author concludes the role of folate in primary and secondary prevention of coronary artery disease is insufficiently known to warrant supplementation or fortification but recommends dietary intake to recommended levels has relevance to coronary artery disease prevention.

Summary on mechanisms

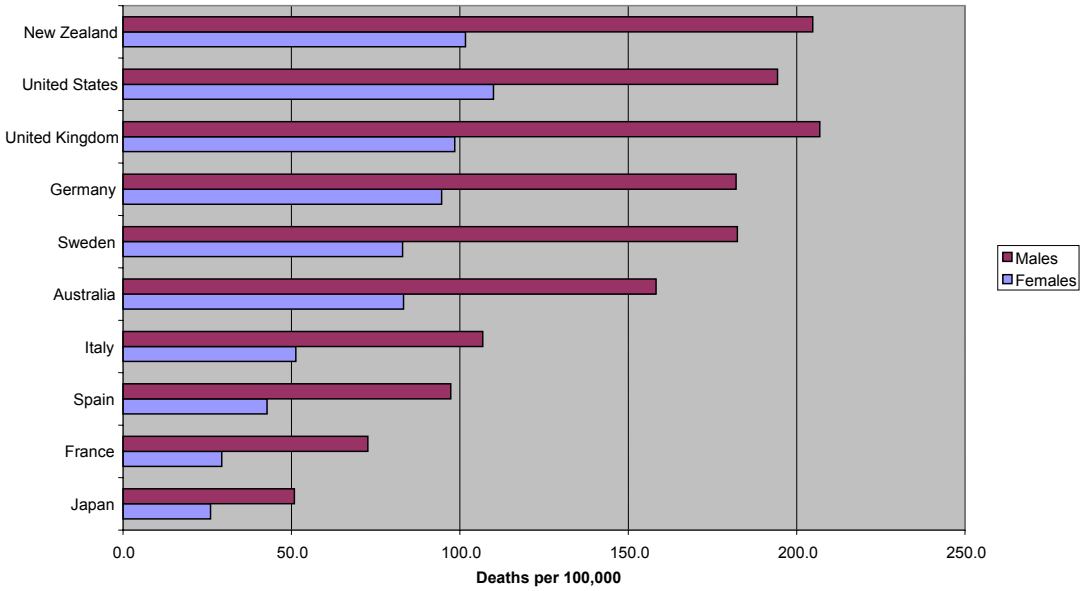
In summary, there is no consensus as to whether all the beneficial substances contained in fruits and vegetables have been identified, nor whether the multiple roles of each of the known bioactive substances in fruits and vegetables have been identified. However the existing evidence does provide biological plausibility to the epidemiological findings of a preventive role for consumption of fruits and vegetables in the development of CHD through one or more of the above mechanisms.

Part 3: Relevance of the findings to Australia and New Zealand

Coronary Heart Disease

Prevention of CHD is relevant to the Australian and New Zealand populations, for whom it is the most common cause of death [83]. Australia and Zealand are not atypical of the countries in which the reviewed studies were conducted. In New Zealand, mortality rates for CHD are slightly higher than in Australia, and similar to rates in the United States (see Figure 1.) The reviewed studies were mostly conducted in the United States and Europe, in countries with both higher and lower mortality rates due to CHD than in Australia.

Figure 1: Death rates for CHD among OECD countries, 1999



Source: Australian Institute of Health and Welfare, 2005. Chronic Diseases and Associated Risk Factors - International Comparisons. [online] http://www.aihw.gov.au/cdarf/data_pages/oecd/index.cfm (10/11/2005)

Consumption of Fruits and Vegetables

Types

The results of the studies apply to types of fruits and vegetables usually available and common in Australia and New Zealand, however they do not necessarily include all of the fruit and vegetable products currently sold in Australia and New Zealand. The main dietary assessment methods in the studies were food frequency questionnaires, 24-hour recall, diet history and weighed records. The few studies that used diet history, recall and weighed record methods would most likely have included any fruit or vegetable (other than those explicitly excluded), whether purchased fresh, frozen or canned and whether consumed raw or cooked, alone or in mixed dishes. These methods collect data about meals consumed and translate this information into estimates of food and nutrient intake, usually disaggregating mixed foods into their constituents. The food frequency questionnaires that were more often used generally

did not specify whether responses were to include fruits and vegetables that were frozen or canned as well as fresh fruits and vegetables, but generally excluded mixed dishes. Additionally, the intervention studies (discussed in Part 4) generally provided participants with minimally processed fruits and vegetables to achieve improvements in biomarkers of CHD. Both studies of CHD risk and biomarkers usually stated or implied whole fruits and vegetables rather than juices of fruits and vegetables. Notable exceptions include Jopshipura et al. [17] who had null findings for citrus juice, and the DASH study interventions which included both fruit and fruit juices in their intervention and had favourable findings. Potatoes, including those consumed as french fries, were often excluded from measures of vegetable intake, and the studies that examined the relationship between potato intake and the risk of CHD reported null findings [17, 27].

Types of vegetables commonly consumed in Australia (Table 2) and New Zealand (Table 4) were typically included in the reviewed studies, except potato which tended to be excluded by studies but is commonly consumed in both countries. The types of fruits typically included in studies were also commonly consumed by Australians and New Zealanders (Tables 3 and 5, respectively); however most of the studies excluded fruit juices, which also are commonly consumed in the region.

Table 2: Types of vegetables consumed by Australian adults (NNS 1995)

	Men	Women	Inclusion in reviewed studies
Potatoes, eg. cooked potato, hot potato chips, mashed potato, potato salad	37%	29%	often excluded
Tomato	14%	15%	tomatoes, tomato juice
Other fruiting vegetables, eg. pumpkin, zucchini, avocado, cucumber, eggplant	10%	13%	eggplant
Other, eg. corn, mushrooms, garlic, onion, mixed vegetables, coleslaw	10%	10%	coleslaw, corn, celery, mushroom, mixed vegetables
Leaf and stalk vegetables, eg. lettuce, spinach, bean sprouts	7%	9%	green leafy vegetables', 'other greens', 'dark green lettuce salad', 'green salad', mustard greens, turnip greens, collards, spinach
Peas and beans, eg. green beans, peas, snow peas	7%	7%	beans (including baked beans), pintos, kidney lima or in chilli, green beans, peas, lentils
Brassicae, eg. cabbage, cauliflower broccoli	7%	9%	broccoli, cauliflower, 'cruciferous vegetables', cabbage, sauerkraut
Carrot and root vegetables, eg. carrot, beetroot, parsnip, radish, sweet potato	8%	8%	'dark-yellow vegetables', sweet potatoes, yam, carrots, mixed veg. with carrots, beet

Source: A NSW Centre for Public Health Nutrition, 2003. Report on the consumption of vegetables and fruit in NSW 2003, State of Food and Nutrition in NSW Series NS NSW Department of Health. [online] http://203.5.110.172/pubs/r/pdf/report_vegies_fruit.pdf (10/11/2005)

Table 3: Types of fruits consumed by Australian adults (NNS 1995)

	Men	Women	Inclusion in reviewed studies
Pome, eg. fresh pears, apples, canned apples	28%	29%	apples, applesauce, pears
Tropical, eg. banana pineapple, mango, pawpaw	21%	21%	banana
Other, eg. melons, grapes, dates, passionfruit	19%	19%	cantaloupe, fruit cocktail
Stone, eg. apricot, cherry, peach, plum	13%	14%	peach
Citrus, eg. orange, canned grapefruit, lemon peel	16%	13%	orange, grapefruit
Dried, eg. sultanas, banana chip, dried peach	2%	2%	raisin, prune
Berry, eg. blackberry, blueberry, strawberry	1%	2%	strawberry, blueberry

Source: A NSW Centre for Public Health Nutrition, 2003. Report on the consumption of vegetables and fruit in NSW 2003, State of Food and Nutrition in NSW Series NS NSW Department of Health. [online] http://203.5.110.172/pubs/r/pdf/report_vegies_fruit.pdf (10/11/2005)

Table 4: Vegetables consumed by New Zealanders aged 15+ years (NNS 1997)

Proportion with at least weekly consumption		Inclusion in reviewed studies
Potato, carrot, tomatoes, lettuce, onions/ leeks, peas, cabbage/ coleslaw	Most (60-100%)	Often excluded potato, sometimes excluded peas. Often included, eg 'dark green lettuce salad', 'green salad', carrot, dark-yellow vegetables, cabbage/ coleslaw Frozen vegetables sometimes excluded
Cauliflower, broccoli, pumpkin, hot chips, frozen mixed vegetables, green beans, cucumber, beans (inc. baked beans and lentils), sweetcorn, mushrooms, courgettes (zucchini, marrow, eggplant, squash), kumara, capsicum	Many (30-60%)	baked beans and lentils sometimes excluded Sometimes included mixed veg. with carrots, beans beans (including baked beans), pintos, kidney lima or in chilli, green beans, peas, lentils, sweet potatoes (kumara), broccoli, cauliflower, 'cruciferous vegetables'
Beetroot, celery, brussel sprouts, avocado, asparagus, sprouts, parsmip	Some (15-30%)	Did not exclude, though did not always mention Sometimes included beet
Other green leafy vegetables, turnips/ swedes, green bananas, yams, watercress, kamo kamo, taro, puha, soybeans/tofu, taro leaf, karengo, Pacific Island yams, cassava, bread fruit	Few (0-15%)	Often did not mention some vegetables specifically (but did not exclude), sometimes excluded soybeans/ tofu Often included 'green leafy vegetables', 'other greens', 'dark green lettuce salad', 'green salad', mustard greens, turnip greens, collards, spinach. Sometimes included yam

Source: 1997 NNS - Russell DG, Parnell WR, Wilson NC et al. 1999. NZ Food: NZ People. Key results of the 1997 National Nutrition Survey. Ministry of Health: Wellington. pp148-154 http://www.moh.govt.nz/moh.nsf/pages/MH852

Table 5: Fruits consumed by New Zealanders aged 15+ years (NNS 1997)

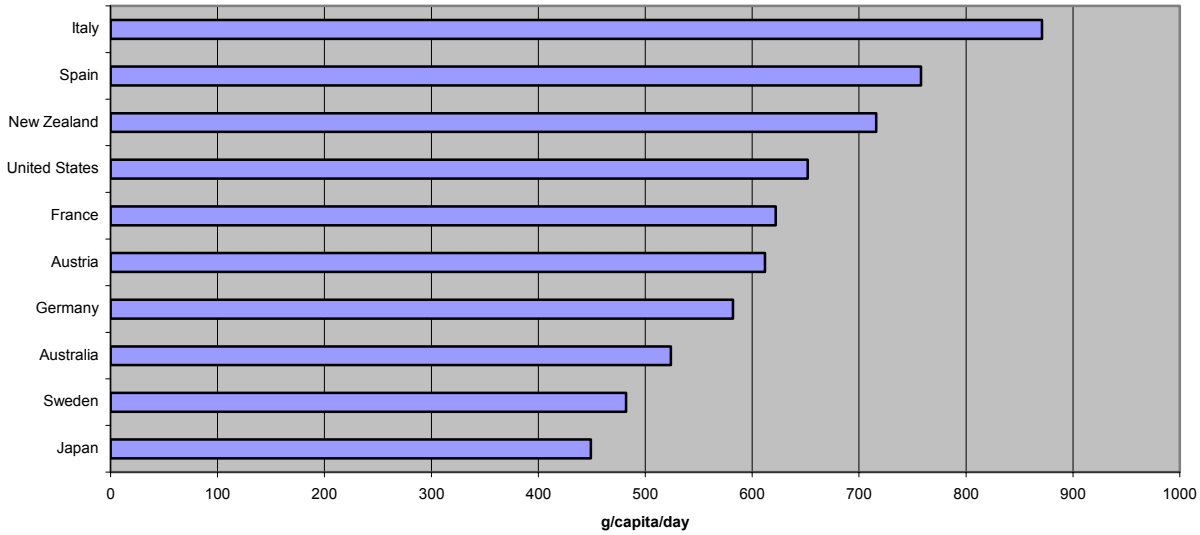
Proportion with at least weekly consumption		Inclusion in reviewed studies
Bananas, apples, oranges	Most (60-100%)	None specifically excluded Sometimes specified apples, applesauce, banana, orange/ citrus
Stone fruit (peach, nectarine, plum, apricot), pears, berry fruits (strawberry, other berries, cherries), kiwifruit, grapes	Many (30-60%)	None specifically excluded Sometimes specified peach, pears, strawberry, blueberry,
Sultanas, feijoas, melon, canned fruit in syrup, other dried fruit, stewed fruit	Some (15-30%)	Sometimes included only fresh fruit Sometimes specified raisin, prune, cantaloupe.
None listed in NNS	Few (0-15%)	

Source: 1997 NNS - Russell DG, Parnell WR, Wilson NC et al. 1999. NZ Food: NZ People. Key results of the 1997 National Nutrition Survey. Ministry of Health: Wellington. pp148-154 http://www.moh.govt.nz/moh.nsf/pages/MH852

Quantities Consumed

Figure 2 compares consumption of fruits and vegetables across OECD countries. New Zealand intakes are high and Australian intakes are more moderate to low relative to many countries. Importantly, reviewed studies that showed a reduction in CHD were conducted in populations with both higher and lower intakes than Australia and New Zealand. In both Australia and New Zealand, average fruit and vegetable intakes are below the dietary recommendations of each country. According to the last national nutrition surveys, two-thirds of New Zealanders met recommended daily intakes (three serves) of vegetables and fewer than half consumed the recommended quantity of fruits (two serves daily) [84]. Australians consumed on average 144g of fruit and 259g of vegetable products and dishes per day [85], which means that intake falls well short of recommended levels for a substantial proportion of the population. The findings of the case-control study conducted in Spain [25], in a population with very high intakes of both fruits and vegetables, showed that the reductions in CHD risk were similar for quintiles consuming on average 300g of fruits, and 550g of vegetables, in comparison with quintiles at higher intakes. At the other end of the spectrum, a study conducted in the US [17] in a population with lower consumption of fruits and vegetables found each daily serve to significantly reduce the risk of CHD. The evidence would suggest that findings from the studies in this review have relevance to Australia and New Zealand: for a substantial proportion of the Australian and New Zealand populations, an increase in dietary intake of fruits and vegetables could decrease the risk of CHD.

Figure 2: Fruit and vegetable intake among OECD countries, 2001
gram/capita/day



Source: Australian Institute of Health and Welfare, 2005. Chronic Diseases and Associated Risk Factors - International Comparisons. [online] http://www.aihw.gov.au/cdarf/data_pages/oecd/index.cfm (10/11/2005)

Lifestyle behaviours

The direction of findings was consistent across most studies, which were conducted in many different nations, with different food cultures and lifestyles. The INTERHEART study [26] showed no evidence for a difference in associations across regions and studied 52 countries across all major continents. Despite countries having different constellations of coronary risk factors, different ways of consuming fruits and vegetables, and likely differences in the nutritional quality of fruits and vegetables, a reduction in risk of CHD was consistently visible. This provides further evidence that the findings suggesting protection against CHD would be relevant to Australian and New Zealand consumers of fruits and vegetables.

Part 4: Relationship of dietary fruit and vegetable intake with relevant biomarkers of disease outcome

In discussing the biological plausibility of the associations between fruit and vegetable consumption and CHD, the potential mechanisms underlying disease development were described (see last section of Part 2). Intermediaries along the potential mechanistic pathways represent alternative outcomes of potential interest, referred to as CHD biomarkers. These include lipid levels (i.e., total cholesterol, LDL, HDL, cholesterol ratios, triglycerides), blood pressure (BP), homocysteine, C-reactive protein, among others. In this Part, we now describe findings from cross-sectional studies that addressed whether a relationship exists between fruit and/or vegetable intake and biomarkers of CHD and experimental studies that addressed whether increased intake of fruits and vegetables favourably alters these CHD biomarkers. The final three Appendices present details on the studies that used various CHD biomarkers as outcomes: Appendix 5 presents intervention studies, whereas Appendix 6 presents longitudinal observational studies, and Appendix 7 presents cross-sectional studies. As in Part 2, studies are numbered (from 1 to 18) and are also labelled with an initial, according to which outcome is being discussed (p=lipids, b=blood pressure, h=homocysteine, and c=c-reactive protein), as many studies addressed more than one biomarker.

CHD biomarkers as outcomes

Intervention Studies

Several intervention studies were conducted that assessed whether following a diet high in fruits and vegetables led to changes in biomarkers for CHD (blood lipids, BP and measures of plasma antioxidants). Since the CHD implications of plasma antioxidant capacity are least established in the literature [40, 41] these will not be discussed here. One study [86] is not considered in this review as it addressed post-prandial rather than typical levels of CHD biomarkers.

Blood lipids

All of the fruit and vegetable interventions included were conducted on adults who were either healthy or had CVD co-morbidities, but none focused on groups with existing CHD and therefore are relevant to addressing the use of fruits and vegetables in a preventive capacity. Of the studies that compared blood lipids thought to increase the risk of CHD (total cholesterol, LDL, HDL:LDL, TC:HDL or triglycerides), three showed reductions in the intervention relative to control groups for at least one subgroup studied, and one of these comparisons reached statistical significance. None of the studies reported a statistically significant increase in these lipids. Unlike the other lipid parameters, an increase in HDL-cholesterol (referred to as HDL for brevity) is thought to reduce risk of CHD. Of the studies that measured HDL, none showed a significant difference in either direction between intervention and control groups.

Biomarker study 1p: In the DASH study, **Obarzanek et al. (2001)** [87] used a single-blinded, randomised, controlled trial (RCT) to establish whether following an

intervention diet of 5.2 serves of **fruit and fruit juices** and 3 serves of **vegetables** daily reduced BP more effectively than following an otherwise similar control diet of 1.6 serves of fruits and fruit juices and 2 serves of vegetables daily for American adults with high BP (not treated by medication). 459 adults participated in this study – 146 were allocated to the fruit and vegetable intervention, 145 to the control group and 145 to another intervention (not discussed in this review). The control diet was used for a three-week run-in period. For men, they found the intervention diet led to significantly greater reductions in total cholesterol (0.18 mmol/L), TC:HDL ratio (0.23) and LDL:HDL ratio (0.19) and non-significantly greater reductions in HDL (0.03 mmol/L), LDL (0.12 mmol/L), and triglycerides (0.1 mmol/L). Among women, they found no significant reductions in lipid parameters relative to the control group; small decreases in triglycerides (0.1 mmol/L) and TC:HDL (0.04 mmol/L) were noted while small increases (0.01 to 0.05 mmol/L) were noted for the other lipid parameters. Subgroup analyses in this study showed the effect may be stronger for those with lower baseline levels of hypertension.

Some methodological issues need to be considered in interpreting these findings. Firstly, the findings may not be entirely generalisable to primary prevention in an Australian/New Zealand context. While this study randomised allocation, it did not use random recruitment, did not report participation rates, purposefully over-sampled African Americans, and did not exclude participants with CVD or CHD in general, only those who had a recent cardiovascular event. Secondly, the intervention diet may have been inadequate to expect beneficial effects. The vegetable content was low (and only 50% greater than vegetable content in the control diet), and there is no description of how much of the fruit was fresh or in juice form, meaning that intake of whole fruits may have been much less than the 5 serves consumed in total. Thirdly, some confounding is possible. The intervention group had a higher proportion of smokers (≈7%) and a lower proportion of persons who consume alcohol (≈11%), although to what extent this would modify a change in blood lipids over eight weeks is not certain. The intervention diet also had extra grains and less sucrose than the control diet, however this is unlikely to have substantially impacted findings. Finally, one cautionary note in interpreting the lack of statistical significance of findings from the DASH study is that a separate and non-comparable DASH diet was the main intervention, and the effect of this DASH diet was the primary hypothesis around which power calculations were made, not the fruit and vegetable intervention diet.

Biomarker Study 2p: Broekmans et al. (2001) [88] used a RCT to determine whether Dutch adults with a habitually low intake of fruits and vegetables (<250g daily) would have greater reductions in blood lipids following a diet high in **vegetables and fruit** (500g/d plus 200mL/d juice) compared with following a diet low in fruit and vegetables (100g/d and 0mL/d juice) for four weeks. Relative to baseline, the 24 people who consumed the diet high in fruits and vegetables had significant reductions in TC and LDL (0.7 and 0.5 mmol/L), a smaller, significant reduction in HDL (0.2mmol/L), and non-significant reductions in triglycerides and LDL/HDL ratio (≈0.2mmol/L and 0.1). Relative to the 23 controls, they had non-significantly greater reductions in all lipids (≤0.2 mmol/L).

Successful randomisation ensured treatment groups were comparable in terms of most important factors and left minimal chance for confounding. However the chance of

confounding from dissimilar baseline diets is not known, as these were not reported and the study did not use a run-in period. Some misclassification of outcome could have slightly biased estimates towards the null, as LDL was calculated rather than measured. A major issue with the way the study was conducted could possibly explain why both intervention and control groups had large significant changes in lipid parameters from baseline and why negligible additional effects of the intervention diet were noted. The fibre content of the intervention diet (56.2 g/d) and the control diet (47.9g/d) were unrealistically high (although it is uncertain whether this was a typographical error in the publication). The study was also not statistically powered to detect the small differences observed between the intervention and control groups. Generally the findings of this study are of questionable use.

Biomarker study 3p: Freese et al. (2002) [89] conducted an unblinded RCT with 72 adult volunteers from a university setting, randomly allocating them to one of four groups: high in either oleic or linoleic acid and either high in **fruits and vegetables** (440g veg, 166g berries, 204g fruit) or low in fruits and vegetables (167g veg, 0g berries, 54g fruit). After the six-week intervention, change in total cholesterol, HDL and LDL were not significantly different across treatment groups. Among those consuming the high linoleic acid diet, the 15 participants who followed a diet high in fruits and vegetables had a 0.39mmol/L greater reduction in total cholesterol, a 0.07mmol/L greater reduction in HDL and a 0.32mmol/L greater reduction in LDL compared with the 13 participants following the diet low in fruits and vegetables. Of those consuming a diet high in oleic acid, the 14 participants who followed the diet high in fruits and vegetables had a 0.02mmol/L greater increase in total cholesterol, a 0.03mmol/L lesser reduction in HDL, and a relative increase in LDL of 0.33 mmol/L, compared with the 15 participants consuming the diet low in fruits and vegetables. While no differences were statistically significant, the findings hint that the effect of consumption of fruits and vegetables may interact with other dietary factors.

Generally, the study was well conducted. Plasma and urinary excretion markers indicated good compliance with the treatment diets. The control group following a self-selected diet also had decreases in total cholesterol and LDL over the six-week trial period, however these were not significant and were small by comparison with the differences found between treatment groups (0.05 and 0.06mmol/L, respectively). The absence of a substantial change in the controls indicates that random or period variation is minimal. Randomisation was largely successful as the even distribution of characteristics across treatment groups left minimal chance for confounding. One exception is that comparison groups varied slightly in their baseline diets, which may have affected their changes in serum lipid measures, particularly as there was no run-in period. The unblinded nature of the study, which is seldom avoidable in dietary interventions, may have provided a small source of bias. Overall there was very wide within-group variation, which reduced the ability of this study to pick up significant effects with small group sizes. This study does not provide strong evidence that increasing fruit and vegetable intake improves lipid profile, however the small sample size and the aforementioned issues mean it also does not provide strong evidence that the intervention does not improve biomarkers for CHD.

Biomarker study 4p: An unblinded RCT by **John et al. (2002)** [53] randomised 690 healthy adults on GP lists who had no CVD or co-morbidities to either a behavioural intervention (n=344) aimed at increasing **fruit and vegetable** intake (without

suggesting any other changes) or a control intervention (n=346) consisting of the same measures and visits but no dietary intervention. After the six-month trial period, the intervention group had daily intake of fruits and vegetables approximately 100g higher than the control group and a slightly lesser reduction in total cholesterol than the control group (0.01mmol/L). The largely successful randomisation of participants and adjustments for baseline values and gender minimised the chance that an imbalance between intervention and control groups accounted for the findings. Generalisability of the study findings to the general population is assisted by the study's sampling design, but is not certain as participation rates were not reported in this paper. One serious weakness in this study is their measurement of non-fasting cholesterol as an outcome, which leaves a greater chance for misclassification bias (ie, the study may have slightly underestimated any effect of the intervention on cholesterol). Also the intensity of the intervention may have been insufficient to achieve a reduction in cholesterol, the study was small, and self-reported measures were used in this unblinded study. As a result of increased dietary awareness, participants in the intervention group may have over-reported their intake relative to controls. As such, the study provides minimal evidence either way as to whether increased fruit and vegetable intake improves lipid biomarkers for CHD.

Blood Pressure

All four fruit and vegetable interventions found a decrease in diastolic BP and three found decreases in systolic BP after the study period relative to a control intervention. In two of the studies, reductions in systolic and diastolic BPs reached statistical significance; no studies found a significant increase in BP. All of these interventions were conducted on adults who were either healthy or had CVD co-morbidities, but none focused on groups with existing CHD, and therefore results are relevant to address the use of fruits and vegetables in primary or secondary prevention.

Biomarker study 5b: As part of the DASH study, **Conlin et al. (2000)** [52] tested the effect of their **fruit and vegetable** intervention diet in relation to BP for a subgroup of 133 DASH participants with hypertension (not currently controlled by medication). After the eight-week trial period, the 49 participants allocated to the intervention group had significantly greater reduction in BP than the 47 participants allocated to the control group (7mmHg systolic and 3mmHg diastolic). The intervention group also had a lower risk (≈30%) of uncontrolled hypertension (systolic BP$\geq$140mmHg and diastolic BP$\geq$90mmHg) and uncontrolled isolated systolic hypertension (systolic BP$\geq$140mmHg diastolic BP<90mmHg) compared with the control group after the intervention, although only the difference in hypertension risk reached statistical significance.

The same methodological considerations previously described for the DASH study apply in interpreting the findings of this study. Randomisation led to similar characteristics between the groups, however the slightly greater proportions of females and African Americans and lower baseline alcohol intake within the intervention group are unlikely to entirely account for findings. Findings support the claim that an increased fruit and vegetable intake can reduce blood pressure in people with hypertension.

Biomarker study 6b: Also as part of the DASH Study, **Moore et al. (2001)** [90] tested the effect of the intervention and control diets described above in 72 American

adults with isolated systolic hypertension. After the eight-week intervention, the 24 participants following the diet higher in **fruits and vegetables** had a non-significantly greater reduction in systolic BP (≈4mmHg) and diastolic BP (≈1mmHg) than the 25 participants in the control group. While the difference between intervention and control groups did not reach statistical significance, it is noteworthy that 50% of participants had normal BP after the intervention diet compared with 24% of participants following the control diet. This comparison was not statistically tested. Methodological issues described above for Biomarker study 1p apply also to this study and the one above (Biomarker study 5b).

Biomarker study 2b: In addition to testing blood lipids, the study by **Broekmans et al. (2001)** [88] tested the effect of a **fruit and vegetable** intervention diet on BP. They noted substantial and significant reductions from baseline in both systolic (≈6mmHg) and diastolic (≈4 mmHg) BP in both the intervention and control groups. Differences in the change from baseline between the intervention and control groups did not reach statistical significance. In the intervention group, the reduction in systolic BP was actually nearly 3 mmHg less than for the controls, and the reduction in diastolic BP was only 0.1 mmHg greater. The same serious methodological issues described earlier, especially those relating to fibre, need to be considered in understanding the findings of this study.

Biomarker study 4b: In the RCT conducted by **John et al. (2002)** [53], BP changes accompanying an increase in **fruit and vegetable** intake were noted. This study found the reduction in BP from baseline was significantly greater in the intervention than control groups (≈4mmHg systolic and ≈1.5 mmHg diastolic BPs). While fat intake was not monitored across the study period, both body weight and cholesterol remained unchanged, which suggests that a concomitant change in fat intake did not account for the reduction in BP that occurred. The measures of BP minimised the chance for misclassification bias that was problematic with the cholesterol measurements. Notably the low 'intensity' of the intervention, an average increase in self-reported intake of 100g/d (which may have been overstated as the study was not blinded), still led to reductions in blood pressure. Overall, this study was well designed to detect changes in BP accompanying advice to increase fruits and vegetables.

Homocysteine

Biomarker study 7h: Appel et al. (2000) [91] conducted an RCT of adults participating in the DASH study, none of whom had hypertension, hyperlipidemia, poorly controlled diabetes, took vitamin or mineral supplements or medications that affect blood pressure, or had evidence of kidney dysfunction. After a three-week run-in, 31 participants were randomised to the control group and 41 to the **fruit and vegetable** intervention group. After the eight-week intervention, serum homocysteine increased for both groups, though non-significantly less so for the intervention group (0.25μmol/L). Quality issues noted for the other DASH studies apply here, and overall the intervention may have been insufficient to expect a reduction in serum homocysteine.

Biomarker study 8h: Silaste et al. (2003) [75] conducted a cross-over intervention study among 37 healthy Finnish female volunteers recruited from the University

Hospital of Oulu. Participants followed a baseline diet for two weeks, then an intervention or control diet for 5 weeks and crossed over treatment arms after a three-week washout period. The baseline and washout diets were the participants' usual at-home diet. The control diet contained only one serve of fresh fruit/vegetable/juice, 200μg folate, <200mg dietary cholesterol and was controlled in fatty acid content with 10% of energy coming from saturated fat,. The intervention diet was otherwise similar but contained in total 600μg /d of folate, 400-500g of raw or steamed **fresh vegetables**, plus 60g of fresh paprika, two serves of **fruit/juice**, plus an unreported quantity of berries. Plasma homocysteine was significantly lower (1.1μmol/L) after the intervention than the control diet. A slightly greater difference in plasma homocysteine may have been detected with a longer washout period, as the washout may have been of insufficient duration since homocysteine was 0.3μmol/L lower after the washout than after the baseline period. This study used appropriate measures that minimised the chance for misclassification bias, monitoring indicated good compliance and the cross-over design eliminated the chance of an imbalance between intervention and control arms confounding results. Overall the findings of this study are trustworthy, however some caution in generalisation is necessary as the study used self-selected volunteers and only females. Also, the focus of this study was folate rather than fruits and vegetables, however the findings are applicable as folate-rich fruits and vegetables were the sole source of folate tested in this study.

Longitudinal Studies

One longitudinal study was located that addressed changes over time in biomarkers for CHD rather than CHD risk (presented in Appendix 6). This study showed evidence that men who consumed diets higher in fruits and vegetables had significantly less annual increase in blood pressure.

Biomarker study 9b: Miura et al. (2004) [92] analysed data collected in the Chicago Western Electric Study, which used a cohort of 2,107 male workers of the Chicago Western Electric Company aged 40-55 years at baseline in 1957-58. Their BPs were measured annually for seven years by trained physicians using mercury sphygmomanometers, and their diet were assessed at baseline and one year later by two nutritionists using the Burke Diet History method. This population consumed few fruits and vegetables, and three categories of intake were compared: low (less than 0.38 serves daily), moderate (0.38-1.125 serves daily) and high (more than 1.125 serves daily). After adjustment for most hypertension risk factors, moderate consumers of **vegetables** (0.38-1.125 serves) had significantly lower annual increases in systolic (0.4mmHg) and diastolic (0.17mmHg) BPs than low vegetable consumers. High consumers of **vegetables** had a non-significantly lower annual increases in systolic (0.28mmHg) and diastolic (0.22mmHg) BPs than low consumers. Compared with low consumers of **fruits**, moderate consumers had reductions in systolic (*0.32mmHg*) and diastolic (0.14mmHg) BPs, and high consumers had similar reductions in systolic (0.27mmHg) and diastolic (*0.22mmHg*) BPs. Only the differences indicated in italics reached statistical significance. Further adjustments for intake of several nutrients and vitamins attenuated these relationships substantially, indicating that part of the inverse associations with BP may stem from the presence or absence of these substances in fruits and vegetables. Dietary measures were appropriate, minimising the chance for misclassification bias. The population was likely representative of the workforce of the times, as the study used a random sample

and had an adequate response rate (67%). However it should also be noted that population intakes of fruits and vegetables were very low compared with modern dietary recommendations and levels of intake. One major limitation of this study is that sodium intakes were not assessed, hence this potential confounder may have accounted in part for the findings.

Cross-sectional Studies

Appendix 7 presents the eight cross-sectional studies that assessed whether fruit and/or vegetable intake was associated with various biomarkers of CHD (total cholesterol, HDL, LDL, TC:HDL, HDL:LDL, triglycerides, systolic or diastolic BP or homocysteine). These studies generally looked at adults, while some looked at men and women separately, and one study focussed on children.

Blood Pressure

Biomarker study 10b: A study by **Beitz et al. (2003)** [93] in Germany of 1,628 women and 1,340 men aged 18 to 79 years showed women's systolic BP was approximately 5mmHg lower for each additional kg/day intake of **fruits and vegetables** (significant). This same study reported there were no significant associations with systolic BP for men, or for diastolic BP in men or women, but did not report the magnitude or direction of any of these observed differences. This study considered most possible factors that could confound a relationship between BP and intake of fruits and vegetables (age, BMI, smoking, SEP, region, season, physical activity, energy intake, sodium, alcohol, coffee, vegetarian diet, and health related-quality of life issues). A small amount of residual confounding is possible as calcium and fatty acid intake were not discussed in this study, and based on findings of other studies, these factors may relate to BP and to fruit and vegetable intake, although they are unlikely to account for the findings. This study used good quality measure of fruit and vegetable intake: a computerised dietary history, which showed good correlations with 24-hr recall and 3-day dietary record, minimising the chance for misclassification bias. Findings are generalisable as the study was population-based, used a stratified random sampling, had an adequate response rate (61.4%), and achieved a representative sample. The findings relate to primary prevention, as the study excluded people with current or previous hypertension or who take medications that alter blood pressure; however findings may not apply to nursing mothers (who were excluded from the study).

Biomarker study 11b: Nagata et al. (2003) [94] studied the relationship between fruit and vegetable intake and BP among 294 men and 330 women who had no history of cancer, angina, heart attack, or diabetes, did not take anti-hypertensive or oral contraceptive medications, and who were recruited from a health check-up program at a general hospital in Japan. They found inverse associations between **fruit** intake and systolic and diastolic BPs for women and systolic BP for men, but a positive association with diastolic BP for men. None of these relationships were statistically significant and all correlations were weaker than $\pm$0.15. The study also addressed **vegetable** intake and found inverse associations with systolic BP and positive associations with diastolic BP both for men and women. All associations were weak (less than $\pm$0.15), and only the reduction in systolic BP for men was statistically significant. A fairly large possibility for bias exists, likely towards the

null, as the study's measure of intake showed poor validity against three-day dietary records for various nutrients and poor repeatability for vitamin C (which is a key component of fruits and vegetables). The generalisabilty of this study is limited as the representativeness of the sample is uncertain. While the response to the survey was high (97.3%), the study was conducted in a non-population setting and no details of the sampling procedure were provided.

Biomarker study 12b: Psaltopoulou et al. (2004) [95] looked at baseline data from a large, prospective cohort study of 20,343 Greek adults aged 20-80 years, and found significant inverse associations between **fruit** intake and both systolic and diastolic BPs. Each additional 200g of fruit intake was associated with 0.4-0.5mmHg reduction in BP (diastolic and systolic). They also found significant inverse associations between intake of **vegetables** and both systolic and diastolic BPs. Each additional 230g of vegetables daily was associated with a reduction in BP of 0.4-0.5mmHg systolic and diastolic. These associations may have been confounded by olive oil intake, as extra adjustment for olive oil intake halved the reduction in diastolic BP (0.2mmHg), which remained statistically significant, and changed the reduction in systolic BP to a non-significant 0.01mmHg. The study used a validated, semi-quantitative, food frequency questionnaire and appropriate BP measurements and considered most potential confounding factors. The main limitation of the study is that it used self-selected volunteers, however these were actively recruited from the general population across Greece, increasing the chance of their representativeness to the Greek population. Also, there is a small possibility for residual confounding, as the paper did not report whether other possible dietary confounding factors were considered; however this is unlikely to explain the entire relationship observed. Overall this study provided good evidence that intake of vegetables is associated with lower BP.

Blood Lipids

Biomarker study 13p: In a multi-centre, population-based study of 4,466 American adults in families with high CHD rates, randomly selected from one of four cohorts, **Djoussé et al. (2004)** [96] found an intake of 5.4 compared with 1.4 serves of **fruit and vegetables** daily was associated with a significant 0.2mmol/L reduction in LDL, and a significant 0.2 reduction in HDL:LDL ratio. This study reported associations with HDL and triglycerides were not statistically significant, but did not report the magnitude or direction of any associations found. Importantly, findings were similar when restricted to subgroups of those with a very high saturated fat intake, and those without baseline CAD or diabetes. This study considered confounding from most possible sources, used appropriate measures for lipid parameters, and employed a semi-quantitative food frequency questionnaire modified from a validated tool. This leaves some chance that misclassification bias reduced the observed relationships, which is lessened by the use of upper and lower intake quintiles for comparison.

Biomarker study 14p: In a study of 95 children aged six to 13years**, Lindquist et al. (2000)** [97] found weak, inverse, non-significant associations between both serum total cholesterol and serum triglycerides and the intake of either **fruits** or **vegetables**. This study adjusted for ethnicity, social class, and intake of other core foods. Some serious methodological issues with this study cast doubt over the findings. Firstly the sample is both small and non-random, which limits the generalisability of the

findings. Secondly, the dietary intake was assessed by three 24-hour recalls which showed very poor correlation between energy intake and expenditure measured by doubly-labelled water (r=0.25) at the individual level. The validity of intake of fruits, vegetables, or any of their key constituents, was not reported. This leaves a substantial chance that estimates were biased towards the null. Overall this study provides minimal evidence for a relationship between intake of fruits and vegetables and lipid biomarkers of CHD.

Biomarker study 15p: Deurenberg-Yap et al. (2001) [98] tested the associations between intake of **fruits** and serum total cholesterol, LDL, TCL:HDL and HDL among 2,408 Singaporean adults, who were randomly sampled from the population using a multistage design that deliberately over-sampled ethnic minorities. They reported that none of the associations were statistically significant but did not report the magnitude or direction of any associations. The study used a validated food frequency questionnaire, however this represented "diet in the last month" and the mean intake of fruits and vegetables was very low (approximately 1.3 $\pm$ 1 serves per day of each). One serious consideration in interpreting these negative findings is that the study relied entirely on the lack of statistical significance of the findings. This could be misleading, as the associations between dietary intake and serum lipids were not the primary hypotheses of this study, and the study design many not have had adequate statistical power to detect such associations.

Biomarker study 16p: In a study of 1,045 Brazilian adults, **Fornés et al. (2000)** [99] reported increasing frequency of **fruit** consumption was associated with a large, significant reduction in LDL (6 mg/dL), and smaller, non-significant reductions in HDL (0.5 mg/dL), after adjusting for age and gender. The relationship with LDL remained significant after adjustment for additional risk and intermediary factors, although the magnitude of adjusted estimates were not reported. Frequency of **vegetable** intake was significantly associated with a large reduction in LDL (3mg/dl) when adjusted for age and gender, and was still significantly associated after adjustment for other factors (magnitude not reported). Intake of **vegetables** was associated with a non-significant reduction (0.13mg/dL) in HDL, which was attenuated to virtually no reduction after adjusting for additional covariates (0.01mg/dL). The study tested frequencies but not quantities of intake, and although the authors reported the food frequency questionnaire was "successfully piloted", they did not report the validity of the measure of fruit and vegetable intake. Therefore, the exact 'dose' of fruits and vegetables associated with these reductions in LDL is unknown. For the lipid parameters, LDL was calculated rather than measured, which leaves some chance for measurement error, and also the storage procedures for blood samples were not described. The net effect of these methodological concerns is that findings are likely to slightly underestimate the true relationships between LDL and fruit and vegetable intake, and cannot rule out that frequent intake of fruits and vegetables might slightly reduce HDL.

Homocysteine

Two cross-sectional studies addressed the relationship between fruit and vegetable intake and plasma homocysteine.

Biomarker study 17h: Chrysohoou et al. (2004) [100] conducted a population-based study in Greece of 1,128 adult men and 1,154 adult women and found plasma homocysteine was significantly inversely associated with both **fruits and vegetables**, although correlations were weak (r=-0.12 and r=-0.15, respectively). These correlations remained significant after adjustment for potential confounding and intermediary factors, but the magnitude of adjusted measurements were not described. Participants who had renal failure, liver disease, chronic obstructive pulmonary disease, or who used drugs that alter homocysteine were excluded from analyses. Measurement error was minimised as the study used appropriate measurement of plasma homocysteine, and used a validated food frequency questionnaire to assess fruit and vegetable intake. Findings are likely to be generalisable as the study used a random population sample and had an adequate response rate (68%). The study is also relevant to primary prevention as it excluded participants with CVD. This study supports an association between both fruit and vegetable intake and plasma homocysteine.

Biomarker study 18h: Gao et al. (2004) [101] conducted a population-based, cross-sectional study in the United States of 445 Hispanic and 154 non-Hispanic white adults aged 60+ years. After considering other potential risk, they found a higher intake of **fruits and vegetables** (averaging 5.5 times daily compared with once daily) is associated with significantly lower plasma homocysteine (≈1μmol/L), and this association followed a dose-response pattern. They also found the odds of having high homocysteine (>10.4μmol/L for women and >11.4 μmol/L for men) were significantly reduced with each additional serve/day of **vegetables** (approximately 20%). (The cut-off point for high homocysteine reflects the 95th percentile for young adults.) Measurement error was minimised in this study as it used a semi-quantitative food frequency questionnaire validated for use in this population and appropriate measurements of homocysteine. Residual confounding factors is unlikely to account for the results observed. Findings are generalisable as the sample was representative of the general population of older adults, however they may not have direct application to primary prevention, as many in this population have a history of one or more CVD conditions, including heart attack, stroke, other heart disease, and hypertension. This study supports the existence of an inverse association between serum homocysteine and fruit and vegetable intake, but on its own provides limited evidence that this relationship holds in the wider, disease-free population and cannot establish direction of the association.

C-reactive protein

Biomarker study 18c: Only the study by **Gao et al. (2004)** [101] addressed the relationship between **fruit and vegetable** intake and serum C-reactive protein, reporting a statistically significant, inverse association between intake of **fruits and vegetables** and C-reactive protein (1mg/L lower in those consuming fruits and vegetables frequently rather than once daily). The odds of clinically elevated C-reactive protein were significantly reduced (≈20%) with each additional serving of fruit and vegetable intake. The cut-off used for high C-reactive protein (<10mg/dL) can predict long-term outcomes after coronary events. The study may have slightly underestimated associations with C-reactive protein, as very low concentrations <0.6mg/L could not be measured. Other limitations as noted above for biomarker study 18 in relation to homocysteine pertain here as well.

Summary of Evidence for an Association with CHD Biomarkers

Studies of fruit and/or vegetable intake that used biomarkers of CHD risk as outcomes were generally supportive of the findings from the case-control and cohort studies that assessed CHD incidence and/or mortality. The findings above should not be considered fully comprehensive, as the search strategy was designed to detect all studies that mentioned CHD or related terms, not each biomarker, therefore any studies that did not mention CHD would not have been included in this review.

The highest level of evidence is provided by experimental studies (summarised in Table 6 below). Randomised controlled trials generally found increased fruit and vegetable intake to result in reductions in BP (which were sometimes statistically significant). None of the randomised controlled trials found a significant reduction in serum cholesterol, LDL, TC:HDL, LDL: HDL, triglycerides nor a significant increase in HDL (relative to control diets). Only two experimental studies were located that measured serum homocysteine. While it was the study of higher quality that found a significant reduction in homocysteine with a diet high in fruits and vegetables, its findings should not be over-generalised as the trial used only a small number of female volunteers.

In addition to the experimental findings, a longitudinal study suggests an inverse association between fruit and vegetable consumption and BP. It found consistently across seven years greater increases in annual BP measurements among men who consumed very few fruits and vegetables, although this cannot be established conclusively as confounding was a strong possibility with this study. Several cross-sectional studies also examined the relationship between fruit and/or vegetable intake and various CHD biomarkers. Although the evidence from these studies is considered of much less value, the findings generally lend support to a beneficial relationship between fruit and vegetable intake and BP, lipids, homocysteine and C-reactive protein. However, findings were not always consistent across all outcome measures and were reported in some population subgroups but not others.

Table 6: Summary of findings from interventions increasing consumption of fruits and vegetables on biomarkers of coronary heart disease

Study	Reference	Duration [a]	n= intervention, control	TC, LDL or ratio	Triglycerides	HDL
1a	Obarzanek et al, 2001	8 weeks	75, 78 men	inv*	inv, ns	inv, ns
			71, 67 women	inv & pos, ns	inv, ns	pos, ns
2a	Broekmans et al, 2001	4 weeks	24,23	inv, ns	inv, ns	inv, ns
3a	Freese et al, 2002	6 weeks	15, 13 (arm 1)	inv, ns		inv, ns
			14,15 (arm 2)	pos, ns	-	pos, ns
4a	John et al., 2002	6 months	344, 346	pos, ns	-	-
				Systolic BP	**Diastolic BP**	
5b	Conlin et al., 2000	8 weeks	49, 47	inv*	inv*	
6b	Moore et al, 2001	8 weeks	24, 25	inv, ns	inv, ns	
2b	Broekmans et al, 2001	4 weeks	24, 23	pos, ns	inv, ns	
4b	John et al., 2002	6 months	344, 346	inv*	inv*	
				Homocysteine		
7c	Appel et al., 2000	8 weeks	41, 39	inv		
8c	Silaste et al, 2003	5 weeks	37 (crossover)	inv*		

[a] Duration of intervention period

* significant at $p<0.05$

Part 5: Conclusions

- Overall, the consistency of findings across studies of CHD morbidity and mortality is most noteworthy. Irrespective of study design and specific outcome, and similarly for study populations differing by age, gender, or nationality, inverse associations were generally reported for fruit and/or vegetable intake and risk of CHD. In the context of this review, statistical significance was considered of secondary importance, because most of the studies were not designed specifically to assess the relationship between fruit and vegetable consumption and CHD risk. Hence many lacked sufficient statistical power for the observed results.

- Evidence points to potentially similar beneficial effects for fruits and vegetables. However, results rarely distinguished between fresh and processed foods (particularly with respect to vegetables). Hence, any emphasis on fresh fruits and vegetables must rely on other sources of information to suggest they might be more beneficial than processed versions.

- The magnitude of the inverse association varied substantially, from less than 10% to more than 50% reductions in risk. A major contribution to this variability is the metric of dietary exposure used in statistical analysis, namely whether associations were in relation to serves per day or top versus lowest quantile (and if the latter, whether tertiles, quartiles, or quintiles, etc, were used). The overall magnitude of consumption in the population studied was also potentially important, and there was some evidence for a threshold effect such that consumption at even higher levels were not necessarily more beneficial. Other study design features similarly may be important to the reported magnitude of the inverse association. Only a pooled analysis or a formal meta-analysis can help sort out what the average effect size might be across these studies.

- Substantial research exists to establish the biological plausibility of a reduced risk of CHD with increased consumption of fruits and vegetables.

- Research on biomarkers related to CHD risk provide early support for some of the hypothetical mechanisms underlying a reduction in CHD risk associated with consumption of fruits and vegetables.
  - Randomised, controlled trials to reduce blood pressure levels among adults with or without hypertension reinforce the inference that increased intake of fruits and vegetables has a beneficial effect.
  - Lipid levels are consistently, inversely associated with fruit and vegetable intake in observational studies, but findings from randomised, controlled trials are less consistent, showing null to moderate improvements.
  - Studies of homocysteine and c-reactive protein are still very limited, but some evidence suggests that levels of these CHD biomarkers may also be reduced with increased fruit and vegetable consumption.

- The epidemiologic evidence accumulating over the past decade is predominantly supportive of an inverse association between fruit and vegetable intake and risk of CHD morbidity and mortality: higher levels of intake generally reduce CHD risk.

There are a very few studies that fail to show such a relationship, and unique study characteristics may account for most of these. Stronger support for a beneficial effect of fruit and vegetable consumption on CHD risk must await large-scale, long-term, randomised, controlled trials focused on incidence of CHD and/or improved understanding of underlying biological mechanisms.

- The inferences from these studies seem applicable to Australia and New Zealand based on the incidence/mortality from CHD, the types of fruits and vegetables consumed, the quantities of intake, and the general, westernised lifestyle characteristic of participants in most of the studies.

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Appendix 1: Search Details

EBSCO Host

Databases searched:
Academic Search Elite, Australia/New Zealand Reference Centre, Biological Abstracts, Pre-CINAHL, CINAHL, MEDLINE, PsycARTICLES, and PsycINFO.

Searches were conducted for the following words in subject terms:

1. Vegetabl* **AND** coronary
2. (Frui* **AND** coronary) **NOT** vegetabl*
3. (Vegetabl* **AND** cardiovascular) **NOT** coronary
4. (Frui* **AND** cardiovascular) **NOT** coronary **NOT** vegetabl*

ProQuest

Databases searched:
Academic Research Library, AMA titles, AMA titles: abstracting and indexing, Health and medical complete, ProQuest dissertations & theses, ProQuest psychology journals, ProQuest science journals, ProQuest social science journals, and ProQuest women's interest.

A search of the default fields was made for:

(fruit **OR** fruits **OR** vegetables **OR** vegetable) **AND** (coronary **OR** cardiovascular **OR** heart)

Blackwell Synergy

Databases searched:
The "all journals" option was selected

A search of abstracts was conducted for:

(fruit **OR** vegetable) **AND** (coronary **OR** cardiovascular)

Appendices 2-7: Review tables for studies from 2000 onwards

Symbols used:
*** $p<0.001$ **$p<0.01$ *$p<0.05$

Abbreviations used:
OR= Odds Ratios
CI = Confidence Interval
AMI= Acute Myocardial Infarction
MI= Myocardial Infarction
CHD= Coronary Heart Disease
LDL= Low Density Lipoprotein
HDL= Hight Density Lipoprotein
TC= Total Cholesterol
RCT= Randomised Control Trial
Apo= ApoLipoprotein
Vit= Vitamin
y= Years
BMI= Body Mass Index (kg/m^2)
etOH= Alcohol
sve= Serve
/d= Per Day

Note:
Serving = USDA servings=1/2 c fruits or vegetables or 1 cup juice or 2 cups leafy salad greens

Appendix 2: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Liu et al., 2000

United States

Women' s Health Study

CHD Study # 1</td>
<td>Cohort study</td>
<td>39127 female health professionals without hx CVD

45-75 yrs

(Dietary study includes 98% of the 39876 Women in the WHS trial)</td>
<td>5 y follow-up (av)

Follow-up of all participants until event or 6 years after baseline (195 647 person-years of follow up)</td>
<td>Fruit intake, vegetable intake (quintiles)

(I serving= USDA serving)

Semi-quantitative FFQ 28 vegetable items, 16 fruit items, 9 responses 'never' to '6+ x/d')

Validated similar populations

Correlation with 7d records: apple 0.80, grapefruit 0.84, tomatoes 0.74, squash 0.50.</td>
<td>Incident CVD, incident MI,

Diagnosis: Incident CVD (MI, stroke, CABG or PTCA, CVD death)
3 cardio-logists, 1 neurologist

CABG/ PTCA: self-report and hospital records

CVD deaths: medical records, autopsy reports, death certificates.

MI: (WHO criteria i.e. symptoms + ECG or enzymes)

Stroke: new neurologic deficit >24h, CT or MRI</td>
<td>Relative Risk (and 95% CI) for CVD incidence
<table>
<tr><th></th><th colspan="5">Quintiles of intake</th><th></th></tr>
<tr><th></th><th>1 (ref) serves/d</th><th>2 serves/d</th><th>3 serves/d</th><th>4</th><th>5</th><th>p for trend</th></tr>
<tr><td>Fruit [a]</td><td>1.00</td><td>0.66 (0.48 – 0.91)</td><td>0.72 (0.52 – 0.97)</td><td>0.82 (0.61 – 1.11)</td><td>0.84 (0.63 – 1.13)</td><td>0.67</td></tr>
<tr><td>Fruit [b]</td><td>1.00</td><td>0.73 (0.52 – 1.01)</td><td>0.70 (0.50 – 0.99)</td><td>0.91 (0.66 – 1.26)</td><td>0.96 (0.70 – 1.33)</td><td>0.69</td></tr>
<tr><td></td><td>0.6</td><td>1.3</td><td>1.9</td><td>2.6</td><td>3.9</td><td></td></tr>
<tr><td>Veg [a]</td><td>1.00</td><td>1.08 (0.80 – 1.44)</td><td>0.81 (0.60 – 1.11)</td><td>0.88 (0.65 – 1.19)</td><td>0.84 (0.61 – 1.14)</td><td>0.11</td></tr>
<tr><td>Veg [b]</td><td>1.00</td><td>1.07 (0.78 – 1.46)</td><td>0.83 (0.59 – 1.16)</td><td>0.91 (0.66 – 1.27)</td><td>0.85 (0.61 – 1.19)</td><td>0.21</td></tr>
<tr><td></td><td>1.5</td><td>2.5</td><td>3.4</td><td>4.6</td><td>6.9</td><td></td></tr>
<tr><td>Both [a]</td><td>1.00</td><td>0.85 (0.63 – 1.15)</td><td>0.82 (0.61 – 1.11)</td><td>0.80 (0.59 – 1.08)</td><td>0.82 (0.60 – 1.10)</td><td>0.18</td></tr>
<tr><td>Both [b]</td><td>1.00</td><td>0.75 (0.54 – 1.04)</td><td>0.83 (0.60 – 1.14)</td><td>0.80 (0.57 – 1.10)</td><td>0.85 (0.61 – 1.17)</td><td>0.45</td></tr>
<tr><td></td><td>2.6</td><td>4.1</td><td>5.5</td><td>7.1</td><td>10.2</td><td></td></tr>
</table>
Relative Risk (and 95% CI) for MI
<table>
<tr><th></th><th colspan="5">Quintiles of intake</th><th></th></tr>
<tr><th></th><th>1 (ref) serves/d</th><th>2 serves/d</th><th>3 serves/d</th><th>4</th><th>5</th><th>p for trend</th></tr>
<tr><td>Fruit [a]</td><td>1.00</td><td>0.66 (0.40 – 1.10)</td><td>0.45 (0.25 – 0.81)</td><td>0.64 (0.38 – 1.09)</td><td>0.57 (0.34 – 0.98)</td><td>0.04</td></tr>
<tr><td>Fruit [b]</td><td>1.00</td><td>0.76 (0.44 – 1.34)</td><td>0.58 (0.32 – 1.09)</td><td>0.82 (0.46 – 1.47)</td><td>0.66 (0.36 – 1.22)</td><td>0.26</td></tr>
<tr><td></td><td>0.6</td><td>1.3</td><td>1.9</td><td>2.6</td><td>3.9</td><td></td></tr>
<tr><td>Veg [a]</td><td>1.00</td><td>0.84 (0.50 – 1.41)</td><td>0.57 (0.32 – 1.01)</td><td>0.78 (0.46 – 1.32)</td><td>0.79 (0.47 – 1.35)</td><td>0.36</td></tr>
<tr><td>Veg [b]</td><td>1.00</td><td>0.94 (0.54 – 1.63)</td><td>0.55 (0.29 – 1.05)</td><td>0.87 (0.49 - 1.55)</td><td>0.88 (0.50 – 1.58)</td><td>0.60</td></tr>
<tr><td></td><td>1.5</td><td>2.5</td><td>3.4</td><td>4.6</td><td>6.9</td><td></td></tr>
<tr><td>Both [a]</td><td>1.00</td><td>0.49 (0.28 – 0.85)</td><td>0.69 (0.42 – 1.14)</td><td>0.50 (0.28 – 0.86)</td><td>0.62 (0.37 – 1.04)</td><td>0.07</td></tr>
<tr><td>Both [b]</td><td>1.00</td><td>0.45 (0.24 – 0.83)</td><td>0.78 (0.46 – 1.33)</td><td>0.51 (0.27 – 0.94)</td><td>0.63 (0.38 – 1.17)</td><td>0.21</td></tr>
<tr><td></td><td>2.6</td><td>4.1</td><td>5.5</td><td>7.1</td><td>10.2</td><td></td></tr>
</table>
Relative Risk (and 95% CI) for CVD amongst those without self-reported diabetes, history of hypertension, history of high cholesterol at baseline
<table>
<tr><th></th><th colspan="5">Quintiles of intake</th><th></th></tr>
<tr><th></th><th>1 (ref) serves/d</th><th>2 serves/d</th><th>3 serves/d</th><th>4</th><th>5</th><th>p for trend</th></tr>
<tr><td>Fruit [a]</td><td>1.00</td><td>0.66 (0.37 – 1.16)</td><td>0.66 (0.37 – 1.16)</td><td>0.68 (0.39 – 1.21)</td><td>0.57 (0.32 – 1.04)</td><td>0.09</td></tr>
<tr><td>Fruit [c]</td><td>1.00</td><td>0.71 (0.39 – 1.26)</td><td>0.37 (0.37 – 1.23)</td><td>0.79 (0.44 – 1.42)</td><td>0.57 (0.30 – 1.09)</td><td>0.15</td></tr>
<tr><td></td><td>0.6</td><td>1.3</td><td>1.9</td><td>2.6</td><td>3.8</td><td></td></tr>
<tr><td>Veg [a]</td><td>1.00</td><td>0.61 (0.35 – 1.08)</td><td>0.53 (0.29 – 0.95)</td><td>0.85 (0.50 – 1.41)</td><td>0.45 (0.24 – 0.84)</td><td>0.06</td></tr>
<tr><td>Veg [c]</td><td>1.00</td><td>0.63 (0.35 – 1.14)</td><td>0.59 (0.33 – 1.09)</td><td>0.96 (0.54 – 1.61)</td><td>0.45 (0.24 – 0.89)</td><td>0.11</td></tr>
<tr><td></td><td>1.5</td><td>2.5</td><td>3.4</td><td>4.5</td><td>6.8</td><td></td></tr>
<tr><td>Both [a]</td><td>1.00</td><td>0.67 (0.38 – 1.20)</td><td>0.67 (0.38 – 1.20)</td><td>0.92 (0.55 – 1.57)</td><td>0.41 (0.21 – 0.80)</td><td>0.04</td></tr>
<tr><td>Both [c]</td><td>1.00</td><td>0.68 (0.37 – 1.22)</td><td>0.77 (0.43 – 1.37)</td><td>0.95 (0.55 – 1.65)</td><td>0.45 (0.22 – 0.91)</td><td>0.09</td></tr>
<tr><td></td><td>2.2</td><td>4.1</td><td>5.4</td><td>7.0</td><td>10.0</td><td></td></tr>
</table>
a adjusted for age, treatment group, smoking b also adjusted for BMI, alcohol, physical activity, vitamin supplements, hx cholesterol, hypertension, diabetes
c adjusted for age, treatment group, smoking, BMI, alcohol, physical activity, supplements</td>
<td>In professional women, after adjusting for most CVD risks - risk CVD onset was non-significantly lower with daily intake of:
• 4 vs 0.6 serves of fruit (by ≈ 4%)
• 7 vs 1.5 serves of vegetables (by ≈ 15%)
• 10 vs 2.6 serves of both (by ≈ 15%)
risk of onset of first AMI was non significantly lower with daily intake of:
• 4 vs 0.6 serves fruit (by ≈ 34%)
• 7 vs 1.5 serves vegetables (by≈ 12)
• 10 vs 2.6 serves of both (by ≈ 37%)
risk of CVD onset for those without prior co-morbidities was:
• ≈ 55% lower with intake of 7 vs 1.5 serves vegetables daily (significant)
• ≈ 43% lower with intake of 4 vs 0.6 serves fruit daily (not significant)
• ≈ 55% lower with intake of 10 vs 2.2 serves of both daily (significant)
Issues:
Did not consider energy intake. (Absence of adjustment for saturated and total fat did not introduce confounding as these were not related to CVD in this study.
Quality rating: A</td>
</tr>
</table>

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Joshipura et al., 2001

Nurses' Health Study and Health Profess-ionals Follow up Study

CHD Study #2

(continued next page)</td>
<td>Cohort study</td>
<td>n=84251 women 34-59y, n=42158 men 40-75y with dietary data eligible: no CVD, cancer, diabetes

Relatively similar SEP within each cohort

Response rate not recorded</td>
<td>8y follow up</td>
<td>Intake of fruits, and vegetables (USDA serves/ day)

Semi-quantitative FFQ (different versions, 11 or 28 vegetable items, 6 or 15 fruit items, 3 potato items)

(preset portion sizes, 9 responses never up to six or more times daily)

Fruit & Vegetable Groupings:
Fruit -
All, citrus & citrus juice
Vegetables –
All, cruciferous, green leafy & potatoes
Fruits & Vegetables
All & Vitamin C rich

"All vegetables" excluded potatoes, tofu & soybeans, dried beans, and lentils</td>
<td>non-fatal MI, or fatal coronary disease

MI by WHO criteria; considered 'probable' if confirmed by interview but no medical records. Confirmed and probable cases were included. Fatal Coronary disease definite if confirmed by hospital record or autopsy or listed as cause of death on certificate, was most plausible cause and evidence of previous coronary disease was available.</td>
<td>RR for CHD by fruit and vegetable intake
<table>
<tr><th>servings/ day
median (Q1 – Q5)</th><th>Q1
(ref)</th><th>Q2</th><th>Q3</th><th>Q4</th><th>Q5</th><th>1 serve/d increase</th></tr>
<tr><td><u>All Fruits & Veges</u></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>women
5.82 (2.93 – 10.15)</td><td>1</td><td>0.91</td><td>0.88</td><td>0.86</td><td>0.80</td><td>0.97</td></tr>
<tr><td>men
5.07 (2.54 – 9.15)</td><td>1</td><td>1.01</td><td>0.95</td><td>0.87</td><td>0.80</td><td>0.96</td></tr>
<tr><td>pooled</td><td>1</td><td>0.95
(0.84 - 1.08)</td><td>0.94
(0.83 – 1.08)</td><td>0.86
(0.75 – 0.99)</td><td>0.80
(0.69 – 0.93)</td><td>0.96
(0.94 – 0.99)</td></tr>
<tr><td><u>Vit C-rich F & V</u></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>women
1.53 (0.54 – 3.08)</td><td>1</td><td>1.00</td><td>0.92</td><td>0.77</td><td>0.95</td><td>0.94</td></tr>
<tr><td>men
1.42 (0.46 – 2.96)</td><td>1</td><td>1.09</td><td>0.89</td><td>0.97</td><td>0.87</td><td>0.93</td></tr>
<tr><td>pooled</td><td>1</td><td>1.04
(0.92 - 1.19)</td><td>0.91
(0.80 – 1.04)</td><td>0.87
(0.70 – 1.07)</td><td>0.91
(0.79 – 1.04)</td><td>0.94
(0.88 – 0..99)</td></tr>
<tr><td><u>All fruits</u></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>women
2.33 (0.86 – 4.54)</td><td>1</td><td>0.84</td><td>0.95</td><td>0.76</td><td>0.85</td><td>0.95</td></tr>
<tr><td>men
2.09 (0.72 – 4.33)</td><td>1</td><td>0.91</td><td>0.94</td><td>0.86</td><td>0.74</td><td>0.92</td></tr>
<tr><td>pooled</td><td>1</td><td>0.87
(0.76 – 0.99)</td><td>0.94
(0.83 – 1.08)</td><td>0.81
(0.70 – 0.93)</td><td>0.80
(0.69 – 0.92)</td><td>0.94
(0.90 – 0.98)</td></tr>
<tr><td><u>Citrus fruit</u></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>women
0.85 (0.08 – 1.80)</td><td>1</td><td>0.94</td><td>0.91</td><td>0.91</td><td>0.88</td><td>0.93</td></tr>
<tr><td>men
0.86(0.08 – 1.88)</td><td>1</td><td>0.91</td><td>0.99</td><td>0.98</td><td>0.87</td><td>0.95</td></tr>
<tr><td>pooled</td><td>1</td><td>0.93
(0.81 – 1.06)</td><td>0.95
(0.83 – 1.08)</td><td>0.94
(0.83 – 1.08)</td><td>0.88
(0.77 – 1.00)</td><td>0.94
(0.87 – 1.01)</td></tr>
<tr><td><u>Citrus juice</u></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>women
0.43 (0.00 – 1.00)</td><td>1</td><td>0.90</td><td>1.05</td><td>0.89</td><td>0.95</td><td>0.95</td></tr>
<tr><td>men
0.43 (0.00 – 1.00)</td><td>1</td><td>1.09</td><td>1.14</td><td>0.91</td><td>1.19</td><td>1.07</td></tr>
<tr><td>pooled</td><td>1</td><td>0.99
(0.82 – 1.20)</td><td>1.09
(0.95 – 1.24)</td><td>0.90
(0.77 – 1.04)</td><td>1.06
(0.85 – 1.32)</td><td>1.01
(0.90 – 1.14)</td></tr>
</table>
all results reported adjusted for age, smoking, alcohol, family history MI, BMI, vitamin supplement use, vitamin E use, physical activity, aspirin use, 2-year follow up period, hypertension, hypercholesterolemia, energy, postmenopausal hormone use.

• vitamin C rich means contains > 30mg/ serve
• Largest change from the results above with additional adjustment for protein, cereal fibre, saturated fat, trans-fatty acids, PUFA, cholesterol, meat intake is estimate for 1 serve/d increase in total fruits & vegetables from 0.96 (0.94 – 0.99) to 0.97 (0.95 – 1.00).
• Results similar for individual fruits and vegetables (not reported)</td>
<td>After consideration of most CVD risk factors, among health professionals with the highest compared the lowest intake (quintiles) the risk of CHD was:
• significantly lower for intake of all fruit and veg, vit C rich fruit and veg, fruit, veg, cruciferous veg, and green leafy veg by between ≈9% for vit c rich fruit and veg to 20% for all fruits & veg
• 12% lower for citrus fruit (not significant)
• 6-15% higher for citrus juice and potatoes (not significant)

After consideration of most CVD risk factors, with each additional serve per day the risk of CHD was:
• significantly lower for all fruit and veg, vit C rich fruit and veg, fruit, veg by 3-7%
• significantly lower by 30% for green leafy veg
• 15% lower, not significantly for cruciferous veg
• 1-14% higher for citrus juice and potatoes (not significant)</td>
</tr>
</table>

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Joshipura et al., 2001 (continued from last page)						RR for CHD by fruit and vegetable intake servings/ day *median (Q1 – Q5)* | Q1 (ref) | Q2 | Q3 | Q4 | Q5 | 1 serve/d increase All veges women | 1 | 0.89 | 0.92 | 0.80 | 0.77 | 0.93 *3.34 (1.60 – 6.21)* men | 1 | 0.96 | 1.00 | 0.94 | 0.87 | 0.97 *2.83 (1.63 – 5.37)* pooled | 1 | 0.92 (0.81 – 1.04) | 0.96 (0.84 – 1.09) | 0.86 (0.73 – 1.02) | 0.82 (0.71 – 0.94) | 0.95 (0.92 – 0.99) Cruciferous veges women | 1 | 0.90 | 0.85 | 0.83 | 0.80 | 0.76 *0.42 (0.14 – 0.95)* men | 1 | 0.87 | 0.95 | 0.86 | 0.93 | 0.96 *0.4 (0.14 – 1.01)* pooled | 1 | 0.89 (0.78 – 1.01) | 0.89 (0.78 – 1.02) | 0.84 (0.74 – 0.97) | 0.86 (0.75 – 0.99) | 0.86 (0.69 – 1.08) Green leafy veges women | 1 | 0.92 | 0.89 | 0.74 | 0.69 | 0.70 *0.73 (0.16 – 1.51)* men | 1 | 0.89 | 0.93 | 0.89 | 0.76 | 0.84 *0.59 (0.16 – 1.36)* pooled | 1 | 0.90 (0.79 – 1.03) | 0.91 (0.80 – 1.03) | 0.81 (0.68 – 0.97) | 0.72 (0.63 – 0.83) | 0.77 (0.64 – 0.93) Potatoes women | 1 | 1.01 | 0.86 | 0.75 | 0.95 | 0.78 *0.43 (0.14 – 0.96)* men | 1 | 1.41 | 1.14 | 1.40 | 1.41 | 1.41 *0.51 (0.14 – 1.02)* pooled | 1 | 1.19 (0.86 – 1.64) | 0.98 (0.75 – 1.30) | 1.03 (0.56 – 1.89) | 1.15 (0.78 – 1.70) | 1.06 (0.59 – 1.89) RR for CHD per 1 serve increase of fruits and vegetables by multivitamin use and smoking status No Multivitamin | Multivitamin | Never/ Past Smokers | Current Smokers All fruits & veges | 0.96 (0.92 – 0.99) | 0.98 (0.94 – 1.02) | 0.96 (0.94 – 0.99) | 0.95 (0.91 – 1.00) All fruits | 0.94 (0.89 – 1.00) | 0.94 (0.87 – 1.00) | 0.95 (0.90 – 1.00) | 0.93 (0.86 – 1.00) All vegetables | 0.92 (0.87- 0.97) | 1.00 (0.93 – 1.06) | 0.97 (0.93 – 1.01) | 0.91 (0.84 – 0.98) Citrus fruit | 0.95 (0.6 – 1.06) | 0.98 (0.86 – 1.11) | 0.95 (0.87 – 1.04) | 0.93 (0.80 – 1.08) Citrus juice | 1.04 (0.90 – 1.20) | 1.01 (0.82 – 1.23) | 1.04 (0.91 – 1.19) | 1.03 (0.72 – 1.47) Cruciferous veges | 0.76 (0.60 – 0.96) | 1.01 (0.57 – 1.79) | 0.87 (0.61 – 1.24) | 0.85 (0.61 – 1.19) Green leafy veges | 0.76 (0.63 – 0.93) | 0.78 (0.56 – 1.07) | 0.77 (0.59 – 1.00) | 0.78 (0.61 – 0.99) Vit C rich F & V | 0.94 (0.87 – 1.02) | 0.97 (0.88 – 1.07) | 0.94 (0.88 – 1.01) | 0.92 (0.82 – 1.03) Potatoes | 0.78 (0.46 – 1.34) | 1.27 (0.93 – 1.73) | 1.12 (0.72 – 1.76) | 0.93 (0.42 – 2.06) RR for CHD (95% CI) with each additional serve/d fruits and vegetables (subgroups) Hypertensive 0.97 (0.93 – 1.02) Normotensive 0.95 (0.93 – 0.98) Diabetic men 0.90 (0.82 – 0.99) Diabetic Women 0.93 (0.85 – 1.02)	Results were • Similar for men and women. • A little stronger in non-users rather than users of multivitamins (cruciferous vegetables & total vegetables only) and in current smokers (only total vegetables) than never or past smokers. • similar for persons with or without hypertension. • Similar for persons with diabetes. Issues: Intake of subgroups of fruits/vegetables small in this population Further adjustments for other nutrients (eg fatty acids, fibre, protein) did not substantially change results. Quality rating: A

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Bazzano et al., 2002

United States NHEFS

CHD Study #3</td>
<td>Cohort study</td>
<td>9608 adults (25-47y at baseline) CHD free

(n=9156 with complete data)

Recruitment: multistage stratified random probability sample

Response rate not recorded</td>
<td>Baseline 1971-1975, average 16.5y follow-up</td>
<td>Frequency of fruit & vegetable consumption (times/ day)

FFQ (3 fruit & vegetable items, assesses intake over last three months)

(Questionnaire asked about F&V of all kinds fresh canned, frozen cooked or raw and juices)

Validation by single 24 hr recall (Spearman r=0.50)</td>
<td>Incidence and mortality from stroke, IHD, CVD

Death or discharge codes (ICD-9;13) Stroke 430-438 IHD 410-414 CVD 390-459</td>
<td>Incidence of Stroke and IHD by intake of fruits and vegetables
<table>
<tr><th></th><th colspan="4">Frequency consumption fruit and vegeables (times/day)</th><th></th></tr>
<tr><th></th><th><1 (ref) n=1094</th><th>1 n=3106</th><th>2 n=3356</th><th>≥3 n=1872</th><th>p for trend</th></tr>
<tr><td>Stroke</td><td>1.00</td><td>0.93 (0.75 – 1.14)</td><td>0.80 (0.65 – 0.99)</td><td>0.61 (0.48 – 0.79)</td><td><0.001[a]</td></tr>
<tr><td></td><td></td><td>1.04 (0.86 – 1.26)</td><td>0.93 (0.76 – 1.13)</td><td>0.73 (0.57 – 0.95)</td><td>0.01 [b]</td></tr>
<tr><td>IHD</td><td>1.00</td><td>0.97 (0.83 -1.13)</td><td>0.84 (0.73 – 0.95)</td><td>0.85 (0.72 – 1.02)</td><td>0.02 [a]</td></tr>
<tr><td></td><td></td><td>1.07 (0.91 – 1.27)</td><td>0.97 (0.83 – 1.14)</td><td>1.01 (0.84 – 1.21)</td><td>0.8 [b]</td></tr>
</table>
Mortality from stroke, IHD and CVD by intake of fruits and vegetables
<table>
<tr><th></th><th colspan="4">Frequency consumption fruit and vegetables (times/day)</th><th></th></tr>
<tr><th></th><th><1 (ref) n=1094</th><th>1 n=3106</th><th>2 n=3356</th><th>≥3 n=1872</th><th>p for trend</th></tr>
<tr><td>Stroke</td><td>1.00</td><td>0.75 (0.55 – 1.01)</td><td>0.67 (0.48 – 0.93)</td><td>0.52 (0.33 – 0.81)</td><td>0.004 [a]</td></tr>
<tr><td></td><td></td><td>0.83 (0.56 – 1.22)</td><td>0.74 (0.48 – 1.12)</td><td>0.58 (0.33 – 1.02)</td><td>0.05 [b]</td></tr>
<tr><td>IHD</td><td>1.00</td><td>0.81 (0.63 – 1.04)</td><td>0.72 (0.57 – 0.91)</td><td>0.66 (0.49 – 0.90)</td><td>0.007 [a]</td></tr>
<tr><td></td><td></td><td>0.89 (0.68 – 1.17)</td><td>0.84 (0.65 – 1.09)</td><td>0.76 (0.56- 1.03)</td><td>0.07 [b]</td></tr>
<tr><td>CVD</td><td>1.00</td><td>0.83 (0.69 – 1.01)</td><td>0.74 (0.62 – 0.87)</td><td>0.63 (0.51 – 0.79)</td><td><0.001[a]</td></tr>
<tr><td></td><td></td><td>0.91 (0.75 – 1.10)</td><td>0.84 (0.70 – 0.99)</td><td>0.73 (0.58 – 0.92)</td><td>0.008 [b]</td></tr>
</table>
a
adjusted for age, race, sex, and energy
b adjusted for age, sex, race, hx diabetes, physical activity, education, alcohol consumption, smoking, supplement use, energy intake</td>
<td>After consideration of most CVD risk factors, intake of fruits and vegetables three or more times daily compared with less than once daily is associated with:
• 1% higher risk of incident IHD (not significant); relationship not dose-response
• 24% reduced risk of mortality from IHD (significant) relationship not dose-response
• 27% reduced risk of mortality from CVD (significant); dose-response relationship

Adjusting only for age, race, sex and energy relationships were significant, inverse and linear for CVD & IHD mortality and IHD incidence

Issues:
classification of dietary exposure by frequency only not quantity

Quality: B</td>
</tr>
</table>

NHEFS: National Health and Nutrition Examination Survey Epidemiologic Follow-up Study

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Appelby et al., 2002

United Kingdom

Health Food Shoppers study

CHD Study #4</td>
<td>Cohort study</td>
<td>n=10741 adults (16-89y at baseline) 'health conscious', no prior cancer (except melanoma)

Recruitment and response rate not reported</td>
<td>Baseline 1973-1979

Follow up: until 31 Dec. 1997 (18-24y)</td>
<td>Frequency of fresh fruit vegetable consumption (1 serve daily vs. less often)

"Diet and Lifestyle Questionnaire"

Validity not reported</td>
<td>Mortality from IHD, all cause mortality

Death codes (ICD-9)

IHD 410-414</td>
<td>RR and 95% CI for All Cause and Ischemic Heart Disease mortality by intake fresh fruit
<table>
<tr><th></th><th colspan="3">RR Mortality by fresh fruit consumption
(At least 1 serve daily versus less often)</th></tr>
<tr><th></th><th>n= events</th><th>IHD mortality</th><th>All cause mortality</th></tr>
<tr><td>Overall n=10741</td><td>612</td><td>0.74 (0.61-0.98)**</td><td>0.81 (0.74-0.89)**</td></tr>
<tr><td>Men n=4325</td><td>326</td><td>0.89 (0.70 – 1.14)</td><td>0.90 (0.79-1.03)</td></tr>
<tr><td>Women n=6418</td><td>286</td><td>0.52 (0.39 – 0.70)**</td><td>0.72 (0.63-0.82)**</td></tr>
<tr><td>Non-smokers n=8675</td><td>427</td><td>0.67 (0.54 – 0.82)**</td><td>0.81 (0.73-0.90)**</td></tr>
</table>
Adjusted for age, sex, smoking (when applicable), consumption of other foods (wholemeal bread, nuts or dried fruit, raw vegetable salads, bran cereals
* p<0.05, **p<0.01</td>
<td>After consideration of some CVD risk factors, people consuming a serve of fruit at least once a day is associated with :
• ≈ 20% reduced risk of death (significant)
• ≈ 25 % lower risk of death from ischemic heart disease (significant)

Subgroup:
• similar or slightly greater risk reduction observed for non-smokers
• greater risk reduction observed for women than men
Issues:
Single cut-off point means that subgroup differences may not reflect biological differences in the effect of fruit but different population distributions of intake

Did not exclude people with a history of CHD

Did not adjust for energy, fat or saturated fat intake

Quality: C</td>
</tr>
</table>

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Steffen et al., 2003

United States ARIC Study

CHD Study #5</td>
<td>Cohort study (multi-centre, population based)</td>
<td>n=11,940 adults (45-64 y at baseline) randomly sampled no CAD, stroke, heart attack, diabetes, cancer

approx 60% participation rate overall

Lower participation by African American men and women (42-49%) than white American men and women (67-68%)</td>
<td>11 y follow up</td>
<td>Intake of fruit and vegetables (USDA serves/d)

66-item FFQ (15 veg categories includes potatoes, excludes fries and vegetables in mixed dishes; 7 fruit categories 1 for juice, 6 for fresh fruits; fresh frozen and canned included).

Modified from 61-item FFQ (validated elsewhere, Willet)</td>
<td>Incident Coronary Artery Disease (CAD), Incident ischemic stroke

Annual phone calls, hospital surveillance, death-certificate registries

Incident CAD: (criteria White et al.) first definite or probable MI, silent MI by ECG definite CAD death, coronary revascularization

Incident Stroke: (criteria National Survey of Stroke) first definite or probable cardioembolic or thrombotic brain infarction - computer algorithm, independent review of medical records and discharge summaries by 1 or 2 physicians</td>
<td>RR for incidence of coronary artery disease across quintiles of intake of fruit and vegetables
<table>
<tr><th>Quintiles</th><th colspan="3">RR (95%CI) for incident CAD</th></tr>
<tr><th>(serves/d)</th><th>Model 1</th><th>Model 2</th><th>Model 3</th></tr>
<tr><td>Q1 (1.5)</td><td>1 (ref)</td><td>1 (ref)</td><td>1 (ref)</td></tr>
<tr><td>Q2 (2.5)</td><td>0.96 (0.74 – 1.25)</td><td>1.08 (0.82 – 1.41)</td><td>1.10 (0.84 – 1.45)</td></tr>
<tr><td>Q3 (3.5)</td><td>1.01 (0.78 – 1.32)</td><td>1.23 (0.93 – 1.61)</td><td>1.21 (0.91 – 1.60)</td></tr>
<tr><td>Q4 (5.0)</td><td>0.82 (0.62 – 1.09)</td><td>1.08 (0.80 – 1.46)</td><td>1.06 (0.78 – 1.44)</td></tr>
<tr><td>Q5 (7.5)</td><td>0.59 (0.42 – 0.81)</td><td>0.85 (0.60 – 1.21)</td><td>0.82 (0.57 – 1.17)</td></tr>
<tr><td>p trend</td><td>0.001</td><td>0.43</td><td>0.29</td></tr>
</table>
RR for incidence of ischemic stroke across quintiles of intake of fruit and vegetables
<table>
<tr><th></th><th colspan="3">RR (95%CI) for incident Ischemic Stroke</th></tr>
<tr><th></th><th>Model 1</th><th>Model 2</th><th>Model 3</th></tr>
<tr><td>Q1</td><td>1 (ref)</td><td>1 (ref)</td><td>1 (ref)</td></tr>
<tr><td>Q2</td><td>1.47 (0.97 – 2.23)</td><td>1.60 (1.05 – 2.44)</td><td>1.55 (1.02 – 2.37)</td></tr>
<tr><td>Q3</td><td>1.04 (0.66 – 1.62)</td><td>1.14 (0.72 – 1.82)</td><td>1.10 (0.69 – 1.76)</td></tr>
<tr><td>Q4</td><td>0.91 (0.56 – 1.45)</td><td>1.09 (0.67 – 1.79)</td><td>1.04 (0.63 – 1.70)</td></tr>
<tr><td>Q5</td><td>0.89 (0.54 – 1.48)</td><td>1.03 (0.59 – 1.78)</td><td>0.94 (0.54 – 1.63)</td></tr>
<tr><td>p trend</td><td>0.21</td><td>0.60</td><td>0.40</td></tr>
</table>
RR for incidence of coronary artery disease across quintiles of intake of fruit and vegetables in White and African Americans
<table>
<tr><th></th><th colspan="2">RR (95%CI) for incident CAD (Model3)</th></tr>
<tr><th></th><th>African Americans
n≈3100</th><th>White Americans
n≈8800</th></tr>
<tr><td>Q1</td><td>1 (ref)</td><td>1 (ref)</td></tr>
<tr><td>Q2</td><td>0.96 (0.57 – 1.59)</td><td>1.11 (0.80 – 1.55)</td></tr>
<tr><td>Q3</td><td>0.70 (0.40 – 1.23)</td><td>1.48 (1.07 – 2.05)</td></tr>
<tr><td>Q4</td><td>0.75 (0.42 – 1.34)</td><td>1.21 (0.84 – 1.75)</td></tr>
<tr><td>Q5</td><td>0.37 (0.17 – 0.80)</td><td>1.13 (0.75 – 1.71)</td></tr>
<tr><td>p trend</td><td>0.01</td><td>0.48</td></tr>
<tr><td colspan="2">p for interaction with race</td><td>0.01</td></tr>
</table>
Model 1: adjusted for age, race, sex, energy intake
Model 2: adjusted as model 1 also smoking, physical activity, alcohol, HRT in women
Model 3: adjusted as model 2 plus BMI, waist-hip ratio, systolic BP, anti-hypertensive medication use, HDL & LDL (CAD model only)</td>
<td>after adjusting for standard CVD risk factors, among healthy adults, consuming around 7.5 compared with 1.5 serves per day of fruits and vegetables was associated with:
• 10-15% lower risk of CAD (non significant); not dose-response
• virtually no difference in risk of stroke.
• 63% lower risk of CAD (significant) in upper vs lowest quintile of intake among African Americans only (linear trend).

Issues:
Confidence intervals wide indicating possibility of small effect in either direction.

Quality Rating: A-B</td>
</tr>
</table>

White et al., (1996) Community surveillance of CHD in the Atherosclerosis Risk in Communities (ARIC) Study: Methods and initial two years' experience *Journal of Clinical Epidemiology*, 49(2):223-233

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Rissanen et al., 2003

Finland Kuopio Ischaemic Heart Disease Risk Factor (KIHD)

CHD Study #6</td>
<td>cohort study</td>
<td>2641 men (1950 men no hx CVD at baseline) 42-60y

population based, random sample (82.9% participated in study)</td>
<td>12.8 y follow up

(baseline 1984-1989)</td>
<td>quintiles of intake of fruits, berries and vegetables

4-d food record

All types of fruits and vegetables (eg fresh, frozen, canned). includes: jams, nectars and juices excludes: potatoes</td>
<td>CVD-Death

Computer linkage to death registry ICD-9 codes 390 – 459)</td>
<td>RR (95%CI) for CVD-related death across quintiles of intake of fruits, berries and vegetables
<table>
<tr><th></th><th>Model 1</th><th>Model 2</th><th>Model 3</th><th>Model 4</th><th>Model 5</th></tr>
<tr><td>Q1 (>133 g/d)</td><td>1.00 (ref)</td><td>1.00 (ref)</td><td>1.00 (ref)</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Q2 (133-214 g/d)</td><td>0.64 (0.39 – 1.06)</td><td>0.71 (0.42 – 1.17)</td><td>0.77 (0.46 – 1.30)</td><td>0.76 (0.45 – 1.30)</td><td>0.76 (0.44 – 1.30)</td></tr>
<tr><td>Q3 (215-293 g/d)</td><td>0.35 (0.19 – 0.64)</td><td>0.42 (0.23 – 0.79)</td><td>0.46 (0.25 – 0.87)</td><td>0.49 (0.26 – 0.91)</td><td>0.50 (0.26 – 0.96)</td></tr>
<tr><td>Q4 (294–408 g/d)</td><td>0.48 (0.28 – 0.82)</td><td>0.59 (0.34 – 1.02)</td><td>0.56 (0.33 – 0.97)</td><td>0.60 (0.34 – 1.03)</td><td>0.62 (0.33 – 1.16)</td></tr>
<tr><td>Q5 (>408 g/d)</td><td>0.43 (0.24 – 0.76)</td><td>0.56 (0.31 – 1.00)</td><td>0.59 (0.33 – 1.06)</td><td>0.61 (0.34 – 1.10)</td><td>0.66 (0.28 – 1.55)</td></tr>
<tr><td>p (Q5 v Q1)</td><td>0.004</td><td>0.050</td><td>0.078</td><td>0.101</td><td>0.342</td></tr>
<tr><td>p (for trend)</td><td>0.001</td><td>0.020</td><td>0.020</td><td>0.037</td><td>0.127</td></tr>
</table>
All models adjusted for energy by residuals method
Model 1: adjusted for age and examination years
Model 2: as 1 also urinary nicotine, alcohol
Model 3: as 2 also BMI, systolic & diastolic BP, diabetes, LDL, HDL, TAG.
Model 4: as 3 also maximal oxygen uptake (a measure of cardiovascular fitness)
Model 5: as 4 and dietary factors (energy adjusted intakes of vit. C, E, β-carotene, lycopene, folate, fibre)</td>
<td>After adjustment for energy, smoking and alcohol intake, among healthy men an intake of 400g per day or more compared with around 130g or less was associated with:
• ≈45% reduced risk of death by CVD (significant); dose-response relationship
• ≈40% reduced risk of death by CVD (not significant) after adjustment for physical activity and possible intermediary factors (BMI, BP, diabetes, lipids); dose-response relationship
• ≈35% reduced risk of CVD death (not significant) after adjustment for some of the nutrients contained in fruits and vegetables

Issues:
Did not adjust for fatty acids – baseline values suggest these were related to fruit and vegetable intake (≈5% (significant) difference in %E from saturated fat between upper and lower quintile of fruit and vegetable intake).
Reduction in the risk ratio after adjustment for nutrients suggests these may partially be responsible for any protective effect of fruits and vegetables. Reduction in risk ratio with introduction of BMI, BP, diabetes and blood lipids suggests some of these may be intermediary processes between an effect of fruit and vegetables on CVD risk).

Quality: A - B</td>
</tr>
</table>

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Genkinger et al., 2004

United States

CLUE Study / Oddessy Cohort

CHD Study #7</td>
<td>Cohort Study</td>
<td>n=6151 adult residents of Maryland (with data) 30-93 y

volunteers from 2 studies (non-random sample)

did not exclude those with baseline histories of CVD or other diseases</td>
<td>Baseline 1974

13y follow-up</td>
<td>Usual consumption fruits and vegetables, cruciferous vegetables (quintiles)

61-item FFQ categories never to 2+ per day

Fruit: apple, applesauce, pear, cantaloupe, orange, grapefruit, other

Vegetables: tomato, broccoli, spinach, mustard greens, green salad, sweet potatoes, yams other (potatoes excluded from analysis)

Validity:
Questionnaire validated against multiple food records. In groups of sex and age correlations were:
0.4-0.6 for energy
0.50-0.57 for vit C.
0.52-0.64 for folate</td>
<td>Mortality from CVD

Death certificates (ICD 9th revision 390 – 459) coded by state nosologists

No follow up status on 3%.

Participants also reported diagnoses in follow up questionnaires</td>
<td>Hazard Ratios for death from CVD by quintiles of intake of fruit and vegetables
<table>
<tr><th></th><th>HR (95%CI)[a]</th><th>HR (95%CI)[b]</th></tr>
<tr><td>Q1 0.89 sve/d</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Q2 1.61 sve/d</td><td>0.76 (0.54 – 1.08)</td><td>0.80 (0.56 – 1.12)</td></tr>
<tr><td>Q3 2.31 sve/d</td><td>0.83 (0.60 – 1.15)</td><td>0.86 (0.62 – 1.20)</td></tr>
<tr><td>Q4 3.21 sve/d</td><td>0.74 (0.53 – 1.02)</td><td>0.79 (0.56 – 1.09)</td></tr>
<tr><td>Q5 4.89 sve/d</td><td>0.71 (0.51 – 0.98)</td><td>0.76 (0.54 – 1.06)</td></tr>
<tr><td>p for trend</td><td>0.07</td><td>0.15</td></tr>
</table>
Hazard Ratios for death from CVD by quintiles of intake of cruciferous vegetables
<table>
<tr><th></th><th>HR (95%CI)[a]</th><th>HR (95%CI)[b]</th></tr>
<tr><td>Q1 0.03 sve/d</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Q2 0.12 sve/d</td><td>0.95 (0.67 – 1.35)</td><td>0.99 (0.70 – 1.43)</td></tr>
<tr><td>Q3 0.17 sve/d</td><td>1.06 (0.77 – 1.46)</td><td>1.17 (0.84 – 1.62)</td></tr>
<tr><td>Q4 0.27 sve/d</td><td>0.94 (0.67 – 1.32)</td><td>1.03 (0.74 – 1.45)</td></tr>
<tr><td>Q5 0.53 sve/d</td><td>0.83 (0.60 – 1.16)</td><td>0.89 (0.64 – 1.25)</td></tr>
<tr><td>p for trend</td><td>0.27</td><td>0.51</td></tr>
</table>
a adjusted for age, energy
b adjusted as a also smoking, BMI, cholesterol

Reported as not confounding results (data not shown) marital status, education, saturated fat intake, diagnosis prior to baseline of diabetes, MI or cancer.

Results similar when stratified by gender, BMI (not shown) and for ever vs never smokers (results not shown.

Consuming 5 or more servings a day of fruits and vegetables associated with CVD HR=1.04 95%CI (0.76 – 1.42)</td>
<td>Considering standard CVD risk factors:
• An intake of fruits and vegetables of around 5 serves per day compared with around one serve per day is associated with around 25-30% reduced risk of cardiovascular disease (significant).
• An intake of cruciferous vegetables of around half a serve per day compared with virtually none is associated with a non-significant reduction in risk of cardiovascular disease of around 10-20%.
• Effect of fruits and vegetables said to be similar irrespective of gender, overweight/ obesity and smoking status.

Issues:
Considered saturated fat intake but not total fat or other fatty acids, physical activity, alcohol.

Quality Rating: B</td>
</tr>
</table>

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Tucker et al 2005

United States

Baltimore Longitudinal Study of Ageing

CHD Study #8</td>
<td>Cohort</td>
<td>n=501 men aged around 30 to 80y at baseline with at least 4d diet records for ≥1 visit, no angina pectoris or MI at baseline

Response rate not recorded</td>
<td>Follow-up of all participants for an average of 18y.</td>
<td>Intake of fruit, vegetables, fruit and vegetables (USDA serves/d)

7-d diet record at 4 time periods 191-1965, 1968-1975, 1984-1991 1993-present (excluded records within 2 years of death or CHD because may affect dietary intake)</td>
<td>Mortality from CHD

3 physician consensus death certificates, hospital and physician records, autopsy data. CHD death: acute MI or sudden coronary death. Time of CHD diagnosis by first Q-wave, nonfatal MI or CHD death.</td>
<td>Risk Ratio (95%CI) for CHD with increasing intake of fruits and vegetables
<table>
<tr><th>(serves/d)</th><th>Model 1
OR (95%CI)</th><th>Model 2
OR (95%CI)</th><th>Model 3
OR (95%CI)</th></tr>
<tr><td>fruit</td><td>0.86 (0.70 -1.05)</td><td>0.97 (0.78-1.20)</td><td>0.97 (0.79 – 1.20)</td></tr>
<tr><td>veg</td><td>0.60 (0.46 - 0.78)</td><td>0.65 (0.50 – 0.85)</td><td>0.73 (0.54 – 0.97)</td></tr>
<tr><td>fruit & veg</td><td>0.79 (0.69 – 0.92)</td><td>0.84 (0.72-0.99)</td><td>0.90 (0.76 – 1.05)</td></tr>
</table>
1) Adjusted for age, energy intake, BMI, etoH, physical activity, supplement use
2) Adjusted as 1 also saturated fat
3) Adjusted as 2 also for secular trend (year of first visit)

Risk Ratios for CHD according to combined fat, fruit and vegetable intake grouping
<table>
<tr><th></th><th>Risk Ratio
Model 4</th><th>Risk Ratio
Model 5</th></tr>
<tr><td>Low FV, Hi %SF</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Low FV, Low %SF</td><td>0.36 (0.15 – 0.84)</td><td>0.41 (0.17 – 0.98)</td></tr>
<tr><td>Hi FV, Hi %SF</td><td>0.33 (0.15 – 0.71)</td><td>0.46 (0.21 - 0.99)</td></tr>
<tr><td>Hi FV, Low %SF</td><td>0.24 (0.11 – 0.52)</td><td>0.37 (0.16 - 0.81)</td></tr>
</table>
Fruit and vegetables Hi FV ≥5 sve/d, Low <5 sve/d
% Energy from saturated fat Hi %SF ≥12 %, Low %SF <12%

4) Adjusted as model 2.
5) Adjusted as model 3.
Additional adjustments for
n-3 fatty acids, PUFA, trans-fat, whole grains did not alter results (not shown).

Note cut-off for % energy as saturated fat was 12% instead of 10% due to insufficient participants with low intake.</td>
<td>In men, after considering most CHD risk factors:

Each additional daily serve of fruit associated with ≈14% reduced risk (not significant) of CHD. Risk reduction negligible after adjusting for saturated fat intake.
Each additional daily serve of vegetables associated with a ≈40% reduced risk of CHD (significant). Risk reduction ≈ 35% after adjusting for saturated fat (significant).
Each additional serve of combined fruit and vegetables associated with a reduction in risk of CHD of around 20% (significant). Risk reduction around 16% (significant) after adjusting for saturated fat and around 10% (not-significant) also considering when subjects were recruited into study
Statistical adjustments for saturated fat may be over-adjustments. Men with both high fruit and vegetable intakes and low saturated fat intakes had ≈63% reduced risk of CHD, while those with one but not the other had a slightly lesser reduction in risk (although this was not statistically significant).

Quality rating: A</td>
</tr>
</table>

Appendix 2 cont'd: Fruits, Vegetables and CHD – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Liu et al., 2001

United States

Physician's Health Study

CHD Study #9</td>
<td>Cohort Study</td>
<td>n=15220 male physicians 40-84 y (at baseline) no heart disease, stroke, cancer enrolled in the Physician's Heath Study RCT which took a random sample of physicians in American Medical Association

response rate not recorded; 69% with complete data included in analyses</td>
<td>12 y follow-up</td>
<td>Intake of vegetables (USDA serves/ day)

Semi quantitative FFQ 'over the last year' incl 8 vegetable items (preset portion sizes, 7 responses 'never' to '2+ times daily)

Validation:
does not claim to be validated per se. Claims ' a similar FFQ was validated' for vegetables
r = .47 to .57 against another FFQ, and
r = .29 to .54 against multiple recall in different populations</td>
<td>Incident MI, First coronary artery bypass graft (CABG) or percutaneous luminal coronary angioplasty (PTCA), Incident CHD (ie. either outcome)

Diagnosis:
PTCA and CABG self-reported

MI classified by WHO criteria. For non-fatal MI used review of medical records. For fatal MI used death certificates, hospital records, and observer reports (when death occurred outside of a hospital setting)</td>
<td>RR (95% CI) for CHD across quintiles of vegetable intake
<table>
<tr><th>Qunitile (serves/d)</th><th>CHD</th><th>MI</th><th>CABG/ PTCA</th></tr>
<tr><td>Model 1</td><td></td><td></td><td></td></tr>
<tr><td>Q1 (<1)</td><td>1.00 (ref)</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Q2 (1-1.49)</td><td>0.93 (0.81 – 1.08)</td><td>1.01 (0.79 – 1.30)</td><td>0.90 (0.75 – 1.08)</td></tr>
<tr><td>Q3 (1.5-1.9)</td><td>0.88 (0.74 – 1.04)</td><td>0.82 (0.61 – 1.12)</td><td>0.90 (0.73 – 1.11)</td></tr>
<tr><td>Q4 (2-2.49)</td><td>0.82 (0.67 – 1.01)</td><td>0.92 (0.65 – 1.30)</td><td>0.77 (0.60 – 1.00)</td></tr>
<tr><td>Q5 (2.5 +)</td><td>0.71 (0.57 – 0.89)</td><td>0.79 (0.55 - 1.16)</td><td>0.67 (0.50 – 0.89)</td></tr>
<tr><td>p trend</td><td>0.001</td><td>0.16</td><td>0.002</td></tr>
<tr><td>Model 2</td><td></td><td></td><td></td></tr>
<tr><td>Q1 (<1)</td><td>1.00 (ref)</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Q2 (1-1.49)</td><td>0.99 (0.85 – 1.15</td><td>1.05 (0.84 – 1.31)</td><td>0.94 (0.87 – 1.14)</td></tr>
<tr><td>Q3 (1.5-1.9)</td><td>0.93 (0.78 – 1.12)</td><td>0.90 (0.59 – 1.16)</td><td>0.99 (0.79 – 1.23)</td></tr>
<tr><td>Q4 (2-2.49)</td><td>0.89 (0.71 – 1.10)</td><td>0.98 (0.67 -1.43)</td><td>0.88 (0.67 – 1.16)</td></tr>
<tr><td>Q5 (2.5 +)</td><td>0.77 (0.60 – 0.98)</td><td>0.81 (0.59 – 1.31)</td><td>0.70 (0.51 – 0.95)</td></tr>
<tr><td>p trend</td><td>0.03</td><td>0.24</td><td>0.03</td></tr>
</table>
(CHD is coronary heart disease defined by either the MI or the CABG/ PTCA outcomes)

RR (95% CI) for CHD (by MI, or CABG/ PTCA) across quintiles of vegetable intake in smokers, and in people with a BMI <u>></u> 25
<table>
<tr><th></th><th>Smokers Only</th><th>BMI <u>></u> 25</th></tr>
<tr><td>Model 1</td><td></td><td></td></tr>
<tr><td>Q1 (<1)</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Q2 (1-1.49)</td><td>0.95 (0.66 – 1.39)</td><td>1.03 (0.84 – 1.25)</td></tr>
<tr><td>Q3 (1.5-1.9)</td><td>1.00 (0.65 – 1.57)</td><td>0.88 (0.70 – 1.11)</td></tr>
<tr><td>Q4 (2-2.49)</td><td>0.84 (0.47 – 1.48)</td><td>0.80 (0.60 – 1.06)</td></tr>
<tr><td>Q5 (2.5 +)</td><td>0.40 (0.18 – 0.86)</td><td>0.73 (0.54 – 0.99)</td></tr>
<tr><td>p for trend</td><td>0.04</td><td>0.01</td></tr>
<tr><td>Model 2</td><td></td><td></td></tr>
<tr><td>Q1 (<1)</td><td>1.00 (ref)</td><td>1.00 (ref)</td></tr>
<tr><td>Q2 (1-1.49)</td><td>1.03 (0.69 – 1.55)</td><td>1.07 (0.86 – 1.32</td></tr>
<tr><td>Q3 (1.5-1.9)</td><td>1.06 (0.65 – 1.73)</td><td>0.93 (0.72 – 1.20)</td></tr>
<tr><td>Q4 (2-2.49)</td><td>0.75 (0.39 – 1.44)</td><td>0.91 (0.67 – 1.23)</td></tr>
<tr><td>Q5 (2.5 +)</td><td>0.41 (0.18 – 0.97)</td><td>0.74 (0.53 – 1.03)</td></tr>
<tr><td>p for trend</td><td>0.06</td><td>0.07</td></tr>
</table>
Model 1: adjusted for age and treatment
Model 2: also adjusted for smoking, alcohol, physical activity BMI, hx diabetes, hx high cholesterol, hx hypertension, use of multivitamins</td>
<td>Among male health professionals, after considering most CVD risk factors, consuming 2.5+ vs <1 serve per day of vegetables is associated with:
• ≈ 20% lower risk of onset of CHD (significant); dose-response relationship
• ≈ 20% lower risk of initial MI (not significant); not dose-response
• ≈ 30% lower risk of CABG/ PTCA (significant); dose-response relationship
• ≈ 60% reduced risk of CHD for smokers (significant); borderline dose-response relationship
• ≈ 25% reduced risk in the overweight and obese (BMI>25) (not significant) borderline dose-response relationship

Issues:
did not adjust for other fatty acids, energy
questionable measurement of dietary exposure

Quality rating: B</td>
</tr>
</table>

Appendix 3: Fruits, Vegetables and CHD – Observational Studies (Case-Control)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Yusuf et al., 2004

INTERHEART Study (52 Countries)

(Europe [Western, Central & Eastern], Middle East, Africa, South Asia, China & Hong Kong, Southeast Asia & Japan, Australia & New Zealand, South America & Mexico, North America)

CHD Study #10</td>
<td>Multi-centre Case Control

Quality – good: dietary measure crude; participation rate unclear</td>
<td>15152 cases & 14820 from 52 countries

Most aged 45-75 yrs, but more inclusive

Recruitment all eligible cases in 262 coronary care units. Hospitals chosen by feasibility. Controls frequency matched for age & sex – hospital and community based.

Response rates not recorded</td>
<td>2000-2002</td>
<td>Daily consumption of fruits & vegetables

measure yes/no or quantitative amounts?

“Structured Questionnaire”

repeatability for consumption of fruits (kappa=0.66)

repeatability for consumption of vegetables (kappa=0.52)</td>
<td>Initial AMI

Diagnosis by symptoms, plus ECG</td>
<td>OR (95% CI) for CHD with daily fruit and vegetable consumption
<table>
<tr><th></th><th>Overall</th><th>Men</th><th>Women</th></tr>
<tr><td>Adjusted 1</td><td>0.70 (0.64 - 0.77)</td><td></td><td></td></tr>
<tr><td>Adjusted 2</td><td>0.70 (0.62 – 0.79)</td><td></td><td></td></tr>
<tr><td>Adjusted 3</td><td></td><td>0.74 (0.66 – 0.83)</td><td>0.58 (0.48 – 0.71)</td></tr>
<tr><td>Adjusted 4</td><td></td><td></td><td></td></tr>
<tr><td>Younger</td><td>0.69 (0.58 – 0.81)</td><td>0.72 (0.59 – 0.88)</td><td>0.62 (0.44 – 0.87)</td></tr>
<tr><td>Older</td><td>0.72 (0.61 – 0.85)</td><td>0.77 (0.64 – 0.93)</td><td>0.55 (0.38 – 0.80)</td></tr>
</table>
Adjusted for
1) age, sex, smoking
2) as ‘1’ also diabetes, hypertension, abdominal obesity, psychosocial variables, exercise, etOH, Apo B/A ratio
3) age, sex, geographic region
4) sex smoking diabetes, hypertension, abdominal obesity, psychosocial variables, exercise, etOH, Apo B/A ratio</td>
<td>Considering most standard CVD risk factors, consuming fruits and vegetables daily is associated with a reduction in risk of onset of heart disease:
• of ≈30% overall (significant)
• of ≈42% in women (significant)
• of ≈26% in men (significant)
• which is greatest in older women and least in older men
• results generally consistent across regions

Issues:
The measure does not capture quantities or distinguish between fruits/ vegetables/ juices. Therefore:
• Dose-response cannot be estimated. The estimated ‘≈30%’ risk reduction applies to the unknown levels of and types of fruit and vegetable consumption in the ‘daily’ compared with ‘not daily’ groups.
• Differences by gender, and age groups may not reflect a biologically different effect of fruit and vegetable consumption. This is reinforced by the large difference gender estimates depending on which other factors were adjusted for.
• Does not adjust for intake of fatty acids, energy
• Effect is cumulative with other health behaviours – authors estimate eating fruits and vegetables, exercising and avoiding smoking could lead to about 80% lower relative risk for MI
Quality: B</td>
</tr>
</table>

Appendix 3 cont'd: Fruits, Vegetables and CHD – Observational Studies (Case-Control)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Martínez-González et al., 2002

Pamplona, Spain

CHD Study #11 part 1</td>
<td>Case control

Design - good Participation rate good, diet & outcome measures good, stats appropriate, adjustment appropriate</td>
<td>171 cases <80y adults

Recruitment: eligible cases admitted to any of three 'third level' hospitals in Pamplona

Hospital based controls matched for age (5 y bands) & sex

95% response rate for both cases and controls</td>
<td>Oct 1999 to June 2000,

Oct 2000 to Feb 2001</td>
<td>Quintiles of fruit intake, quintiles of vegetable intake

Semi-quantitative 136-item FFQ

expanded from FFQ, validated for intakes of energy, protein, CHO, fats (saturated, mono- and poly-unsaturated), alcohol, cholesterol, fibre, vitamin A & vitamin C)</td>
<td>Initial AMI (non-fatal)

Diagnosis: 2+ ECG, ECG probable changes plus cardiac enzymes, symptoms plus enzymes</td>
<td>Odds Ratios and 95% CI for CHD across quintiles of consumption of fruits and vegetables (adjusted for energy by residuals method)
<table>
<tr><th></th><th>Q1</th><th>Q2</th><th>Q3</th><th>Q4</th><th>Q5</th><th>p for trend</th></tr>
<tr><td colspan="7">Fruit consumption</td></tr>
<tr><td>Adj 1</td><td>1 (ref)</td><td>0.74 (0.35 – 1.56)</td><td>0.46 (0.22 – 0.96)</td><td>0.57 (0.26 – 1.26)</td><td>0.52 (0.23 – 1.14)</td><td>0.19</td></tr>
<tr><td>Adj 2</td><td>1 (ref)</td><td>0.27 (0.09 – 0.78)</td><td>0.30 (0.11 – 0.79)</td><td>0.30 (0.11 – 0.82)</td><td>0.29 (0.10 – 0.83)</td><td>0.16</td></tr>
<tr><td>Adj 3</td><td>1 (ref)</td><td>0.28 (0.08 – 0.95)</td><td>0.24 (0.08 – 0.79)</td><td>0.27 (0.08 – 0.89)</td><td>0.20 (0.05 – 0.78)</td><td>0.14</td></tr>
<tr><td>Adj 4</td><td>1 (ref)</td><td>0.28 (0.08 – 0.95)</td><td>-</td><td>0.25 (0.08 – 0.74)</td><td>-</td><td>-</td></tr>
<tr><td>Median (g/day)</td><td>95</td><td>209</td><td>303</td><td>451</td><td>751</td><td></td></tr>
<tr><td colspan="7">Vegetable consumption</td></tr>
<tr><td>Adj 1</td><td>1 (ref)</td><td>0.60 (0.30 – 1.20)</td><td>0.70 (0.35 – 1.40)</td><td>0.82 (0.42 – 1.60)</td><td>0.60 (0.29 – 1.25)</td><td>0.35</td></tr>
<tr><td>Adj 2</td><td>1 (ref)</td><td>0.43 (0.18 – 1.04)</td><td>0.40 (0.16 – 0.98)</td><td>0.59 (0.26 – 1.34)</td><td>0.42 (0.17 – 1.03)</td><td>0.15</td></tr>
<tr><td>Adj 3</td><td>1 (ref)</td><td>0.64 (0.24 – 1.75)</td><td>0.67 (0.22 – 2.00)</td><td>1.44 (0.47 – 4.43)</td><td>0.80 (0.20 – 3.13)</td><td>0.79</td></tr>
<tr><td>Adj 4</td><td>1 (ref)</td><td>0.64 (0.24 – 1.75)</td><td></td><td>0.92 (0.35 – 2.41)</td><td></td><td>-</td></tr>
<tr><td>Median (g/day)</td><td>278</td><td>411</td><td>550</td><td>657</td><td>950</td><td></td></tr>
</table>
1) matched for hospital, age & sex
2) as '1' also adjusted for smoking, BMI, hypertension, History of elevated cholesterol, diabetes, physical activity, marital status, occupation, education (multivariable)
3) as '2' also adjusted for %energy from alcohol, saturated fat, trans-fat, energy-adjusted glycaemic load, folic acid intake, energy-adjusted olive oil intake (additional)
4) quintiles 3-5 are one category, adjusted as '3'.</td>
<td>After adjusting for CVD risk factors:
• a high intake of fruits is associated with ≈ 80% reduction in odds of CHD onset (sig).
• A high consumption of vegetables associated with a non-significant reduction in odds of onset of CHD (≈20%)

Threshold (not linear) effect for fruits suggested by similar OR in quintiles 3, 4 & 5.

Issues:
Results likely influenced by very high intakes in this population relative to other study populations.

Quality rating: A</td>
</tr>
</table>

Appendix 3 cont'd: Fruits, Vegetables and CHD – Observational Studies (Case-Control)

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Martínez-González et al., 2002 Pamplona, Spain **CHD Study #11 part 2**	Case control Design - good Participation rate good, diet & outcome measures good, stats appropriate, adjustment appropriate	n=171 cases n=171 controls <80 y men and women Recruitment: eligible cases admitted to any of three 'third level' hospitals in Pamplona Hospital-based controls matched for age (5 y bands) & sex 95% response rate for cases & controls	Oct 1999 to June 2000, Oct 2000 to Feb 2001	Quintiles of fruit intake, quintiles of vegetable intake quintiles 2-5 vs 1 (post-hoc cut offs) Semi-quantitative FFQ (136 items), expanded from FFQ, validated for intakes of energy, protein, CHO, fats (saturated, mono- and poly-unsaturated), alcohol, cholesterol, fibre, vitamin A & vitamin C)	Initial AMI (non-fatal) Diagnosis: 2+ ECG, ECG probable changes plus cardiac enzymes, symptoms plus enzymes	OR (95% CI) of initial MI according to intakes of fruits, and vegetables **Fruit *(g/d)*** Q1 <175: 1 (ref) Q2 175-252: 0.28 (0.08 – 0.91) Q3 252-364: 0.38 (0.13 – 1.10) Q4 365-570: 0.32 (0.11 – 0.97) Q5 >570: 0.43 (0.14 – 1.34) Q3,4,5 vs Q1: 0.37 (0.14 – 0.96) [Additionally adjusted [b]: 0.65 (0.16 – 2.61)] Q2-5 vs Q1: 0.35 (0.14 – 0.89) **Veg *(g/d)*** Q1 <347: 1 (ref) Q2 347-482: 0.42 (0.16 – 1.08) Q3 482-583: 0.36 (0.13 – 0.98) Q4 583-744: 0.61 (0.23 – 1.57) Q5 >744: 0.42 (0.15 -1.20) Q3,4,5 vs Q1: 0.46 (0.21 – 1.04) [Additionally adjusted [b]: 0.71 (0.23 – 2.17)] Q2-5 vs Q1: 0.45 (0.21 – 0.98) a) matched for age, hospital, gender adjusted for energy intake, smoking, BMI, hypertension, high cholesterol, diabetes, physical activity, SES b) matched and adjusted as 'a' also adjusted for other food(s)/ food groups (olive oil, fibre, fish, alcohol, meat/ meat products, white bread + rice + pasta)	After adjusting for CVD risk factors, odds of onset of CHD were: • ≈63% lower with intake of at least 250g fruit relative to less than 175g daily (significant) – smaller (≈35%) and not significant after adjustment for other food groups • ≈54% lower with intake of at least 480g vegetables relative to less than 350g daily (smaller (≈30% and not significant after adjusting for other food groups). Using the post-hoc determined cut-offs, and not adjusting for other food groups, odds of CHD onset were: • ≈65% lower with intake of at least 175g of fruit daily (significant) • ≈55% lower with at least 350g vegetables daily (significant) Issues: Confidence intervals were quite wide with additional adjustments (perhaps dietary variables highly correlated, and relatively small sample size) Quality rating: A

Appendix 3 cont'd: Fruits, Vegetables and CHD – Observational Studies (Case-Control)

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Rastogi et al., 2004 India **CHD Study #12**	case-control study multi-centre	n=350 cases, n=700 controls matched for age, sex, hospital (aged 21-74y av.52 ± 11y, 88% males) Cases: 84% participation rate. Eligible all first AMI in 8 hospitals not pregnant, no previous MI or IHD, cancer, chronic kidney, GI, thyroid disease, prior (4wks) acute viral infection. Controls: 99-100% participation rate. With minor ailments from (eye, ear, nose & throat, dermatology, orthopaedics, surgery, general medicine, gynaecology, other wards/clinics). Selected by1 of 2 methods. 1. Research assistant followed one physician and invited his/her patients who were eligible to participate. 2. Screened & invited to participate based on queue number.	approx Jan 1999 to Dec 1999	Daily intake fruits, vegetables (excludes potatoes), green leafy vegetables, beans, potatoes, fruits New Delhi: 149-item FFQ based on previous 24h recalls Bangalore: 141-item FFQ based on previous 24h recalls Each 21 vegetable items, 16 fruit items, 5 bean items common veg were potatoes, tomatoes cucumber, onion, green leafy veg, gourds, okra, carrots common fruits were: bananas, mangoes, citrus fruit, papaya apples	First AMI Diag-nosis by clinical exam, ECG, and cardiac enzymes		After adjustment for other CVD risk factors and dietary factors, risk of onset of ischemic heart disease was: • ≈70% lower with >3 vs ≤1 serves of vegetables daily (significant) (dose-response) • ≈70% lower with >3 vs ≤1 serves of green leafy vegetables daily (significant) (dose-response) • ≈20% lower with >3 vs ≤1 serves of potatoes daily (not significant) • ≈2x higher for with >3 vs ≤1 serves of fruit daily (significant) Issues: No adjustment for energy but energy sources did not confound *Generalisability* - some fruits & vegetables not typical in Australia/ N.Z. Population tendency to undernutrition (India) vs over-nutrition (Aust & N.Z) esp. important regarding fruit & bean findings – could reflect lack of protein/energy Quality Rating: A

	Model 1	Model 2	Model 3	Model 4
Vegetables				
≤1 serve/d	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 serve/ d	0.85 (0.60 – 1.21)	0.81 (0.54 – 1.21)	0.73 (0.45 – 1.19)	0.73 (0.44 – 1.20)
2-3 serve/ d	0.79 (0.53 – 1.19)	0.78 (0.49 – 1.25)	0.63 (0.35 – 1.22)	0.62 (0.34 – 1.12)
>3 serve/ d	0.59 (0.32 – 1.08)	0.36 (0.18 – 0.73)	0.27 (0.11 – 0.64)	0.33 (0.13 – 0.82)
p for trend	*0.09*	*0.01*	*0.006*	*0.006*
Green leafy				
≤1 serve/d	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 serve/ d	1.12 (0.75 – 1.66)	0.93 (0.59 – 1.50)	0.83 (0.47 – 1.44)	0.85 (0.48 – 1.52)
2-3 serve/ d	0.78 (0.49 – 1.23)	0.71 (0.45 – 1.26)	0.60 (0.32 – 1.14)	0.55 (0.28 – 1.06)
>3 serve/ d	0.61 (0.37 – 0.98)	0.43 (0.24 – 0.75)	0.33 (0.17 – 0.64)	0.34 (0.17 – 0.69)
p for trend	*0.002*	*0.0002*	*0.0001*	0.0002
Potatoes				
≤1 serve/d	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 serve/ d	0.81 (0.55 – 1.19)	0.82 (0.53 – 1.27)	1.05 (0.64 – 1.71)	1.08 (0.64 – 1.80)
2-4 serve/ d	1.15 (0.77 – 1.72)	1.27 (0.80 – 2.00)	1.37 (0.79 – 2.35)	1.47 (0.83 – 2.60)
>4 serve/ d	1.06 (0.65 – 1.71)	0.87 (0.50 – 1.51)	0.75 (0.39 – 1.45)	0.86 (0.42 – 1.75)
p for trend	*0.7*	*0.8*	*0.3*	*0.7*
Fruit				
≤1 serve/d	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 serve/ d	1.10 (0.82 – 1.48)	1.16 (0.82 – 1.62)	1.26 (0.84 – 1.91)	1.45 (0.46 – 1.25)
2-3 serve/ d	0.88 90.58 – 1.33)	1.15 (0.72 – 1.83)	1.21 (0.68 – 2.14)	1.29 (0.71 – 2.35)
>3 serve/ d	1.46 (0.89 – 2.39	1.96 (1.11 – 3.46)	2.11 (1.03 – 4.32)	2.46 (1.15 – 5.25)
p for trend	*0.4*	*0.04*	*0.06*	*0.03*

Model 1: adjusted for age, sex, hospital
Model 2: adjusted as model 1 also smoking (cigarettes, bidis)
Model 3: adjusted as model 2 also BMI, waist-hip ratio, physical activity, hx hypertension, hx diabetes, hx high cholesterol, family hx IHD, alcohol, education, household income, Hindu religion
Model 4: adjusted as model 3 also cereal intake, green leafy veges (except total vegetable and green leafy analysis), mustard oil in cooking, mustard oil use in frying

(note – other fats used not associated wth IHD in this study)

Appendix 3 cont'd: Fruits, Vegetables and CHD – Observational Studies (Case-Control)

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Tavani et al., 2004 Northern Italy **CHD Study #13**	combined 3 case-control studies	1713 cases, 2317 hospital controls <75 (1179 men 558 women) Cases: first AMI in study hospitals Controls: same hospital, diagnosis unrelated to CVD and major CVD risks (response over 95% in all studies) Exclusions: do not know family hx	1983-1992 1988-1999 1995-1999	Frequency intake vegetables (portions/wk) Questionnaire administered by interviewer Reproducible for vegetable and fruit items, validated for nutrients. Fruit (fresh only) Vegetables (did not distinguish fresh, frozen, canned but excluded potatoes, legumes and vegetable soups)	Incident non-fatal MI Diagnosis ICD9 410.0 by WHO criteria	OR (95%CI) for initial non-fatal AMI according to intake of vegetables **OR (95%CI)** All subjects (n=4054) Vegetables <7.5 vs ≥7.5 portions/ wk 1.26 (1.08 – 1.46) Family History IHD (n=1094) Vegetables <7.5 vs ≥7.5 portions/ wk 1.35 (1.01 – 1.80) No Family History IHD (n=2960) Vegetables <7.5 vs ≥7.5 portions/ wk 1.29 (1.07 – 1.55) All models adjusted for study centre, age, sex, education, serum cholesterol, hx diabetes, hx hypertension, physical activity, BMI, smoking, consumption of coffee, alcohol, coffee fish Did not consider saturated fat intake or olive oil Despite approximately equal numbers for the overall analysis and the subgroup analysis by family history, the global effect is smaller than the effect for both subgroups. Suggestions: could be an error. Unsure whether this is a typographical error in the publication.	Considering most CVD risk factors, the risk of onset of heart disease is: • increased by ≈26% with consuming <7.5 portions per week of vegetables (significant) • ≈ 35% for those with, and ≈ 29% for those without family history of heart disease (both significant) • not differently affected by vegetable intake for people with and without a family history (statistically) Issues: Unsure of size of one portion therefore difficult to describe magnitude of effect Fatty acids not considered Quality: B

Appendix 4: Fruits, Vegetables and CHD – Meta-analysis

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Anderson et al., 2000	meta-analysis	7 studies (vegetables) 8 studies (fruits) Wolk et al 1999 Campbell et al 1998 Pietnen et al 1996 Rimm et al 1996 Knekt et al 1994 Fehily et al 1993 Gramenzi et al 1993 McGee et al 1984 Kahn et al 1984	studies from 1984-1999	consumption of fruits, vegetables (unsure what units – guessing quintiles)	CHD risk CHD risk or mortality, CAD, IHD	Pooled Relative Risks (95%CI) Vegetables 0.78 (0.39 – 0.89) (primary adjusted) 0.77 (0.70 – 0.86) (secondary adjusted) Fruits 0.85 (0.74 – 0.98) (primary adjusted) 0.86 (0.77 – 0.96) (secondary adjusted) Primary adjustments: age, energy, other dietary factors (protein, various micronutrients incl vit B6), smoking Secondary: vit B6, gender, smoking, BMI, alcohol, family history, BP or hypertension, , cholesterol or hx heart disease, physical activity HRT or menopausal status, dietary factors, supplement use, education, use of aspirin	Risk of CHD (or related diseases): • Is reduced ≈20% with a high intake of vegetables (significant) • Is reduced ≈15% with a high intake of fruits (significant) • is shown to be reduced by a similar amount whether studies adjusted only for age, energy, smoking and 'other dietary factors' or also adjusted for use of supplements, family history, presence of many traditional coronary risks, physical activity, menopausal status, education, or use of aspirin. Issues: Pooled RR is based on risks in the upper vs lower categories of intake, which are not quantified. Notably, quintiles reflect actual intakes achieved by segments of the populations studied, which were largely from the U.S and Europe, and therefore are comparable to achievable levels in the Australian/ New Zealand diet.

Appendix 5: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Obarzanek et al, 2001 United States DASH **Biomarker Study #1**	RCT Single-blinded Not clinical setting	459 adults ≥ 22 y high BP no HT medication (exclusion: very high cholesterol) 146 - intervention recruited from 4 centres; participation rates not reported	8 wk intervention 3wk run in (control diet)	3 diets: 1) 'control', 3) F&V F&V diet: 5.2 servings/d fruit and juices, 3.0 servings veg DASH diet: also high in fruits 5.6 sves and vegetables 5.2 but also uses low fat dairy products, whole grains, poultry, fish nuts, and has less fats, red meat, sweets, sugary beverages Control Diet: 1.6 servings/d fruit and juices, 2.0 serves veg F&V diet otherwise similar to controls (same macronutrients) however also slightly extra grains and less sucrose than the control diet. Food prepared by research team, consumed at home	Plasma TC, LDL, HDL, trygliceride s, TC:HDL, LDL:HDL Used controlled storage and processing procedures	Change in blood lipids from end of run-in period in F&V diet group after intervention (net of control) Δ blood lipids (mmol/L) Δ TC -0.10 (-0.23 – 0.04) Δ LDL -0.05 (-0.17 – 0.07) Δ HDL -0.005 (-0.04 – 0.03) Δ TAG -0.09 (-0.19 – 0.002) Δ TC:HDL -0.14 (-0.29 – 0.02) Δ LDL:HDL -0.10 (-0.23 – 0.04) Change in blood lipids from end of run-in period in F&V diet group after intervention (net of control) Change in blood lipids (mmol/L) Δ TC women 0.03 (-0.16 – 0.22) Δ TC men -0.18 (-0.36 – 0.00)[a] Δ TC 'higher baseline' -0.12 (-0.29 – 0.05) Δ TC 'lower baseline' -0.01 (-0.21 – 0.18) Δ LDL women 0.05 (-0.12 – 0.23) Δ LDL men -0.12 (-0.29 – 0.05) Δ LDL 'higher baseline' -0.01 (-0.16 – 0.15) Δ LDL 'lower baseline' -0.08 - -0.25 – 0.10) Δ HDL women 0.01 (-0.04 – 0.07) Δ HDL men -0.03 (-0.08 – 0.02) Δ HDL 'higher baseline' 0.03 (-0.02 – 0.08) Δ HDL 'lower baseline' -0.04 (-0.09 – 0.01) Δ TAG women -0.10 (-0.24 – 0.04) Δ TAG men -0.07 (-0.20 – 0.06) Δ TAG 'higher baseline' -0.02 (-0.15 – 0.12) Δ TAG 'lower baseline' -0.15 (-0.29 - -0.02)[b] Δ TC:HDL women -0.04 (-0.26 – 0.19) Δ TC:HDL men -0.23 (-0.44 - -0.01)[b] Δ LDL:HDL women 0.02 (-0.24 – 0.04) Δ LDL:HDL men -0.19 (-0.39 – 0.00)[b] [a] p=0.053 [b] p<0.05 controlled for race, sex and baseline lipids by adjustment or stratification	Compared with controls, people with high BP who followed a diet with an increase in fruit (3 serves daily) and vegetable (1 serve daily) content for 8 weeks: • had slightly greater reductions (0.03 to 0.19) in TC, HDL, LDL, and LDL:HDL ratio in *(men)* (only TC significant & LDL:HDL ratio borderline significant) • had slightly lesser reductions 0.01-0.05 in TC, HDL, LDL, and LDL:HDL ratio *(women)* (none significant) • had slightly greater reductions in TAG ≈0.1mmol/L *(men & women)* (not significant) • had greater reductions in TC:HDL ratio (≈0.2mmol/L, significant *(men)*) (≈ 0.04 mmol/L, significant *(women)*) *Subgroup* Data suggest any effect might be larger for men than women. Data suggest effect may vary with baseline levels, but not consistently across parameters Issues: Fruit and Vegetable intervention diet NOT primary hypothesis Vegetable content of the intervention diet was quite low, and was minimally different from the control diet. Fruit included juices but unsure how much was juice, how much whole fruit intervention group higher proportion smokers (≈7%) lower proportion drink alcohol (≈11%) than control group Quality rating: B

Johnson et al (1996) Comparison of multiple-pass 24-hour recall estimates of energy intake with total energy expenditure determined by the doubly labelled water method in young children *J Am Diet Ass* 96: 1140-4

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Broekmans et al., 2001

Netherlands

Biomarker Study #2</td>
<td>RCT</td>
<td>n=48 healthy adults 40-60y with low usual fruit and veg intake <250g/d, and not taking supplements or vitamins

volunteers recruited through newspaper ad

1 non-complier excluded from analysis

23 'low' group
24 'high' group</td>
<td>Sept 1997 to Oct 1997

4 week diet period (no run in)</td>
<td>2 diets:
1/ low in fruit and vegetables (100g/d veg, 0mL/d juice)
2/ 'high' in vegetables & fruit (500g/d plus 200 ml/d juice)

Evening meal consumed on-site, rest taken home

Diet controlled for energy, fat (total and fatty acid composition), protein and carbohydrates. (≈30%E from fat, ≈<15%E from saturated fats, ≈15%E from protein, ≈55%E from CHO for both diets.)

Both diets contained 47.9 and 56.2g of fibre daily – more than recommendations in Australia & New Zealand and typical population intake in the Netherlands (25.2g/day in the Seven Countries study) [102]</td>
<td>Serum triglycerides, TC, HDL, LDL; systolic BP

LDL was calculated.

Used controlled storage & processing procedures</td>
<td>Difference between high and low group in change in serum lipids after intervention (post – pre)
<table>
<tr><th></th><th>Δ from baseline (high – low)</th><th>Δ (low)</th><th>Δ (high)</th></tr>
<tr><td>TAG</td><td>-0.04 (-0.2 – 0.2)n.s</td><td>-0.1±0.4</td><td>-0.2±0.5</td></tr>
<tr><td>TC</td><td>-0.2 (-0.5 - 0.03) n.s</td><td>-0.5±0.5*</td><td>-0.7±0.7*</td></tr>
<tr><td>HDL</td><td>-0.1 (-0.2 – 0.02) n.s</td><td>-0.1±0.2*</td><td>-0.2±0.2*</td></tr>
<tr><td>LDL</td><td>-0.2 (-0.4 – 0.1) n.s</td><td>0.3±0.5*</td><td>-0.5±0.6*</td></tr>
<tr><td>LDL/HDL</td><td>-0.03 (-0.3– 0.3) n.s</td><td>0.02±0.6</td><td>-0.1±0.6</td></tr>
<tr><td>SBP</td><td>2.8 (-2.6 – 8.1) n.s</td><td>-7.7±8.4*</td><td>-5.8±11.2*</td></tr>
<tr><td>DBP</td><td>-0.1 (-3.1 - 2.8) n.s</td><td>-3.9±6.1*</td><td>-4.4±6.4*</td></tr>
</table>
Reported as mean (95% CI) and means ± standard deviation

Note: all but HDL: LDL ratio and TAG were significantly reduced from baseline in both groups.

groups equal for age, gender, smoking, baseline fruit and vegetables, body weight, BMI, height
90% power to detect change of
0.45 mmol/L TC, 9.4 mmHG systolic pressure

For 11MJ diet, fibre contents were high in both intervention and control groups. Fibre contents of 'low' diet: 47.9 g/d (low fruit and vegetable diet) 56.2 g/d (high fruit and vegetable diet)</td>
<td>Compared with controls consuming only 100g of fruits and vegetables daily, habitually low vegetable and fruit consumers who consumed a diet high in fruits and vegetables (approx 500g/d):
• had non-significantly greater reductions in all lipids (≤0.2mmol/L)
• had similar reductions in systolic (2.8mmHg) and diastolic (0.1mmHg) BP (neither significant)
Both intervention and control groups had reductions from baseline in:
• in systolic and diastolic BP (≈5mmHg), TC, LDL, and HDL (ranging from 0.1 to 0.7 mmol/L), (significant)
• TAG and in LDL/HDL (not significant)

Issues:
Study had insufficient power to detect the observed difference in TC between the groups, or systolic BP. LDL calculated, not measured leading to some potential misclassification bias. Monitored intakes suggested good compliance with diets. Intervention was short and lacked a run in period. Large significant metabolic improvements from baseline in both intervention and control groups may be due to both intervention and control diets being improvements over baseline diets (meeting macronutrient recommendations and having a high fibre content) and may mask any additional effect of vegetables above other dietary components. The very high fibre content of both diets negates the ability of the study to detect any effect of fruits and vegetables attributable to their fibre content.
Quality Rating: C</td>
</tr>
</table>

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Freese et al., 2002

Bio-marker Study #3</td>
<td>RCT</td>
<td>n=77 healthy men & women 19-52y and 19 healthy volunteers 22-50y

Volunteer staff and students of university; randomly allocated to treatment groups (stratified by sex); controls recruited separately

Blinded for fat type but not fruits and vegetables

Diet groups
1a n=13
1b n=15
2a n=15
2b n=14
control n=15</td>
<td>6 week intervention (no run in)</td>
<td>4 treatment diets (1a,1b,2a,2b)

1) high linoleic acid or 2) high oleic acid

a) Low or b) high in vegetables (167g vs 440g), berries (0g vs 166g), & fruits (54g vs 204g)

1 control diet (self-selected; instructed to keep as per normal) baseline intake 287 ± 149g vegetables, 257 ± 224 g fruit, 35 ± 49 g berries</td>
<td>Plasma TC, HDL, LDL, Trigly-cerides, LDL oxidation, LCAT activity, APO-A, APO-B

Used controlled storage and processing procedures

LDL calculated (not measured)</td>
<td>Change from baseline in plasma lipids, apolipoproteins, and oxidation after intervention
<table>
<tr><th></th><th colspan="2">Low veg, fruit, berries</th><th colspan="2">High veg, fruit, berries</th><th></th><th>Control</th></tr>
<tr><th></th><th>Linoleic</th><th>Oleic</th><th>Linoleic</th><th>Oleic</th><th>p</th><th></th></tr>
<tr><td>ΔTC [a]</td><td>0.11 ± 0.49</td><td>0.02 ± 0.59</td><td>-0.28 ± 0.53</td><td>0.04 ± 0.69</td><td>0.18</td><td>-0.05 ±0.52</td></tr>
<tr><td>ΔHDL[a]</td><td>-0.04 ±0.19</td><td>-0.09 ± 0.19</td><td>-0.11 ± 0.21</td><td>-0.06 ±0.17</td><td>0.71</td><td>0.00 ± 0.11</td></tr>
<tr><td>ΔLDL [a]</td><td>0.13 ±0.43</td><td>-0.11 ± 0.57</td><td>-0.19 ± 0.48</td><td>0.14 ± 0.66</td><td>0.18</td><td>-0.06 ±0.56</td></tr>
<tr><td>ΔTAG [a]</td><td>0.04 ± 0.31</td><td>-0.01 ± 0.26</td><td>0.04 ± 0.49</td><td>-0.07 ± 0.28</td><td>0.75</td><td>0.02 ± 0.53</td></tr>
<tr><td>ΔAPO-A[b]</td><td>0.02 ± 0.20</td><td>-0.05 ± 0.19</td><td>-0.05 ± 0.19</td><td>0.01±0.21</td><td>0.61</td><td>0.10 ± 0.17</td></tr>
<tr><td>ΔAPO-B[b]</td><td>0.07 ± 0.11</td><td>0.02 ± 0.15</td><td>0.00 ± 0.11</td><td>-0.00 ± 0.09</td><td>0.22</td><td>-0.00 ±0.09</td></tr>
<tr><td>ΔLCAT[c]</td><td>4.4 ± 10.2</td><td>3.4 ± 13.1</td><td>1.5 ±12.9</td><td>3.6 ± 9.3</td><td>0.89</td><td>-1.0 ± 16.3</td></tr>
<tr><td>ΔTBARS[d]</td><td>0.21 ± 0.35</td><td>0.16 ± 0.37</td><td>-0.16 ± 0.79</td><td>0.17 ± 0.42</td><td>0.41</td><td>-0.25 ±0.37</td></tr>
<tr><td>ΔLDLlag [e]</td><td>9.9± 40.9</td><td>12.8 ± 40.4</td><td>1.7 ± 19.5</td><td>24.5 ±51.5</td><td>0.32</td><td>9.1 ±40.2</td></tr>
<tr><td>ΔLDLmax [f]</td><td>-0.2 ± 17.6</td><td>-4.8 ± 18.3</td><td>8.9 ± 17.5</td><td>-7.8 ± 21.1</td><td>0.046</td><td>-6.4 ±17.1</td></tr>
</table>
Measures presented changes in means ± standard deviations
a lipid measures (TC, HDL, LDL, TAG) in mmol/L
b APO-A & APO B apolipoproteins A and B in g/L
Measures of lipid peroxidation:
c TBARS (plasma): (Thiobarbiutric aicd-reactive substances in plasma) malondialdeide in μmol/L
d LDL lag – LDL lag phase in minutes (measure of susceptibility of LDL to oxidation)
e LDL max – maximum diene production in nmol/ mgLDL/ min (measure of formation of dienes during copper induced oxidation, where greater values reflect a faster oxidation of LDL, that is a lesser antioxidant capacity)

Diet at baseline 'similar' across groups with approx 200-300g vegetables, 130-250g fruit 12-41g berries. Fruit higher in control group 257g (others 130-200) berries higher in oleic acid low vegetable group and control group (35-40g vs 12-15g).

Plasma and urinary measures show good compliance with diets. Plasma and urinary measures of biomarkers suggest control group did not change fruit and vegetable intake from baseline.</td>
<td>No significant differences among the four treatment groups in all markers of lipids and peroxidation except LDL max.

Differences in changes from baseline between those following high and low F&V diets (within fat types) were mostly very small. Largest differences were:
• 0.88 mmol/ L for TC, HDL, LDL, & TAG.
• 0.07g/L for apolipooproteins
• 11 minutes in LDL lag phase
• 3 nmol/ mgLDL/ min LDLmax (significant). (High linoleic acid & high F&V diet was associated with most oxidisability of LDL (LDLmax)
Control group had no large significant changes in absence of study intervention – no period effect.

Issues:
Short study without run in on volunteers with baseline diets relatively high in fruits, vegetables and berries (maybe health conscious and non generalisable).
Baseline diets similar but not equal and no run-in.
Not blinded.
Quality rating: B</td>
</tr>
</table>

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>John et al 2002

United Kingdom

Biomarker Study #4</td>
<td>RCT</td>
<td>n=690 healthy adults 25-64y
no CVD (except hyper-tension), no GI disease, cancer, serious psychiatric disorder, hyper-cholesterolaemia, recent traumatic event, unable to give informed consent

Participants randomly selected from 2 GP lists, random allocation to intervention (smoking stratified)

Intervention n=344
Control n=346</td>
<td>6-month</td>
<td>Intervention: Behavioural intervention aimed to increase fruit and vegetable intake (no other changes suggested)

Control: same measures taken and visits but no diet intervention

DINE FFQ modified to assess fruit and vegetables</td>
<td>Systolic and diastolic BP, Total Cholesterol

BP
(mean of 2 readings measured by electronic sphygmomanometer, taken by research nurses) at baseline and 6 month follow-up visit

Total cholesterol (plasma)
non-fasting sample, used controlled storage and processing procedures,
(Beckman Syncrhon clinical chemistry analyser)</td>
<td>Change from baseline in intervention and control groups in intakes and clinical markers
<table>
<tr><th></th><th>Change from Baseline</th></tr>
<tr><td>Δ F& V (portions)</td><td></td></tr>
<tr><td>I</td><td>1.4 (1.7)</td></tr>
<tr><td>C</td><td>0.1 (1.3)</td></tr>
<tr><td>difference I – C</td><td>1.3 (1.1 – 1.6)</td></tr>
<tr><td>difference I – C (adj)</td><td>1.4 (1.2- 1.6)</td></tr>
<tr><td>Δ Systolic BP (mmHg)</td><td></td></tr>
<tr><td>I</td><td>-2.0 (13.5)</td></tr>
<tr><td>C</td><td>1.4 (14.6)</td></tr>
<tr><td>I – C</td><td>-3.4 (-1.3 – -5.5)</td></tr>
<tr><td>difference I – C (adj)</td><td>-4.0 (-2.0 – -6.0)</td></tr>
<tr><td>Δ Diastolic BP (mmHg)</td><td></td></tr>
<tr><td>I</td><td>-1.6 (8.7)</td></tr>
<tr><td>C</td><td>-0.3 (8.7)</td></tr>
<tr><td>I – C</td><td>-1.4 (-0.1 - -2.7)</td></tr>
<tr><td>difference I – C (adj)</td><td>-1.5 (-0.2 – -2.7)</td></tr>
<tr><td>Δ TC (mmol/L)</td><td></td></tr>
<tr><td>I</td><td>-0.018 (0.87)</td></tr>
<tr><td>C</td><td>-0.036 (0.56)</td></tr>
<tr><td>I – C</td><td>0.018 (-0.092 – 0.128)</td></tr>
<tr><td>difference I – C (adj)</td><td>0.010 (-0.097 – 0.116)</td></tr>
</table>
adj: adjusted for baseline value and sex</td>
<td>After intervention to increase fruit and vegetable intake, the intake of the intervention group was approximately 100g /d higher than the control group. This resulted in:
• A significantly greater reduction in BP in the intervention group (≈ 4mmHg systolic, ≈ 1.5mmHg diastolic).
• Virtually no change in total cholesterol (0.01mmol/L, non-significant)
• Cholesterol samples were non-fasting, leaving a chance for misclassification bias.
• Body weight constant, and unchanged cholesterol suggests results not due to participants lowering their fat intake

Issues:
Used non-fasting cholesterol which leaves greater chance for misclassification (bias towards the null)

Quality Rating: A – BP
C - cholesterol</td>
</tr>
</table>

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Conlin et al., 2000

United States

DASH Study

Bio-marker Study #5</td>
<td>RCT
Single-blinded
Not clinical setting

Quality issues:
Overall OK, intention to treat</td>
<td>n=133 adults ≥22y with Stage I hypertension, no poorly controlled diabetes/ hyperlipidemia, no pregnancy/ lactation, no supplements/ antacids, no renal insufficiency, no cardio-vascular event in last 6 months, BMI <35, etOH ≤14 drinks/wk

recruited from 4 centres by mass mailing and community screening (non-random); deliberate aim to over-recruit African American participants</td>
<td>8 week intervention diet (3 week run-in)</td>
<td>3 diets:
1) 'control', 2) 'DASH', 3) F&V

Control Diet: 1.9 servings/d fruit and juices, 2.0 serves veg

F&V diet: 5.2 servings/d fruit and juices, 3.0 servings veg – compared with control slightly extra grains , less sucrose but macronutrients as per control

DASH diet: also high in fruits 5.6 serves and vegetables 5.2 but also uses low fat dairy products, whole grains, poultry, fish nuts, and has less fats, red meat, sweets, sugary beverages

Run in diet = control diet

Food prepared by research team, consumed at home</td>
<td>Systolic & Diastolic BP

Random-zero sphygmomanometer by trained certified staff, paired measurements taken weekly in run-in and intervention times

Baseline: average of screening and 4 paired measurements at run-in.

End: average of 5 pairs taken in last 13 days of intervention

Control of hypertension: SBP <140 mmHg and DBP <90 mmHg.

Control of Isolated Systolic Hyptertension: SBP <140 mmHg</td>
<td>Relative Risk for hypertension and Isolated Systolic Hypertension after the 8 week intervention diet
<table>
<tr><th></th><th>RR (95%CI) hypertension</th><th>RR (95%CI) ISH</th></tr>
<tr><td>Control</td><td>1 (ref)</td><td>1 (ref)</td></tr>
<tr><td>F & V</td><td>0.72 (0.52 – 0.97)*</td><td>0.71 (0.46 – 1.09) n.s.</td></tr>
<tr><td>DASH</td><td>0.39 (0.23 – 0.65)***</td><td>0.29 (0.13 – 0.64)***</td></tr>
</table>
Reduction in BP after three eight-week intervention diets
<table>
<tr><th></th><th>Δ SBP</th><th>Δ DBP</th></tr>
<tr><td>F & V[a] minus control</td><td>-7.2 (-10.9 to -3.6)***</td><td>-2.8 (-5.1 to -0.6)*</td></tr>
<tr><td>F & V[b] minus control</td><td>-7.0 (-10.7 to -3.4) ***</td><td>-3 (-5.3 to -0.7)**</td></tr>
<tr><td>DASH minus F& V</td><td>-4.1 (-8.0 to -0.2)*</td><td>-2.6 (-5.0 to -0.2)*</td></tr>
<tr><td>DASH vs minus F & V</td><td>-4.5 (-8.4 to -0.7)*</td><td>-2.9 (-5.3 to -0.5)*</td></tr>
</table>
n.s. p≥0.05, * p<0.05, ** p<0.01, ***p<0.001
[a] adjusted for clinical centre
[b] adjusted as a, also for gender, race, age, etOH, baseline SBP</td>
<td>Considering most CVD risks, compared with people following an otherwise equal control diet, people with Stage I hypertension who followed a diet with an additional 3 serves of fruit/ juice and 1 serve of vegetables:
• had ≈7mmHg greater reduction in systolic BP (significant) after the 8 week diet period
• ≈3mmHg greater reduction in diastolic BP (significant) after the 8 week diet period
• ≈30% lower risk of uncontrolled hypertension (significant) and ISH (not significant) after the 8 week diet period

Those who followed a high fruit and vegetable diet including 2 extra serves of vegetables (DASH):
• had greater reductions in systolic (≈5mmHg) and diastolic BP (≈3mmHg) (both significant) than people following the fruits and vegetables diet
• had ≈60% reduction in risk of hypertension and ≈70% reduction in risk of ISH (both significant) after the trial relative to the control group

Issues:
volunteers
deliberate overinclusion of African Americans
F&V diet NOT the primary hypothesis (power calculations based on DASH diet expectations)
Other dietary modifications, and/or a greater 'dose' of vegetables can achieve even greater reductions in BP and risk of hypertension, and isolated systolic hypertension
Very low intervention intensity (1 serve vegetable increase)
Quality: B</td>
</tr>
</table>

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Moore et al., 2001

United States

DASH study

Bio-marker Study #6</td>
<td>Multi-centre randomised clinical trial not blinded

Quality – generally OK</td>
<td>72 DASH participants who had Isolated Systolic Hypertension SBP 140 to 159 mmHg (47 ± 22y)

recruited from 4 centres; non-random recruitment from bulk mailout and screening; randomised to interventions</td>
<td>8-week diet period (3 week run in)</td>
<td>3 diets:
1) 'control',
2) 'DASH'
3) 'F&V'
Control Diet: 1.6 servings/d fruit and juices, 2.0 serves veg

DASH diet:
also high in fruits 5.6 sves and vegetables 5.2 but also uses low fat dairy products, whole grains, poultry, fish nuts, and has less fats, red meat, sweets, sugary beverages

F&V diet: 5.2 servings/d fruit and juices, 3.0 servings veg – compared with control slightly extra grains , less sucrose but macronutrients as per control

Food prepared by research team, consumed at home</td>
<td>BP (systolic, diastolic & 24h ambulatory)

Sphyngomanometer - common protocol

Baseline: average of 3 measures taken during last 2 weeks of run-in

Post: average measures taken on of 5 of last 13 days of intervention diet.

Ambulatory BP by Spacelabs monitor (end of run in and end of intervention)</td>
<td>Change in BP from baseline after 8 week intervention diet
<table>
<tr><th></th><th>DASH diet (n=23)</th><th>F&V diet (n=24)</th><th>Control diet (n=25)</th></tr>
<tr><td>Δ SBP</td><td>-11.8 ± 9.3 ***</td><td>-3.8 ± ? n.s.</td><td>-0.6 ± ? n.s.</td></tr>
<tr><td>Δ DBP</td><td>-3.5 ± 6.3 **</td><td>-1.3 ± ? n.s.</td><td>1.0 ± ? n.s.</td></tr>
<tr><td>Δ 24h SBP</td><td>-9.4 *</td><td>-4.1 n.s.</td><td>-0.6 n.s.</td></tr>
<tr><td>Δ 24h DBP</td><td>n.s.</td><td>n.s</td><td>n.s.</td></tr>
<tr><td>≤140mmHg SPB post</td><td>18 of 23</td><td>12 of 24</td><td>6 of 25</td></tr>
</table>
(Full details not given)
n.s not significant (p>0.05) * p<0.05

Baseline (end of run- in) values were
147 ± 5 (DASH) 146 ± 5 (F&V) 146 ± 6 (control)</td>
<td>Participants with baseline systolic hypertension, after consuming a diet high in fruits and vegetables for 8 weeks:
• had reductions of systolic and diastolic BP ≈4 and ≈1mmHg respectively (neither significant).
• Had a greater reduction in SBP (≈3.2mmHg) than participants following the control diet (not significant).
• Had a greater reduction in DBP (≈2.3mmHg) than participants control diet (not significant).
• had normal systolic BP (<140mmHg) [50% of participants]

The DASH diet which included other dietary modifications and 2 serves more vegetables than the fruit and vegetable diet significantly improved all measures of BP from baseline, and relative to the control group.

Issues:
volunteers
no blinding
small vegetable content of the high fruit and vegetable diet (inadequate dose?)
cannot separate the effect of the additional vegetable in DASH diet from other dietary modifications
Fruit and vegetable diet NOT primary hypothesis

Quality rating B:</td>
</tr>
</table>

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Appel et al, 2000 United States DASH trial **Bio-marker Study #7**	RCT feeding study single blinded quality issues:	n=118 adults ≥22y, no hypertensive medication, normal BP, no poorly controlled diabetes, no hyperlipidemia, no vitamin/ mineral supplements, no medications that affect BP, GFR <50 ml/min recruited from 4 centres; non-random recruitment ; randomised to interventions	8 week intervention (3 week run in)	3 diets 1/ Control (high fat, 1.9 sv fruit (incl. juices, 2.1 sv vegetables) 2/ fruits & vegetables diet (as control 5.6 sv fruit incl juices, 3.3 sv vegetables) 3/ combination diet (lower fat, lower saturated fat, 5.3 sv fruit incl. juices, 5.2 sv vegetables) run in diet = control diet 1 meal at centre per weekday rest taken home	serum homocysteine HPLC (8% between run coefficient) controlled collection and storage procedures	Change in homocysteine: Δ homocysteine (μmol/L) Control +0.46 (-0.04 – +0.96) Fruits & veg +0.21 (-0.27 - +0.69) Combination -0.34 (-0.84 – +0.16) Compared with the control diet the combination diet produced a significantly different change in homocysteine [-0.8 μmol/L (-1.51 -= -0.1)] but the fruit and vegetable diet did not [-0.25 μmol/L (-0.94 - +0.44)].	Among adults, after an 8 week dietary intervention, the change in plasma homocysteine levels: • a slight increase ≈0.5μmol from baseline (not significant) in those consuming the high fat control diet was • a slight increase from baseline (≈0.2μmol/L) (not significant), that was less than the increase in the control group (by 0.25μmol/L) in those following a diet similar to the control but with an additional serve of vegetables and 3 serves of fruit/ juice • was a ≈0.3μmol/L reduction from baseline levels (non-significant), that was 0.8 μmol/L different (statistically significant) from the increase in the control group among those who followed a diet lower in fat and saturated fat, and with an additional 3 serves of vegetables and 3 serves fruit/ juice Issues: Use of volunteers F&V diet NOT the primary hypothesis Intervention diet increased vegetable content by small amount (1 serve) "all else being equal" violated for combination diet - cannot distinguish between effect of extra vegetable content, reduction in fat and reduction in saturated fat Quality rating: C

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Silaste et al., 2003 Finland **Biomarker Study #8**	crossover intervention	n=37 healthy female volunteers (workers at University Hospital of Oulu) Eligibility: BMI 20-29, no GI/ renal/ hepatic disease, no alcoholism, no use of supplements, no food allergy, normal lipids and glucose, not pregnant/ lactating	2 week baseline, 5 week interventions, 3 week washout	Diets: Baseline & Washout 'usual at home diet' Low folate/ fruit and vegetable <200g dietary cholesterol, sat. fat 10% energy, fatty acid controlled, one serve fresh vegetables/ fruit/ juice [total 200 μg/ d folate]. High folate/ fruit/ vegetable As the low fruit/vegetable/ folate intervention diet in terms of fats and energy, but with 60g fresh paprika, 400-500g raw or steamed fresh vegetables, 2 serves fruit/ juice, plus berries (quantity not reported) [total 600 μg/ d folate] Food prepared at hospital kitchen, food taken home for weekend. Compliance observed for lunches and by self-report – no major non-compliance noted. Body weight maintained by nutritionist adjusting energy to daily body weight.	plasma total homocysteine Overnight fasting blood samples at baseline, end of washout and intervention periods. Used controlled storage/ processing procedures. Total Homocysteine by immunoflourometric IMX method (interassay CV 3.2%, Nordic quality assurance system (mean bias for seven sera -3.5%)	Mean plasma homocysteine after each intervention period Homocysteine (μmol/ L) Baseline 8.1 (1.9) Low fr/veg/folate 8.0 (1.4) Washout 7.8 (1.5) High fr/veg/folate 6.9 (1.5) * * different from low fr/veg/folate diet p<0.001	Healthy volunteers had significantly lower plasma homocysteine (1.1 μmol/ L, 13%) after following a diet high in folate-rich fruits, vegetables and berries than when following a diet low in folate-rich fruits, vegetables and berries. Issues: Although baseline and washout homocysteine measures not statistically different, the washout period was too short for some other markers used. Decreases in plasma homocysteine may have been slightly greater if the washout period were longer. Quality Rating: A

Appendix 6: Fruits, Vegetablse and CHD Biomarkers – Observational Studies (Prospective Cohort)

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Miura, et al., (2004)

United States

Chicago Western Electric Study

Biomarker Study #9</td>
<td>cohort study

quality not sure

sampling good, methods/ measures good. However lack of control for Na^{+} intake important (also Mg^{++} K^{+}, fibre) lack of fineness in intake categories</td>
<td>2107 men aged 40-55y at baseline employed at least 2y by Chicago Western Electric Company, Illinois 1710 w/ sufficient data for analysis

67% participation rate</td>
<td>annual follow up for 7y

Baseline Oct 1957 – Dec 1958</td>
<td>Intake of vegetables, fruits

2x Burke's diet history by 2 nutritionists 1 year apart. Mean of both values used.

195 specific food cross-check</td>
<td>BP (systolic, diastolic)

mercury sphygmomanometer, trained clinicians</td>
<td>Annual change in systolic BP over 7 years across categories of fruit and vegetable intake
<table>
<tr><th></th><th>Model 1 ΔmmHg / yr</th><th>Model 2 ΔmmHg / yr</th><th>Model 3 ΔmmHg / yr</th><th>Model 4 ΔmmHg / yr</th><th>Model 5 ΔmmHg / yr</th></tr>
<tr><td>Veg (sv/d)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td><0.38</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td></tr>
<tr><td>0.38- 1.125</td><td>-0.46**</td><td>-0.48**</td><td>-0.40**</td><td>-0.40**</td><td>-0.29</td></tr>
<tr><td>>1.125</td><td>-0.33</td><td>-0.38</td><td>-0.24</td><td>-0.28</td><td>0.08</td></tr>
<tr><td>Fruit (sv/d)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td><0.38</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td></tr>
<tr><td>0.38- 1.125</td><td>-0.44***</td><td>-0.44***</td><td>-0.35**</td><td>-0.32*</td><td>-0.29*</td></tr>
<tr><td>>1.125</td><td>-0.40*</td><td>-0.42*</td><td>-0.28</td><td>-0.27</td><td>-0.22</td></tr>
</table>
Annual change in diastolic BP over 7 years across categories of fruit and vegetable intake
<table>
<tr><th></th><th>Model 1 ΔmmHg / yr</th><th>Model 2 ΔmmHg / yr</th><th>Model 3 ΔmmHg / yr</th><th>Model 4 ΔmmHg / yr</th><th>Model 5 ΔmmHg / yr</th></tr>
<tr><td>Veg (sv/d)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td><0.38</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td></tr>
<tr><td>0.38- 1.125</td><td>-0.18*</td><td>-0.19*</td><td>-0.17*</td><td>-0.17*</td><td>-0.11</td></tr>
<tr><td>>1.125</td><td>-0.21</td><td>-0.25*</td><td>-0.22</td><td>-0.22</td><td>-0.06</td></tr>
<tr><td>Fruit (sv/d)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td><0.38</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td><td>ref</td></tr>
<tr><td>0.38- 1.125</td><td>-0.17*</td><td>-0.18*</td><td>-0.16*</td><td>-0.14</td><td>-0.13</td></tr>
<tr><td>>1.125</td><td>-0.25*</td><td>-0.25*</td><td>-0.24*</td><td>-0.22*</td><td>-0.19</td></tr>
</table>
*** p<0.001 ** p<0.01 *p<0.05

Model1: adjusted for age
Model 2: adjusted as 1 also weight per year
Model 3: adjusted as 2 also height education, cigarettes per day, alcohol intake, energy intake
Model 4: as 3 also intake other foods (fruits, vegetables, fish, beef-lamb-veal, pork, poultry)
Model 5: adjusted as model 4 also nutrient intakes (CHO, protein, sat. fat, PUFA, dietary cholesterol, iron, B1, B2 B3 vit C beta-carotene, retinol

No adjustment for Na^{+} intake important (also Mg^{++} Ca^{++} K^{+}, fibre which may be important)</td>
<td>After adjustment for most risk factors for hypertension and other foods, compared with those consuming less than 0.4 serves per day, annual increase in BP was:
• 0.4mmHg less (systolic) (significant) and 0.17 mmHg less (diastolic) (significant) for those consuming 0.4 - 1.1 serves of vegetables (significant)
• 0.28mmHg less(systolic) (not significant) and 0.22mmHg less (diastolic) for those consuming more than 1.1 serve vegetables (not significant)
• 0.32mmHg less (systolic) (significant) and 0.14 mmHg less (diastolic) (not significant) for those consuming 0.4 – 1.1 serves of fruits
• 0.27mmHg less (systolic) (not significant) and 0.22 mmHg less (diastolic) (significant) for those consuming more than 1.1 serves of fruits.

Issues:
Adjustment for nutrients reduced observed relationships, indicating either confounding by some factors (eg fats) or that some of the protective relationship is related to the vitamins which were included in the model.

Unmeasured dietary factors (especially salt).

Quality rating: C</td>
</tr>
</table>

Appendix 7: Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Beitz et al., 2003

Germany German Nutrition Survey

Bio-marker Study #10</td>
<td>population based cross-sectional</td>
<td>n= 1628 women & n=1340 men aged 18-79y,

response rate 61.4%

representative sample with complete data

Exclusions: current or hx hypertension, medications which raise or lower BP, nursing mothers</td>
<td>Oct 1997-Mar 1999</td>
<td>Vitamin C intake (dietary & total), fruit & vegetable intake

Computerised Dietary History of usual diet (by trained nutritionists) plus interview re: supplements

validated against 3d record and 24hr recall. Adjusted Spearman's correlations w/ 3d record: energy 0.74, fibre 0.69, vegetable protein 0.59, carbohydrate 0.67</td>
<td>BP (systolic & diastolic)

Mercury sphygmomanometer 3 measurements, average of last two used</td>
<td>Increase in systolic BP by intake of Vit C. and fruits and vegetables among women
<table>
<tr><th></th><th>B (std. error)</th><th>Standard B</th></tr>
<tr><td>Dietary Vit C. (g/d) (model 1)</td><td>-7.44 (4.71) n.s.</td><td>-0.03</td></tr>
<tr><td>Total Vit C. (g/d) (model 2)</td><td>-4.28 (2.61) n.s.</td><td>-0.03</td></tr>
<tr><td>Fruit & veg (kg/d) (model 3)</td><td>-5.38 (1.55)***</td><td>-0.07</td></tr>
</table>
Increase in systolic BP by intake of Vit C. and fruits and vegetables
<table>
<tr><th></th><th colspan="2">Women</th><th colspan="2">Men</th></tr>
<tr><th></th><th>B (std. error)</th><th>Standardized B</th><th>B (std. error)</th><th>Standardized B</th></tr>
<tr><td>Hi F&V, hi vit C</td><td>-2.33 (0.89)**</td><td>-0.06</td><td>1.82 (0.94) n.s.</td><td>0.05</td></tr>
<tr><td>Hi F&V, low vit C</td><td>-2.99 (1.37)*</td><td>-0.05</td><td>-1.30 (1.44) n.s.</td><td>-0.02</td></tr>
<tr><td>Low F&V, hi vit C</td><td>0.71 (1.02) n.s.</td><td>0.02</td><td>-0.74 (1.12) n.s.</td><td>-0.02</td></tr>
<tr><td>Low F&V, low vit C</td><td>ref</td><td></td><td>ref</td><td></td></tr>
</table>
Increase in diastolic BP by intake of Vit C. and fruits and vegetables
<table>
<tr><th></th><th colspan="2">Women</th><th colspan="2">Men</th></tr>
<tr><th></th><th>B (se)</th><th>Standardized B</th><th>B (se)</th><th>Standardized B</th></tr>
<tr><td>Hi F&V, hi vit C</td><td>-0.34 (0.56) n.s.</td><td>-0.01</td><td>0.19 (0.64) n.s.</td><td>0.01</td></tr>
<tr><td>Hi F&V, low vit C</td><td>-1.00 (0.86) n.s.</td><td>-0.03</td><td>-1.17 (0.97) n.s.</td><td>-0.03</td></tr>
<tr><td>Low F&V, hi vit C</td><td>0.89 (0.65) n.s.</td><td>0.03</td><td>0.06 (0.75) n.s</td><td>0.002.</td></tr>
<tr><td>Low F&V, low vit C</td><td>ref</td><td></td><td>ref</td><td></td></tr>
</table>
n.s. p>0.05, *p≤0.05, **p≤ 0.01, *** p≤ 0.001
all models adjusted for age, BMI and smoking status (considered but did not need to adjust for SEP, smoking, physical activity, alcohol, coffee, vegetarian diet, health-related quality of life issues, region, season, and energy intake

Fruit and vegetables: hi ≥600g/d low <600g/d
Total Vitamin C (includes supplements): hi ≥0.15 g/d low < 0.15 g/d

Results for systolic BP (men) and diastolic BP (women, men) by f&v intake, dietary vit. C, total vit. C reported as 'not significant' but not shown

later considered sodium – did not alter results; later did sub-group analyses by smoking status</td>
<td>After considering most CVD risk factors, BP was:
• ≈ 5mmHg lower (systolic) with each additional kg/d fruits and vegetables (significant) for women
• reported as "not significant" for men (systolic)
• reported as "not significant" in either gender (diastolic)

Compared with those consuming less than 600g/d of fruits and vegetables and less than 0.15 g/d vit. C, those consuming 600g or more:
• systolic BP 2 or 3 mmHg lower (significant) for women or 1 or 2 mmHg lower (not significant) for men)
• diastolic BP less by around 1mmHg (not significant) for men and women with low vit C intakes or similar to within half a mmHg (non-significant) for men and women with high vit C intakes

Issues:
Some dietary factors were not considered which may relate to both f&v intake and BP (fatty acid intake, calcium)

Quality Rating: B</td>
</tr>
</table>

Definitions: B is the regression coefficient (slope) from the linear regression model. It represents the increase in the outcome variable which occurs with every increase of unit in the independent variable of interest. Standardized B: the increase in the standardized value of the outcome variable which occurs with every increase of one standardized unit in the independent variable of interest

Appendix 7 cont'd: Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Nagata et al., 2003

Japan

Biomarker Study #11</td>
<td>Cross sectional</td>
<td>n=294 men
n=330 women

Sampling: participants in a health check-up program at a general hospital no data on sampling method, response to survey 97.3%

exclusions: use of anit-hypertensives, oral contraceptives, HRT, cancer, angina/ MI, diabetes</td>
<td>September 1996 and Aug 1997</td>
<td>Fruit intake g/d,
Vegetable intake g/d

Semi-quantitative 169-item FFQ

validated against 3d records
Correlation coefficients with from 0.15 to 0.54 in males and from 0.18 to 0.47 in females for various nutrients. F&V intake estimates 30-45% higher by FFQ than diet record.

Repeatability: ICCs from 0.46 to 0.78 in men and from 0.36 to 0.67 in women Vit C ICC very low in women</td>
<td>BP

digital recorder, between 8&9am by same observer</td>
<td>Correlation between fruit and vegetable intake and BP among men and women
<table>
<tr><th></th><th colspan="2">Systolic</th><th colspan="2">Diastolic</th></tr>
<tr><th></th><th>Veg</th><th>Fruit</th><th>Veg</th><th>Fruit</th></tr>
<tr><td>Men [a]</td><td>-0.14*</td><td>-0.08</td><td>-0.07</td><td>0.01</td></tr>
<tr><td>Men [b]</td><td>-0.12*</td><td>-0.05</td><td>-0.05</td><td>0.06</td></tr>
<tr><td>Premenopausal women [a]</td><td>0.003</td><td>-0.10</td><td>0.04</td><td>-0.10</td></tr>
<tr><td>Premenopausal women [b]</td><td>0.002</td><td>-0.12</td><td>0.06</td><td>-0.11</td></tr>
<tr><td>Peri-post menopausal women [a]</td><td>0.03</td><td>-0.09</td><td>-0.01</td><td>-0.12</td></tr>
<tr><td>Peri-post menopausal women [b]</td><td>0.09</td><td>-0.04</td><td>0.05</td><td>-0.10</td></tr>
</table>
* p<0.05
a) adjusted for age, energy
b) adjusted as 'a' also BMI, alcohol, salt, seaweed
Potential confounders considered in study: also include marital status, exercise, age at menarche, number of births</td>
<td>Correlations between BP and intake of vegetables were:
• all weaker than ±0.15
• inverse for men (systolic BP significant, diastolic non-significant)
• very nearly zero for women (not significant)

Correlations between BP and intake of fruits were:
• all weaker than ±0.15 and non significant
• negative, (except for with diastolic pressure in men)

Issues:
Sample representativeness uncertain
Questionable validity of dietary exposure assessment leaving potential for misclassification with probable bias towards the null

Quality: B</td>
</tr>
</table>

Appendix 7 cont'd : Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Psaltopoulou et al., 2004 Greece EPIC study **Biomarker Study #12**	Cross-sectional	20, 343 volunteers, 20-86y normal BP Volunteers actively recruited from across Greece.	Baseline 1994-1999	Usual intake of fruit and veg over last year Semi-quantitative 150 item FFQ Good validity (Gnardellis et al., 1997)	Arterial BP 2 readings of mercury sphygmo-manometer by trained physician	Increase in BP (mmHg) per SD of fruit and vegetable intake Systolic Blood Pressure: β (95%CI) / p; Diastolic Blood Pressure: β (95%CI) / p Vegetable: -0.5 (-0.7 - -0.2) / <0.001; -0.4 (-0.5 - -0.2) / <0.001 Vegetable [a]: -0.01 (-0.3 - 0.3) / 0.95; -0.2 (-0.4 - -0.0) / 0.02 Fruit: -0.5 (-0.8 – -0.3) / <0.001; -0.4 (-0.5 - -0.2) / <0.001 adjusted for age, place of residence, education, BMI, waist-hip ratio, energy, physical activity [a] also adjusted for olive oil intake 1 SD vegetable intake = 233.0 (men), 236.7 (women) 1 SD fruit intake = 212.6 (men), 212.2 (women)	After adjustment for most CVD risk factors, each additional SD of intake of fruits, and of vegetables was associated with: • ≈0.5mmHg reduction in systolic BP (both significant, linear) • ≈0.4mmHg reduction in diastolic BP (both significant, linear) • a lesser reduction in BP with vegetable intake after adjustment for olive oil ≈0mmHg *(diastolic*) to 0.2mmHg *(systolic)* (non significant) Issues: Self-selected volunteers Quality Rating: A

NHEFS: National Health and Nutrition Examination Survey Epidemiologic Follow-up Study EPIC: European Prospective investigation into Cancer and Nutrition

Gnardellis C., Trichopoulou, A., Katsouyanni, K., Polychronopoulous, E., Rimm, E.B., (1997) Reproducibilty and validity of an extensive semi-quantitative food frequency questionnaire using dietary records and chemical markers among Greek Schoolteachers. Int J Epidemiol 1997; 26(suppl 1):s118-27

Appendix 5 cont'd: Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Djoussé et al., (2004)

United States

National Heart, Lung, and Blood Institute Family Study

Biomarker Study #13</td>
<td>Cross-sectional (multi-centre population based)

Quality good</td>
<td>n=4466 adults (enrolled in one of 4 cohort studies)

Randomly selected within cohorts – screened, 588 'families' chosen randomly, 657 chosen for elevated CAD rates in family members</td>
<td></td>
<td>Usual consumption fruits and vegetables

Semi-quantitative 100-item FFQ (6 fruit items, 11 vegetable items)

Modified from validated FFQ (Willett et al.)</td>
<td>Serum LDL, HDL: LDL ratio, HDL and triglycerides

Blood samples: fasting samples taken.

Used controlled storage and processing procedures</td>
<td>Adjusted LDL cholesterol according to daily fruit and vegetable intakes in men and women
<table>
<tr><th>Intake F& V serves/d</th><th colspan="2">LDL Adjusted means ± SE</th></tr>
<tr><th></th><th>Model 1</th><th>Model 2</th></tr>
<tr><td>Men</td><td></td><td></td></tr>
<tr><td>1.4 (0-1.9)</td><td>3.36 ± 0.04</td><td>3.36 ± 0.04</td></tr>
<tr><td>2.5(2.0-2.9)</td><td>3.35 ± 0.04</td><td>3.35 ± 0.04</td></tr>
<tr><td>3.4 (3.0 – 3.9)</td><td>3.26 ± 0.04</td><td>3.26 ± 0.04</td></tr>
<tr><td>5.4 (4.0 – 15.2)</td><td>3.17 ± 0.06</td><td>3.17 ± 0.06</td></tr>
<tr><td>p for trend</td><td><0.0001</td><td>0.0002</td></tr>
<tr><td>Women</td><td></td><td></td></tr>
<tr><td>1.4 (0-1.9)</td><td>3.35 ± 0.05</td><td>3.36 ± 0.05</td></tr>
<tr><td>2.5(2.0-2.9)</td><td>3.22 ± 0.04</td><td>3.23 ± 0.04</td></tr>
<tr><td>3.4 (3.0 – 3.9)</td><td>3.21 ± 0.04</td><td>3.21 ± 0.04</td></tr>
<tr><td>5.4 (4.0 – 15.2)</td><td>3.11 ± 0.04</td><td>3.11 ± 0.04</td></tr>
<tr><td>p for trend</td><td><0.0001</td><td><0.0001</td></tr>
</table>
Adjusted HDL:LDL ratio according to daily fruit and vegetable intakes in men and women
<table>
<tr><th>Intake F& V serves/d</th><th colspan="2">LDL:HDL Adjusted means ± SE (Model 2)</th></tr>
<tr><th></th><th>Men</th><th>Women</th></tr>
<tr><td>1.4 (0-1.9)</td><td>3.21 ± 0.05</td><td>2.52 ± 0.05</td></tr>
<tr><td>2.5(2.0-2.9)</td><td>3.19 ± 0.05</td><td>2.40 ± 0.04</td></tr>
<tr><td>3.4 (3.0 – 3.9)</td><td>3.16 ± 0.05</td><td>2.42 ± 0.04</td></tr>
<tr><td>5.4 (4.0 – 15.2)</td><td>3.03 ± 0.05</td><td>2.36 ± 0.04</td></tr>
<tr><td>p for trend</td><td>0.006</td><td>0.020</td></tr>
</table>
All corrected for clustering
Model 1: adjusted for age, age squared, field centre, risk group (random v CAD high risk), BMI, energy intake, smoking status, dietary cholesterol, history of CAD and diabetes
Model 2: adjusted as model 1 also education, physical activity, intakes of saturated fat, polyunsaturated fat, total fat</td>
<td>After considering most risk factors for CVD, an intake of 5.4 vs 1.4 serves per day was associated with:
≈0.2 mmol/L lower LDL for men & women (significant); dose-response relationships
reduction in HDL: LDL ratio of ≈0.2 for men & women (significant); dose response relationship
"no significant change" in HDL (p for trend=0.57 (men), 0.97 (women)) or triglycerides (p for trend =0.83 (men), 0.60 (women))" (magnitudes not reported)
Results unchanged with exclusion of baseline CAD, diabetes; similar when restricted to 75th percentile saturated fat intake
Issues:
magnitude of change in HDL and triglycerides not reported

Quality: A</td>
</tr>
</table>

Appendix 7: Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Lindquist et al., 2000 United States **Biomarker Study #14**	Cross-sectional Quality – used non-random sample, small sample size, misclassification	n=95 children 6.5 – 13 y, 54 white and 41 African American Recruited (non-random)		USDA servings fruit, vegetables per day 3 x 24 hr recalls over 2 weeks (1 weekend day, 2 weekday) validated for energy by doubly labelled water (Johnson et al., 1996) At group level, mean energy very similar by both methods. At individual level, correlation between both measures show low validity (r=0.25, p=0.24) limits of agreement show 24 hr recalls from 4600 kJ lower to 3400kJ higher than doubly labelled water method.	Serum total cholesterol, triglycerides (fasting) Used controlled storage and processing procedures	Increase in lipids with increase in daily fruit and vegetable intake Cholesterol (log mmol/L): β = -0.019, p = 0.22 Triglycerides (log mmol/L): β = -0.008, p = 0.85 Fruit (log ~servings) Cholesterol (log mmol/L): β = -0.02, p = 0.60 Triglycerides (log mmol/L): β = -0.048, p = 0.64 Vegetable (log ~servings) adjusted for ethnicity, social class, intake of other core foods (added sugar, discretionary fat, dairy, grain)	After adjustment for some potential confounding factors, among children: • serum cholesterol had a weak negative associations with intake of fruits (non significant) and vegetables (non significant) • serum triglycerides had weak negative associations with intake of fruits (non significant) and vegetables (non-significant) Issues: Poor validity of dietary exposure assessment with potential misclassification of dietary exposures gives probable bias towards null. Sample is both non-random and small Quality rating: B

Appendix 7 cont'd: Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Deurenberg-Yap et al., 2001 Singapore National Health Survey 1998 **Biomarker Study #15**	cross-sectional	n=4723 adults (64% Chinese, 21% Malays, 15% Indians), Sampling: multistage, with oversampling of Malays and Indians Response rate 64%	1998	intake of fruits and vegetables, vegetables 159-item FFQ 'usual intake over last month' in language subject most familiar with, sometimes interpreters; frequency per day week or month – used visual serving size aids validated	plasma TC, HDL, LDL, TC:HDL ratio (calculated) used controlled collection, storage and processing procedures overnight fasting blood samples – same day separation and use of plasma TC (enzymatic method), HDL (homogeneous enzymatic test), LDL (homogeneous turbidimetric method)	Mean total cholesterol of sex-ethnic groups correlated with mean vegetable intakes (r=-0.82). Associations with other outcomes and associations for fruit not reported. RR of elevated TC (>6.2 mmol/L), LDL (>4.1 mmol/L), TCHDL >4.4 mmol/L) and low HDL (<0.9 mmol/L) not significantly associated with quintiles of fruit and vegetable intake adjusting for age, BMI and WHR. (Actual estimates not presented). Typical intakes (servings/ day) Vegetables 1.29 ± 0.79 (F), 1.36 ± 0.90 (M). Fruits 1.27 ± 0.99 (F), 1.35 ± 1.09 (M)	Among healthy Singaporean residents: • Intake of vegetables strongly inversely associated with total cholesterol. • (without adjustments) strong negative correlation (-0.82) between group vegetable intake and group total cholesterol • Intake of fruits and vegetables not associated with risk of elevated blood lipids. Issues: mean vegetable intakes very low (much below Singaporean recommendations) Diet in the last month may not be the most relevant dietary exposure Actual magnitude of results, and many analyses not presented. Different primary hypothesis. Quality: C

Appendix 7 cont'd: Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Fornés et al 2000

Brazil

Biomarker Study #16</td>
<td>cross-sectional</td>
<td>n=1045 adult men and women 20 y+

representative sample</td>
<td></td>
<td>Intake of fruits, vegetables, beans

FFQ (44 Brazilian foods, 7 response categories from never to once per day)

assesses diet over last year

validity – successfully piloted

Fruits – banana, orange, apple, papaya, watermelon, melon, pear, tangerine

Vegetables– watercress, lettuce, endive, chicory, cabbage, pumpkin, summer squash, beetroot, carrot, chayote, fruit of the jiloeiro, corn, cucumber, okra, tomato, French bean, kale, cauli, radish swill chard, wild chicory, spinach potato, sweet potato, cassava</td>
<td>Serum HDL, LDL

12h fasting blood samples,

storage not described

LDL calculated (not measured)</td>
<td>Increase in serum LDL and HDL with increasing frequency of consumption of fruits and vegetables (linear model)
<table>
<tr><th></th><th>β (95% CI) for cholesterol mg/dL
Fruits</th><th>
Vegetables</th></tr>
<tr><td>LDL 1</td><td>-5.49 (-9.61 - -1.37)**</td><td>-3.21(-5.61 - -0.82)**</td></tr>
<tr><td>LDL 2</td><td>-</td><td>-</td></tr>
<tr><td>LDL 3</td><td>-</td><td>-</td></tr>
<tr><td>HDL 1</td><td>-0.47 (-1.79 – 0.85)</td><td>-0.13 (-0.88 – 0.62)</td></tr>
<tr><td>HDL 2</td><td>-0.17 (-1.48 – 1.14)</td><td>-0.25 (-1.04 - 0.46)</td></tr>
<tr><td>HDL 3</td><td>-0.13 (-1.48 – 1.22)</td><td>0.01 (-0.79 – 0.81)</td></tr>
</table>
Frequency of consumption continuous measure of times per day (assuming units of 1 x per day)
1) adjusted for age and gender
2) adjusted as '1', also BMI, WHR, education, income, physical activity, smoking, alcoholism
3) adjusted as '2' also consumption of other food groups
* p<0.05, **p<0.01, p<0.001

Note no description given of typical intakes in this population

Appears that for models 2 and 3 the HDL results were erroneously typed in place of the LDL results</td>
<td>In Brazilian adults, increasing frequency of consumption of fruits was associated with:
• LDL level weakly (r=-0.05) (significant) at the crude level; HDL not associated (not significant)
• Large significant reduction in LDL cholesterol (approx 6mg/dL) when adjusted only for age and gender
• Reduction still significant after adjustment for all other factors (magnitude not reported).
• Small non-significant reduction in HDL (approx 0.5 mg/dL), reduced further (0.13 mmol/L) when additionally adjusted for other variables

In Brazilian adults, increasing frequency of consumption of vegetables was associated with:
• LDL level weakly (r=-0.11) (significant) at the crude level
• Large significant reduction in LDL cholesterol (approx 3mg/dL) when adjusted only for age and gender
• Reduction still significant after adjustment for all other factors (magnitude not reported).
• Small (approx 0.13mg/dL), non-significant reductions in HDL
• Virtually no change (approx 0.01mg/dL increase) (not significant) in HDL when additionally adjusted for other non dietary variables

Issues:
Calculation of LDL – potential misclassification with bias towards null
Units for frequency of fruit and vegetable intake unclear
Magnitude of adjusted estimates unknown

Quality rating: B</td>
</tr>
</table>

Appendix 7 cont'd: Fruits, Vegetables and CHD Biomarkers – Cross-sectional Studies

Study	Design	Participants	Duration	Dietary Measures	Outcome	Results	Comments
Chrysohoou et al., 2004 Greece ATTICA study **Biomarker Study #17**	Cross sectional population based	n=1128 adult men and 1154 women Sampling: multistage, random stratified by age and gender 68% participation rate, representative sample Exclusions: renal failure, liver disease, CVD, chronic obstructive pulmonary disease, use of drugs that alter homocysteine	2001-2002	intake of fruits, vegetables FFQ validated for fat, etOH, and protein; also vegetables (test-retest) Included: Fresh & frozen vegetables, fresh fruit Excluded: mixed dishes, canned foods	plasma homocysteine 12h fasting used controlled collection, storage and processing procedures Total homocysteine by fluorescence polarization immunoassay.	Correlations between plasma homocysteine and intake of: fruit (r=-0.12, p=0.006) vegetables (r=-0.15, p=0.02)	In adult men and women, plasma homocysteine levels were correlated: • weakly and inversely with fruits (r=-0.12) (significant), although weakly • weakly and inversely with vegetables (r=-0.15, p=0.02) (significant), although weakly • inversely and significantly (p<0.05) after adjustment for potential confounding factors [age, smoking, education, income, alcohol and coffee intake, BMI, systolic & diastolic BP, glucose, serum cholesterol] Issues: Magnitude of adjusted estimates unclear. Results apply to types of fruits (fresh) and vegetables (fresh and frozen) in study Quality: A

Appendix 7 cont'd: Fruits, Vegetables and CHD Biomarkers – Intervention Studies

<table>
<tr><th>Study</th><th>Design</th><th>Participants</th><th>Duration</th><th>Dietary Measures</th><th>Outcome</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>Gao et al 2004

United States

MHAES

Biomarker Study #18</td>
<td>Cross-sectional</td>
<td>445 Hispanic and 154 Non Hispanic white adults ≥60y in Massachusetts (Hispanic and non Hispanic from same neighbourhoods)

Note many had history of one or more CVD conditions – heart attack stroke, other heart disease, hypertension

Representative sample</td>
<td>1993 - 1997</td>
<td>Fruit and vegetable intake (quartiles)

Semi-Quantitative FFQ

Validated for use in this population

Fruits: apples, pears, bananas, peaches, cantaloupe, watermelon, strawberries, mangoes, oranges grapefruit other fruit, orange or grapefruit juice, other 100% juice.

Vegetables: tomatoes, string beans, peas, broccoli, cauliflower, spinach, mustard greens, cole slaw, carrots, green salad, avocado, winter squash other vegetables, dried beans, beans with rice, chili with beans, peas with rice, vegetable soups and homemade soups</td>
<td>Plasma C-reactive protein, total plasma homocysteine, clinically elevated C-reactive Protein>10mg/dL, high homocysteine >10.4μmol/L for women & >11.4μmol/L for men

12h fasting blood samples,

used controlled collection nad storage procedures</td>
<td>Adjusted Mean ± SEM of Plasma CRP and Hcy in Elderly Hispanic Men and Women by quartile of intake of fruits and vegetables
<table>
<tr><th>Quartile (median intake)</th><th>C-reactive protein (mg/L)</th><th>Homo-cysteine (μmol/L)</th></tr>
<tr><td>Q1 (1.4 times/d)</td><td>4.8 ± 1.1</td><td>11.6 ± 1.0</td></tr>
<tr><td>Q2 (2.7 times /d)</td><td>4.8 ± 1.0</td><td>10.8 ± 1.0</td></tr>
<tr><td>Q3 (3.8 times /d)</td><td>4.5 ± 1.1</td><td>11.0 ± 1.0</td></tr>
<tr><td>Q4 (5.5 times /d)</td><td>3.9 ± 1.1*</td><td>10.5 ± 1.0*</td></tr>
<tr><td>p trend [a]</td><td>0.017</td><td>0.049</td></tr>
<tr><td>p trend [b]</td><td>0.010</td><td>0.033*</td></tr>
</table>
a adjusted for age, sex, ethnicity, BMI, diabetes, hypertension, smoking, alcohol, vitamin supplement use, energy intake, frequency consumption meat, cereal, dairy products, use of aspirin, NDAIDs, antihypertensives, diuretics, or CVD medications, hormones
b C-Reactive Protein model also adjusted for Homocysteine and vice versa
Means and SEM presented are adjusted as b</td>
<td>Considering other risk factors, in an elderly population, frequent intake (av. 5.5 times/d vs 1.4 times/d of fruits and vegetables) is associated with:
• ≈ 1mg/L lower plasma C-reactive protein (significant); dose response relationship
• ≈ 1μmol/L lower plasma homocysteine (significant); dose response relationship
• For each serving intake adjusted odds ratio for high plasma CRP was 0.79 (0.65-0.79) (ie reduced risk approx 20%)
• For each serving intake adjusted odds ratio for high plasma homocysteine was 0.83 (0.72-0.96) (ie reduced risk approx 20%)

Issues:
could not measure very low <0.6mg/L CRP concentrations – likely effect to underestimate, not overestimate associations
cut off for high C-reactive protein can predict long term outcomes after coronary events
cut off for high homocysteine based on 95th percentile for young adults

Quality Rating: A</td>
</tr>
</table>