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Article (Accepted Version)

Alissa, Eman M and Ferns, Gordon A (2015) Dietary fruits and vegetables and cardiovascular diseases risk. *Critical Reviews in Food Science and Nutrition*, 57 (9). pp. 1950-1962. ISSN 1040-8398

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Dietary Fruits and Vegetables and Cardiovascular Diseases Risk

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Abstract

Diet is likely to be an important determinant of cardiovascular disease (CVD) risk. In this article, we will review the evidence linking the consumption of fruit and vegetables and CVD risk.

Efforts were initially focused on individual protective nutrients, such as vitamin E, vitamin C, and β -carotene, in an attempt to identify putative intervention strategies. However these have generally proven to be disappointing when tested in clinical trials. The evidence now suggests that a complicated set of several nutrients may interact with genetic factors to influence CVD risk. Therefore, it may be more important to focus on whole foods and dietary patterns rather than individual nutrients to successfully impact on CVD risk reduction.

The initial evidence that fruit and vegetable consumption has a protective effect against CVD came from observational studies. However uncertainty remains about the magnitude of the benefit of fruit and vegetable intake on the occurrence of CVD and whether the optimal intake is five portions or greater. Results from randomized controlled trials do not show conclusively that fruit and vegetable intake protects against CVD, in part because the dietary interventions have been of limited intensity to enable optimal analysis of their putative effects.

The protective mechanisms of fruit and vegetables may include some of the known bioactive nutrient effects dependent on their antioxidant, anti-inflammatory and electrolyte properties, but also include their functional properties, such as low glycemic load and energy density. Taken together, the totality of the evidence accumulated so far does appear to support the notion that increased intake of fruits and vegetables may reduce cardiovascular risk. It is clear that fruit and vegetables should be eaten as part of a balanced diet, as a source of vitamins, fiber, minerals and phytochemicals.

A clearer understanding of the relationship between fruit and vegetable intake and cardiovascular risk would provide health professionals with significant information in terms of public health and clinical practice.

Introduction

Dietary factors play an important role in the development and progression of cardiovascular disease (CVD) (Din,2002, Hu & Willett, 2002), and dietary optimization must be considered an important lifestyle intervention for the management of existing CVD and its prevention (Ferrari et al.,2004).

The importance of diet in maintaining optimal health has emerged from several lines of evidence including observations that the rates of particular diseases differ significantly between cultures; with dietary variation being an obvious factor contributing to these differences (Key et al.,1996, Ness & Powles,1997). In contrast to the literature on cancer risk (Steinmetz & Potter,1991), most studies of diet and CVD have reported associations of risk with specific nutrients rather than with entire foods (Willett,1993). Nonetheless, several studies have reported on the role of overall dietary patterns in predicting cardiovascular risk (Hu,2002). In these studies, high intakes of fruits, vegetables, legumes, whole grains, poultry, and fish were associated with lower cardiovascular risk whereas a western pattern diet, characterized by high intakes of red and processed meat, sweets, fried food, and refined grains were associated with higher cardiovascular risk independent of lifestyle factors (Hu et al.,2000, Fung et al.,2001). The utility of adopting a whole diet approach (e.g., the Dietary Approaches to Stop Hypertension (DASH), Mediterranean, and Portfolio diets), with emphasis on antioxidant-rich fruits and vegetables and whole grains, in CVD prevention has been widely supported (De Waart et al.,2001; Sacks et al.,2001; Trichopoulou et al.,2003; Witztum,1994).

Diets rich in antioxidants derived from fruits and vegetables are thought to be protective against reactive oxygen species, and this, provides a potential mechanism through which they may prevent cancer and CVD. This has, in part, been the rationale for the WHO and other health authorities to recommend the consumption of these foods (Committee on Diet and Health, National Research Council,1989, WHO,2003). Dietary advice for CVD prevention is complex for a number of reasons. Fruit and vegetables differ qualitatively and quantitatively in their content of specific antioxidants; particularly the relative balance between these antioxidants and their bioavailability. Furthermore it is unclear what the optimal number of portions should be consumed per day and how many portions should be taken as fresh, processed, or dried fruit. It is also unclear whether the benefits

of a diet rich in fruit and vegetables would be more evident in primary versus secondary prevention (WHO,2003). A clear understanding of the relationship between fruit and vegetable intake and cardiovascular risk would provide health professionals with significant information in terms of public health and clinical practice (Stephoe et al.,2003). The American Heart Association (AHA) has had a longstanding commitment to provide information about the role of nutrition in CVD risk reduction (Krauss et al.,2000). Nutritional advice is often easier to understand in the context of foods rather than the individual nutrients they contain. The AHA previously recommended a diet that contains more than 5 servings of fruit and vegetables daily (Kromhout,2001).

Nevertheless, there remains a low intake of fruits and vegetables worldwide (Hall et al.,2009). An ecological study examined associations between fruits and vegetables consumption in the population and six health outcomes (ischemic heart diseases, stroke and cancers of the stomach, oesophagus, colon, rectum and lung) using global data. Data were obtained from national representative dietary surveys and the Food and Agriculture Organization of the United Nations, with analyses being stratified by 14 geographical regions. The analysis suggested that 2.6 million deaths worldwide and 31% of coronary heart disease and 19% of ischaemic stroke may be due to a suboptimal consumption of fruits and vegetables (Lock et al.,2005). More recently, dietary guidelines for Americans recommend that most people should eat at least 9 servings (4 1/2 cups) of fruits and vegetables a day, 4 servings (2 cups) of fruits, and 5 servings (2.5 cups) of vegetables, based on a 2000 kcal diet (United States Department of Agriculture 2010).

Often, dietary guidance emphasizes the importance of eating whole fruit and vegetables whilst reducing the consumption of foods high in fat and sodium, i.e., french fries. Indeed fruits and vegetables are generally have a low energy density but the nutritional contribution of standard servings of fruits and vegetables varies widely (Hornick & Weiss,2011). Moreover, the content of phytochemicals also varies greatly and is often not listed in nutrient databases (Song et al.,2010).

Many constituents of fruits and vegetables (fibre, fat and water soluble vitamins, sterols and phytochemicals) have also been associated with reduced risk for CVD, which supports the notion that a high fruit and vegetable consumption might protect against cardiovascular events via a number of different mechanisms (Knekt et al.,1996; Rimm et

al.,1996; Liu et al.,2001; Padayatty et al.,2003; Genkinger et al.,2004; Pereira et al.,2004). These nutrients act through a variety of mechanisms, such as reducing antioxidant stress, improving the plasma lipoprotein profile, lowering blood pressure, improving insulin sensitivity, and hemostasis regulation (Van Duyn & Pivonka,2000; Bazzano et al.,2003, Wannamethee et al.,2006). The benefit of a diet rich in fruits, vegetables, and grains is attributed to the complex mixture of micronutrients, phytochemicals, and fiber (Samman et al.,2003). Therefore, the additive and synergistic effects of these nutrients in fruits and vegetables may be responsible for their potent antioxidant and anticancer activities (Wolfe et al.,2008, Song et al.,2010). This may partially explains why no single antioxidant can replace the combination of nutrients in fruits and vegetables in achieving the observed health benefits.

Overall, several reviews and meta-analyses examining intakes of fruits and vegetables have reported an inverse association between intake of fruit and vegetables and CVD occurrence, although epidemiological evidence for causality is controversial (Dauchet et al.,2005; Dauchet et al.,2006; He et al.,2006; Agudo et al.,2007; He et al.,2007; Wang et al.,2014). The magnitude of the favorable association remains uncertain because of differences in methodological approaches, analytical techniques, and the definition of outcomes.

In this article, we will review the evidence concerning the link between fruits and vegetables consumption and the risk of CVD.

Health Effects of Fruits and Vegetables

The protective effects of fruits, vegetables, and whole grains against several chronic diseases are well-documented, however, the evidence is linked to whole foods, rather than supplements of individual dietary constituents, hence the actions of individual dietary constituents do not fully explain the observed health benefits of diets rich in fruits and vegetables. Supplementation with individual antioxidants has been investigated in randomized clinical intervention trials, but have not consistently shown a benefit (Ommen et al.,1996, Stephens et al.,1996, Yusuf et al.,2000). Isolated components of the diet may either lose their bioactivity, may not behave the same way as they do in whole

foods, or may require other constituents of the whole food for their full functional activity (Hennekens et al.,1996).

Fruit and vegetables differ in their nutrient content, the manner in which they are prepared and eaten, and the amounts in which they are eaten daily. Such differences have important implications for the analysis of fruit and vegetable intake and their consequential impact on chronic disease risk. Other factors, potentially confounding the interpretation of fruit and vegetable intake on cardiovascular risk, include the wide variety of fruit and vegetables consumed globally, each with very different micro- and macronutrient, contents, and their potential interactions with other dietary components. These make the interpretation of studies of fruit and vegetable consumption on human health in prospective cohort studies or randomized controlled trials. Few studies have investigated the intake of fruits and vegetables separately as in the European Prospective Investigation into Cancer and Nutrition (EPIC) study (Sargeant et al.,2001). Furthermore the inclusion of legumes and potatoes in the vegetable category as in the Atherosclerosis Risk in Communities (ARIC) Study (Steffen et al.,2003) has not been consistently applied.

The role of fruit or vegetable juices as distinct from that of the whole fruit and vegetable has not been well studied. Fruit and vegetable juices are obviously different in form from whole fruit or vegetables, and often lack much of fiber of the latter. In addition, they are often sweetened with a variety of substances or added sugar, which contribute to their energy density but not necessarily to their protective function against chronic diseases. However, the beneficial health effects of consuming fruits are partially attributable to their antioxidant activity, which is present in fruit juices. For example, the vitamin C content of citrus juices and polyphenols content of pomegranate juice are considered protective against oxidative stress and atherogenesis (Aviram et al.,2002, Vinson et al.,2002). Nevertheless it is worth noting that the antioxidant content of juices, measured in vitro, does not necessarily translate into bioavailable antioxidant activity, as the absorption of some antioxidants is poor (Vinson et al.,1995).

Whilst fresh, cooked, and processed fruits and vegetables including frozen and canned, 100% fruit juices, 100% vegetable juices, and dry fruits are all considered as servings of fruits and vegetables a day, processing of fruit and vegetables alters their structure and

induces significant changes in chemical composition, nutritional value, and bioavailability of bioactive compounds. These may all have an impact on their potential protective effects. Fruit juices, for example, whilst containing less fiber than whole fruit, may be a good source of phytochemicals (Ruxton et al.,2006). Vegetables are often cooked before consumption, which causes a significant loss of water-soluble and heat-sensitive bioactive compounds (Ruiz-Rodriguez et al.,2008). In contrast, processing can enhance the availability of some bioactive compounds. It has been shown for example that heat treatment improves the bioavailability of lycopene from tomatoes (Rao & Agarwal,1999) and carotenoids from carrots (Hornero-Me´ndez & Mi´nguez-Mosquera,2007). Furthermore, processing could convert folate polyglutamate in vegetables into monoglutamate, which has better bioavailability (Melse-Boonstra et al.,2004).

Much attention has been paid to antioxidant vitamins found in fruits and vegetables, yet these foods are also rich in fiber (Smith & Tucker,2011) and nitrate (Lidder & Webb,2013) and, therefore, a diet rich in fruits and vegetables will also be rich in a complex mixture of micronutrients, phytochemicals and fibre, with the exact combination dependent on the range of fruits and vegetables consumed.

The protective effect of dietary fibre on cardiovascular risk is biologically plausible, and there are many potential mechanisms through which fibre may act on individual coronary risk factors. Soluble, viscous fibre types can affect absorption from the small intestine because of the formation of gels that attenuate postprandial changes in blood glucose and lipoproteins (Lunn & Buttriss,2007). The formation of gels also slows gastric emptying, maintaining levels of satiety and contributing towards less weight gain (Lunn & Buttriss,2007). Soluble fibre and resistant starch molecules are additionally fermented by bacteria in the large intestine, producing short chain fatty acids, which help reduce circulating cholesterol levels (Slavin et al.,1999).

It has been suggested that an increased intake of salicylate from foods might have contributed to the decline in CVD (Ingester & Feinleibl,1997). However, the impact of salicylate content of foods remains controversial. Salicylates per se do not affect thromboxane B2 formation or platelet aggregation. It is acetylsalicylate that is able to

inactivate cyclo-oxygenase and subsequently affect cardiovascular risk (Janssen et al.,1996).

Other nutrients in fruits and vegetables, for example fructose, are thought to be dietary risk factors for CVD. Because of its low glycemic index, fructose was suggested as an alternative sweetener for diabetic patients (Henry et al., 1991). However, in patients with overt hyperglycemia and insulin deficiency, fructose may be associated with a greater increase in blood glucose concentrations than in diabetic patients with less elevated blood glucose concentrations (Swanson et al., 1992). Moreover, fructose induced hypertriglyceridemia in non-diabetic subjects susceptible to elevated serum triglycerides has also been a matter of concern (Henry et al., 1991). This is particularly relevant in patients with diabetes, among whom hypertriglyceridemia is the most common lipid abnormality and may increase the risk of CVD.

Bioactive Components of Fruits and Vegetables

A number of mechanistic hypotheses involving specific constituents of fruits and vegetables have been proposed. The antioxidant hypothesis suggests antioxidant vitamins such as C and E, β -carotene and other carotenoids; antioxidant minerals such as selenium and zinc and other antioxidant compounds such as flavonoids are protective (Witztum,1994). The lipid oxidation hypothesis, proposes that LDL cholesterol is modified to oxidized LDL by free radicals, and this is a potently pro-atherogenic lipoprotein particle (Witztum & Berliner 1998). When circulating LDL molecules are present at high levels in blood, they infiltrate the artery wall and increase intimal LDL, which can then be oxidized by free radicals. This oxidized LDL is more atherogenic than native LDL and serves as a chemotactic factor in the recruitment of circulating monocytes and macrophages. Since oxidized LDL plays a key role in the initiation and progression of atherosclerosis, giving dietary items with antioxidant activity capable of preventing LDL oxidation has been a putative therapeutic approach. Dietary micronutrients provided as antioxidants may be incorporated into LDL and may then be preferentially oxidized when the LDL is exposed to free radicals; this may occur before any extensive oxidation of the sterol or polyunsaturated fatty acids can occur (Sanchez-

Moreno et al., 2000). Therefore, dietary micronutrients might retard the progression of atherosclerotic lesions by reducing the formation of oxidized LDL.

Inflammation is thought to promote the initiation and progression of atherosclerosis, and is also involved in the acute thrombotic complications of atherosclerosis (Libby et al., 2002). The relationship between high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation, and the total antioxidant capacity (TAC) of the diet has reported that dietary TAC is independently and inversely correlated with hs-CRP (Brighenti et al.,2005). The authors suggest that this could be one of the mechanisms behind the protective effects of fruits and vegetables.

The relationship between dietary fruit and vegetables and atheroma formation has been explored in animal models and in vitro cell culture systems. However, the relevance of these models to human disease remains contentious (Wolfe et al.,2008; Song et al.,2010; Wang et al.,2014).

The benefits of a diet rich in fruits and vegetables may also be due to the displacement of other less healthy foods. The presumptive protection afforded by antioxidants present in fruits and vegetables (Knekt et al., 1994; Kok et al.1987; Losonczy et al.,1996; Yochum et al.,2000) was originally based on the measurement of several biochemical markers as an index of fruit and vegetable intake (vitamin E, carotenoids, vitamin C, and phytochemicals). However, dietary phytochemicals have been shown to have roles in the regulation of prostaglandin synthesis, reduction of vascular tone, inhibition of platelet aggregation, regulation of cholesterol synthesis and absorption, and reduction of blood pressure and these may not be reflected by the measurements of the biomarkers that have been used previously.

Of the many nutrients present in fruit and vegetables; dietary vitamins have been evaluated most extensively in literature. Despite the abundance of observational epidemiological data supporting their role in CVD prevention (Marchioli et al.,2001; Knekt et al.,2004; Ye & Song,2008), intervention trials with folate, carotenoids, and vitamin C have failed to demonstrate convincingly that these vitamins have a role in preventing CVD and cardiovascular mortality (Vivekananthan et al.,2003; Cook et al.,2007; Albert et al.,2008; Sesso et al.,2008). In this context, the U.S. Preventive Services Task Force concluded that the current evidence is insufficient to assess the

balance of benefits and harms of the use of single- or paired-nutrient supplements (except β -carotene and vitamin E that have proven to be harmful) for the prevention of CVD and cancer (Moyer, 2014).

Moreover, a meta-analysis evaluating the effects of combinations of multivitamin and mineral supplements on prevention of CVD found no evidence of a protective effect (Huang et al.,2006). Factors that might have affected the results include inappropriate dosing, insufficient duration of intake, or the stage of disease at which the intervention was introduced. A further possibility is that CVD prevention trials might have recruited participants not likely to benefit from treatment, such as those with a satisfactory vitamin status. In order to translate the knowledge of molecular and cellular mechanisms of atherosclerosis more effectively into clinical benefit, the methodological issues of the current clinical trials must be interpreted with caution.

The tendency of researchers to pursue hypotheses based on single nutrients, may underestimate the potential benefits of chemically complex as foods. The outcomes of the β -carotene and α -tocopherol trials may be particularly instructive in this regard (Greenberg & Sporn,1996, Rowe,1996).

Observational Studies

Table (1) shows a summary of observational studies on the relationship between fruit and vegetable consumption and prevention of CVD, which had ecological, case-control, or prospective cohort designs. Given the extreme variability of observational studies design, trend analyses of dose-response relationships do not often reveal statistically significant associations, as shown in Table (2). These studies have varied considerably in their inclusion criteria, recruitment methods, sample size, duration of follow-up, definition of events and adjustment factors, and presentation of results. More attention will be required on the design of appropriate long-term prospective studies because they offer the most useful methodological compromise for studying associations between nutritional factors and CVD, particularly for primary prevention.

Methodological differences among observational studies include dietary assessment methods, the variety of fruit or vegetables being investigated, the definition of the reference group, and the choice of exposure categories. Measurement error in dietary

assessment is a persistent problem when assessing associations between diet and diseases (Horner et al., 2002). The bias can be large and in either direction. In addition, where errors exist in the measurement of confounders, the bias would work in the direction of confounding, which may either exaggerate or dilute the estimated association (Willett,1990). Other potential biases cannot be ruled out, such as those due to misclassification of fruit and vegetable intake by the dietary assessment method, the number of groups of fruit and vegetable consumed, and various reference categories between individual studies (Lee and Niemen, 2003). Findings from validation studies using biomarkers suggest that measurement error in dietary exposures will often result in attenuated estimates, which may often be quite severe (Willett,1990).

Therefore, more objective and accurate indices of fruit and vegetable intake, such as nutritional biomarkers, are becoming popular. Such biomarkers of intake need to be able to discriminate between differences in intakes should be non-invasive or minimally invasive, reproducible, easily measured, and highly responsive to the intervention being carried out (Crews et al., 2001).

Data from dietary assessments in prospective studies were often unadjusted for total calorie intake and only assessed at a single time-point (baseline). Moreover, the reliability and accuracy of these methods to assess long-term nutritional habits are limited and have not always been robustly validated. Therefore, heterogeneity complicates estimation of the magnitude of effects, and the comparison of results between studies (Dauchet et al., 2009).

It is possible that variation between study populations on the types of fruits and vegetables and whether cooked or raw vegetables are most commonly consumed may introduce heterogeneity. Sometimes heterogeneity is introduced by including vegetables that contain more protective nutrients, which yield a stronger association in comparison with weaker association resulting from including heavily processed vegetables. Stratified analysis for fruits and vegetables separately may reduce the heterogeneity. Moreover, adjustment for major confounding factors in the studies that have been included should reduce the potential bias due to these other dietary and lifestyle factors.

Limitations of observational studies include the problem of residual confounding, which also remains when meta-analyses of these studies are undertaken. Exploration of

confounding factors, through meta-regression, has not yet confirmed that adjustment for such confounders sufficiently explained the strength or direction of associations observed (Lawlor et al.,2004).

Risk reduction estimates from cohort studies have generally been less impressive than initially observed in ecological or case–control studies. Several reasons for this are possible including an improvement of the dietary habits in the comparator population over the duration of these studies, which could affect the outcomes of prospective follow-up. Also, the lack of comparability of dietary assessment methods combined with inaccurate measurements makes it difficult to detect associations or weaken these associations. Indeed meta-analyses of observational studies report relative risks indicative of a protective effect of fruit and vegetables, but they also show substantial inter-study heterogeneity, which might be explained by methodological issues or publication bias (Dauchet et al.,2006, He et al.,2007).

The observation of a favorable association between fruit and vegetable consumption and cardiovascular risk does not necessarily indicate causality; hence, fruit and vegetable intake might simply indicate a reduced intake of unhealthy nutrients, such as saturated fats and salt. Furthermore, social and behavioral factors may often be associated with a health-conscious lifestyle, and the impact of these cannot always be measured precisely or might not be appropriately considered in statistical analyses. Therefore, the confounding effects in observational studies might not be fully appreciated.

Interventional Studies

Because observational studies do not control for unmeasured confounders, the causal relationship remains to be established using randomized controlled trials. Although nutritional prevention trials provide the most robust estimate of causal effects, they also have some limitations that may make their interpretation difficult. The success of these studies relies on compliance of the participants for the duration of the study, which can be over several years. Obviously, a double-blind controlled trial is not feasible in the context of interventions with food items, leaving the possibility for biases in the assessment of end-points. Secondly, increased consumption of fruit and vegetables might induce changes in other components of the diet. For instance, in the DASH trial, fruit and

vegetables were substituted by snacks to achieve equivalence of energy intake in the control and experimental diet. Thirdly, results of tightly controlled intervention trials cannot easily be extrapolated to a free-living general population, or to public health policies. Finally, the difficulty of achieving a substantial modification of diet in long-term studies hampers the interpretation of the results.

Not surprisingly, given their methodological difficulties, few randomized nutritional trials have been conducted to evaluate the effect of high fruit and vegetable intake on the incidence or recurrence of CVD (reviewed by Brunner et al.,2006). It must also be emphasized that changes in fruit and vegetable intake achieved in these studies have been generally modest in these clinical studies.

In the Indian heart study, a semi-vegetarian diet enriched with fruits, vegetables, whole grains, and nuts reduced coronary death and nonfatal myocardial infarction (Singh et al.,1992). A vegetarian diet is associated with a lower blood cholesterol concentration, a lower incidence of stroke, and a lower risk of mortality from stroke or ischemic heart disease (Bazzano et al.,2002, Bazzano et al.,2003) when compared to a mixed diet. However, vegetarians often have a higher consumption of whole grains, soy, and nuts, all of which have significant cardio-protective effects (Kelly & Sabate,2006, Mellen et al.,2008).

In the Diet and Reinfarction Trial (DART) II, recommendations aimed at promoting increased consumption of fruit, vegetables, fiber, and orange juice had no effect on cardiac death in patients with angina pectoris (Burr et al.,2003). However, the small difference in fruit and vegetable intake between study and control participants (~20 g per day) may have contributed to the lack of significant benefit (Ness et al.,2004, Burr, 2007).

In the Women's Health Initiative (WHI) Randomized Controlled Dietary Modification Trial, fruit and vegetable intake in the group that received nutritional advice had increased only slightly (by 1.2 servings per day), compared with the control group and the two groups did not differ significantly in the rate of major CVD events (Howard et al.,2006a).

The Lyon study showed major benefits of a Mediterranean Diet but given the complexity of this diet and the use of margarines rich in α -linolenic fatty acid, these benefits cannot be attributed to fruit and vegetable consumption alone (de Lorgeril et al.,1999).

Similarly, the Prevencio'n con Dieta Mediterránea (PREDIMED) study has demonstrated that adherence to the Mediterranean Diet is associated with an increase in serum markers of atheroma plaque stability which may partly explain the cardio-protective role of Mediterranean Diet (Casas et al.,2014).

Nevertheless, when the short-term effects of Mediterranean diets versus those of a low-fat diet were investigated on intermediate markers of cardiovascular risk, significant dietary changes were only seen in olive oil, nuts and red meat within the intervention groups (Estruch et al.,2006). Therefore, any clinical benefit of the Mediterranean Diet cannot be attributed to a change in fruits and vegetables intake.

Fruits and Vegetables and Cardiovascular Risk Factors

Investigating the effects of fruit and vegetable intake on classical cardiovascular risk factors is crucial in determining biological plausibility of a causal relationship between fruit and vegetable consumption and CVD.

Hypertension

Fruit and vegetables are good sources of magnesium and potassium, which have been inversely associated with hypertension in previous studies (Zhang et al.,2012). The Dietary approaches to stop Hypertension (DASH) study showed that fruit and vegetable consumption was associated with a significant decrease in blood pressure, in which the effect was more pronounced in hypertensive patients than in normotensive participants (Broekmans et al.,2001, Sacks et al.,2001). This might be attributed to an increase in the intake of potassium, magnesium, or some other component in fruits and vegetables, or alternatively a reduced intake of sodium (Hord et al., 2009). A number of intervention studies have given conflicting results on the beneficiary effect of increased fruits and vegetables intake on blood pressure (Appel et al.,1997; McCall et al.,2009; Berry et al.,2010). This is a consequence of the potential differences in study designs, participant

characteristics (in terms of degree of hypertension at baseline) and methods used to achieve an increase in fruits and vegetables intake.

Results from a large randomized controlled trial, to increase daily consumption of fruit and vegetables to at least five portions among healthy study participants randomly selected from primary health care centers, have shown that increased consumption of fruit and vegetables can contribute to a small decrease in blood pressure (John et al.,2002).

Hyperlipidemia

Evidence for the effects of increased fruits and vegetables intake on hypercholesterolaemia is limited to date, with many studies either not being specifically designed to test the effects on hypercholesterolaemia, or where increased fruits and vegetables was combined with some other dietary intervention, such as reduced fat intake. The large randomized controlled trial, which demonstrated a positive effect of increased fruits and vegetables intake on blood pressure, showed no effect on blood levels of total cholesterol (John et al.,2002).

Several components of fruit and vegetables have cholesterol-lowering properties, particularly dietary fiber (Satiya & Hu,2012). Similarly, ingestion of large quantities of phytosterols (>2 g per day) lowers plasma LDL-cholesterol levels (Thompson & Grundy,2005). Since the available evidence demonstrates that viscous fibres, plant sterols, vegetable proteins and nuts can each independently lower serum cholesterol by 5–10% (Anderson et al.,2000). The Portfolio Diet was introduced as a dietary approach based on current dietary recommendations that is feasible, accessible, and has maximized the metabolic advantages of plant-based foods. It is in compliance with the dietary recommendations of the Adult Treatment Panel III of the National Cholesterol Education Program. The major recommendations are that <7% energy should come from saturated fat, dietary cholesterol intake should be <200 mg/d, dietary fibre intake should be 20–30 g/d, and 50–60% energy should come from carbohydrate, 15% energy from protein and 25–35% energy from fat (NCEP, 2001). It is assumed that the effect of combining these components may be additive and as such leads to clinically-significant reductions in

serum cholesterol and consequently lower the risk of developing CVD (Jenkins et al.,2002).

Diabetes

The relationship between fruit and vegetable intake and the risk of type II diabetes has not been established and cohort studies have produced conflicting results (Bazzano et al.,2008, Villegas et al.,2008). The discrepancies between studies could be explained by confounding factors and by the possible antagonistic effects of the sugars, fiber, and antioxidants in fruit and vegetables. The results from a few prospective studies regarding fruit and vegetable intake and risk of type 2 diabetes mellitus are not entirely consistent. For instance, an inverse relation between intake of vegetables, but not of fruits, and risk of type 2 diabetes mellitus was observed in the Nurses' Health Study (Colditz et al.,1992). An inverse relation between intake of fruit and vegetables and diabetes mellitus risk in women, but not for men in the National Health and Nutrition Examination I Follow-up Study (Ford & Mokdad,2001). The Iowa Women's Health Study did not show an association between fruit and vegetable consumption and incident diabetes (Meyer et al.,2000). Also, none of these studies examined different types of fruits and vegetables in relation to the risk of type 2 diabetes mellitus.

Compared with carbohydrate-dense foods, fruits and vegetables (with the exception of potato) generally contribute to a lower glycemic load (a measure of the postprandial glucose-raising potential of dietary carbohydrates). In prospective studies, a diet low in glycemic load has been associated with lower risks of type 2 diabetes mellitus and CVD (Liu et al.,2001, Liu et al.,2000b). The rich fiber content of whole fruits and vegetables may be partly responsible for this response, but other factors such as the physical structure of fruits and vegetables, antioxidants, carotenoids, flavonoids, and many enzyme inhibitors may play important roles as well (Liu et al.,2001, Ylonen et al.,2003).

A link has been proposed between increased consumption of green leafy vegetables and reduced incidence of diabetes, albeit is based on a limited number of studies (Carter et al.,2010, Cooper et al.,2012), with other classes of fruits and vegetables, such as root vegetables, also potentially playing a role.

Randomized prevention trials have demonstrated the effectiveness of complex lifestyle interventions (including physical activity and body weight reduction) on reducing the risk

of diabetes, but the contribution of fruit and vegetables per se in these studies is difficult to estimate given the complexity of the interventions (Pan et al.,1997, Tuomilehto et al.,2001).

Obesity

It has been reported that replacing energy-dense foods with fruit and vegetables could help to decrease calorie intake and, therefore, body weight (Epstein et al.,2001). Accumulating evidence indicates that the combination of increased fruit and vegetable intake, together with other dietary recommendations, might promote satiety and weight loss in overweight individuals (Tohill,2005). Although the effect of fruit and vegetable consumption on weight control has been scarcely investigated in clinical settings, it has been found that decreased fat intake combined with increased fruit and vegetable intake results in greater weight loss when compared with low-fat diets alone (Howard, et al.,2006b).

Multiple pathways are expected to be involved, high water and fiber content and low-energy density of whole fruits and vegetables may promote weight control by reducing total energy intake through increasing satiety or by substituting for foods with high-energy densities (Pereira & Ludwig, 2001). Multiple enzymatic inhibitors in fruits and vegetables may also affect body weight through other pathways, including the modulation of metabolic efficiency and/or insulin secretion or action (Liu et al.,2003).

Epidemiological Studies and their Limitations

There is still considerable scientific uncertainty about the relationship between specific dietary components and cardiovascular risk. Reasons for this uncertainty include the high doses of nutrients used in intervention trials that may have resulted in unexpected effects including pro-oxidant activity of excessive doses of a single antioxidant, and residual confounding in cohort studies due to imperfect classification of factors such as smoking that may have biased the results. Since the effect of antioxidants on mortality may vary by oxidative burden, associations between fruits and vegetables and CVD may be affected by cigarette smoking and body mass index, both sources of oxidative stress.

Interactions between the numerous nutrients found in fruit and vegetables are difficult to evaluate in biological experiments. Moreover, different varieties of fruit and vegetables,

growing techniques, industrial processing, and storage and cooking methods can strongly affect the composition and properties of the nutrients. Perhaps the most effective combination of vitamins or the optimal biochemical structure for synthetic molecules used for CVD prevention have not been identified.

Responsiveness of biomarkers may be altered by lifestyle and other factors, such as age, smoking behavior, physical activity, and the presence of low-grade inflammation. Such factors may have affected the response of biomarkers in intervention studies, but the studies are too heterogeneous in terms of design, level of control of overall dietary intake, duration, and the nature of the control group to allow exploration of these issues. The level of nutrients in food will directly affect biomarker response and may vary by individual variety of fruit or vegetable (Kurilich & Juvik, 1999), production conditions (Kopsell and Lefsrud, 2006), and processing, storage, and cooking methods, particularly for vitamin C (Castenmiller et al., 1999).

The current literature does not allow us to examine the association of cardiovascular risk with specific fruits and vegetables or with fruit and vegetable groups, or to examine the effect of seasonal deficits in intake; nor could we examine the effects of secular changes in diet. International collaboration for food based analyses of existing cohort studies, where the data is available, could help refine the current state of knowledge. It will be important to address this in future studies to provide clarification regarding the optimum protective level of fruits and vegetables intake, and therefore be as directly relevant to dietary recommendations and policy as possible.

Future Prospects

Because of the physical and biochemical complexity of whole foods, much uncertainty remains regarding the direct relation between fruit and vegetable intake and CVD risk. As fruits and vegetables are a complex food group, judging biological plausibility of the association demonstrated in observational studies is extremely challenging, so is defining their exact protective mechanism in interventional trials.

Results from the WHO MONICA study show that inter-population differences are very inadequately explained by the classical cardiovascular risk factors, suggesting that the search for new determinants of vascular risk should be explored further (WHO MONICA

project, 1994). A recent randomized clinical trial shows that fruit and vegetable intake in hypertensive patients resulted in a dose-dependent improvement in endothelium dependent forearm blood flow (McCall et al.,2009). Nevertheless, this is in contrast to other negative ascorbic acid supplementation studies (Chen et al.,2006). Thus, increasing fruit and vegetable consumption is likely to have beneficial effects due to synergistic effects of bioactive compounds that improve the vascular phenotype but may not be readily detected by routine clinical or biochemical examination. Endothelial function is known to be a surrogate measure of vascular function with known diagnostic and prognostic values.

As we continue to identify the roles of nutrients in foods in the complex pathways that contribute to CVD risk and/or prevention, scientists are focusing on genetic modulation of these pathways so that better guidance may be developed for subsets of the population at differential risk. Thus, the use of biological and genetic markers in future studies may help to clarify whether a causal relationship exists between fruit and vegetable intake and CVD risk and/or prevention in a subset of the population in whom interventions would be best targeted. It has recently been shown that there may be genetic variation in the metabolic response to intake of compounds found in fruits and vegetables, such as vitamin C (Timpson et al.,2010) and carotenoids (Borel,2012).

A recent human nutrigenomics study also confirmed the importance of whole food instead of a single phytochemical (Milenkovich et al.,2011). The effects of orange juice and pure citric phytochemical hesperidin were investigated on the expression of genes in leukocytes in healthy volunteers after consumption of orange juice, hesperidin, or placebo for 4 weeks. Global gene expression profiles were determined using human whole genome cDNA microarrays. Both orange juice and hesperidin consumption significantly affected leukocyte gene expression. Orange juice consumption induced changes in the expression of 3422 genes, hesperidin intake modulated the expression of 1819 genes, and 1582 genes were in common in both groups. Many of the protein products of these affected genes were involved in chemotaxis, adhesion, infiltration, and lipid transport, suggesting lower recruitment and infiltration of circulating cells to vascular wall, lower lipid accumulation, and formation of the atherosclerotic plaque.

Conclusions

Many cardiovascular risk factors, including hyperlipidemia, hypertension, obesity and diabetes are substantially influenced by dietary factors. **There is good evidence that a high consumption of vegetables and fruit is associated with a reduced levels of adiposity and consequently a lower risk of hypertension, and type 2 diabetes mellitus.** Understanding the relation between fruit and vegetable consumption and CVD is important for guiding consumer education and informing dietary guidelines to reduce cardiovascular risk. The key is to increase the total up to above five daily servings of fruits and vegetables in all forms. Fruit and vegetables should be eaten as part of a balanced diet, as a source of vitamins, fiber, phytochemicals, and water. People should obtain bioactive micronutrients from a wide variety of whole foods for optimal nutrition and health well-being, not from dietary supplements, or from foods enriched in cereal or vegetable derived fiber.

Early efforts focused on identifying protective nutrients, for example vitamin E, vitamin C, and β -carotene, have proven to be disappointing when tested in clinical trials. This is almost certainly owing to the complexity of fruit or vegetables and the large number of bioactive compounds present, and also because of other dietary sources containing these bioactive compounds. Rather, the evidence now suggests that a complicated set of many nutrients interact to influence CVD risk. Therefore, it is important to focus on whole foods and dietary patterns to impact on CVD risk reduction.

Evidence that fruit and vegetable consumption has a protective effect against CVD comes mainly from observational studies. Uncertainty remains about the real magnitude of the relationship between fruit and vegetable intake and the occurrence of CVD. Results from randomized controlled trials do not show conclusively that fruit and vegetable intake protects against CVD, in part because the dietary interventions have not been intensive enough to enable optimal analysis of their putative effects.

Protective mechanisms may involve not only known bioactive nutrients such as fiber and potassium, but also functional aspects of fruit and vegetable intake such as low glycemic load and energy density. Taken together, the totality of the evidence accumulated so far does appear to support the notion that increased intake of fruits and vegetables may reduce cardiovascular risk.

The effect of specific classes of fruits and vegetables combined, fruit or vegetables alone, the effect of quantity versus variety of fruits and vegetables consumed and the degree of processing on demonstrated associations remains uncertain. Some of these uncertainties could be answered in future pooling projects.

References

- Agudo A, Cabrera L, Amiano P, et al. Fruit and vegetable intakes, dietary antioxidant nutrients, and total mortality in Spanish adults: findings from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). *Am J Clin Nutr* 2007;85:1634-42.
- Albert, C. M. Cook NR, Gaziano JM, et al. effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. *JAMA* 2008;299:2027–2036.
- Anderson JW, Allgood LD, Lawrence A, et al. Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: meta analysis of 8 controlled trials. *Am J Clin Nutr* 2000;71:472–479
- Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* 1997;336:1117–1124.
- Aviram M, Dornfeld L, Kaplan M, et al. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. *Drugs Exp Clin Res.* 2002;28(2-3):49-62.
- Bazzano LA, He J, Ogden LG, et al. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutrition examination survey epidemiologic follow-up study. *Am J Clin Nutr.* 2002;76:93–9.
- Bazzano, L. A., Li, T. Y., Joshipura, K. J., Hu, F. B. intake of fruit, vegetables, and fruit juices and risk of diabetes in women. *Diabetes Care* 2008;31:1311–1317.
- Bazzano LA, Serdula MK, Liu S. Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Curr Atheroscler Rep.* 2003;5:492–9.
- Berry SE, Mulla UZ, Chowienzyk PJ, et al. Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomized controlled trial. *Br J Nutr* 2010;104:1839–1847.
- Borel P. Genetic variations involved in inter-individual variability in carotenoid status. *Mol Nutr Food Res* 2012;56:228–240.
- Brighenti F, Valtuena S, Pellegrini N, et al. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. *Br J Nutr* 2005;93(5):619–25.
- Broekmans, W. M. Klöpping-Ketelaars WA, Kluft C, et al. Fruit and vegetables and cardiovascular risk profile: a diet controlled intervention study. *Eur. J. Clin. Nutr.* 2001;55:636–642
- Brunner, E. J., Thorogood, M., Rees, K., Hewitt, G. Dietary advice for reducing cardiovascular risk: Cochrane systematic review. *Int. J. Epidemiol.* 2006;35:538–540

Burr, M. L. secondary prevention of CHD in UK men: the Diet and reinfarction Trial and its sequel. *Proc. Nutr. Soc.* 2007;66:9-15.

Burr, M. L. Ashfield-Watt PA, Dunstan FD, et al. Lack of benefit of dietary advice to men with angina: results of a controlled trial. *Eur. J. Clin. Nutr.* 2003;57:193–200

Carter P, Gray LJ, Troughton J, et al. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *BMJ* 2010;341:c4229.

Casas R, Sacanella E, Urpí-Sardà M, et al. The effects of the mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease. A randomized trial. *PLoS One.* 2014;12;9(6):e100084.

Castenmiller, J. J.M., West, C. E., Linssen, J. P.H., et al. The food matrix of spinach is a limiting factor in determining the bioavailability of β -carotene and to a lesser extent of lutein in humans. *J. Nutr.* 1999;129:349–355.

Chen H, Karne RJ, Hall G, et al. High-dose oral vitamin C partially replenishes vitamin C levels in patients with type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. *Am J Physiol Heart Circ Physiol.* 2006;290:H137–H145.

Colditz G, Manson J, Stampfer M, et al.: Diet and risk of clinical diabetes in women. *Am J Clin Nutr* 1992, 55:1018–1023.

Committee on Diet and Health, National Research Council. *Diet and Health: Implications for Reducing Chronic Disease Risk.* National Academy Press, Washington DC, 1989

Cook, N. Albert CM, Gaziano JM, et al. A randomized factorial trial of vitamins C and e and beta carotene in the secondary prevention of cardiovascular events in women: results from the women's Antioxidant Cardiovascular study. *Arch. Intern. Med.* 2007;167:1610–1618.

Cooper AJ, Forouhi NG, Ye Z, et al. Fruit and vegetable intake and type 2 diabetes: EPIC-InterAct prospective study and meta-analysis. *Eur J Clin Nutr* 2012;66:1082–1092.

Crews, H., Alink, G., Anderson, R., et al. A critical assessment of some biomarker approaches linked with dietary intake. *Br. J. Nutr.* 2001;86(Suppl 1): S5–S35.

Dauchet L, Amouyel P, Dallongeville J. Fruit and vegetable consumption and risk of stroke: a meta-analysis of cohort studies. *Neurology.* 2005;65:1193–7.

Dauchet L, Amouyel P, Dallongeville J. Fruits, vegetables and coronary heart disease. *Nat Rev Cardiol* 2009;6:599–608

Dauchet, L., Amouyel, P., Hercberg, S., Dallongeville, J. Fruit and vegetable consumption and risk of coronary heart disease: a metaanalysis of cohort studies. *J. Nutr.* 2006;136:2588–2593

- Dauchet L, Ferrieres J, Arveiler D, et al. Frequency of fruit and vegetable consumption and coronary heart disease in France and Northern Ireland: the PRIME study. *Br J Nutr* 2004; 92:963–972
- de Lorgeril, M, Salen P, Martin JL, et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart study. *Circulation* 1999;99:779–785.
- De Waart FG, Schouten EG, Stalenhoef AFH, Kok FJ. Serum carotenoids, {alpha}-tocopherol and mortality risk in a prospective study among Dutch elderly. *Int J Epidemiol*. 2001;30(1):136-43.
- Din R. Diet and cardiovascular disease. *Am J Public Health*. 2002;92(7):1050-1.
- Epstein LH, Gordy CC, Raynor HA, et al. Increasing fruit and vegetable intake and decreasing fat and sugar intake in families at risk for childhood obesity. *Obes Res* 2001;9:171–178.
- Estruch R, Martí´nez-Gonza´lez MA, Corella D, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med* 2006;145:1–11.
- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285: 2486–2497.
- Fehily AM, Yarnell JW, Sweetnam PM, Elwood PC. Diet and incident ischaemic heart disease: the Caerphilly Study. *Br J Nutr*. 1993;69(2):303-14.
- Ferrari R, Guardigli G, Mele D, et al. Oxidative stress during myocardial ischaemia and heart failure. *Curr Pharm Des* 2004;10:1699-1711
- Ford ES, Mokdad AH. Fruit and vegetable consumption and diabetes mellitus incidence among U.S. adults. *Prev Med* 2001, 32:33–39.
- Fraser GE, Sabaté J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. *Arch Intern Med*. 1992;152(7):1416-24.
- Fung T, Willett W, Stampfer M, et al. dietary pattern and risk of coronary heart disease in women. *Arch Intern Med* 2001;161:1857-62
- Gale C R, Martyn C N, Winter P, Cooper C. Vitamin C and risk of death from stroke and coronary heart disease in a cohort of elderly people. *Br Med J* 1995; 310: 1563–66.
- Gaziano J M, Manson J E, Branch L G, et al. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 1995; 5:255–60
- Genkinger JM, Platz EA, Hoffman SC, et al. Fruit, vegetable, and antioxidant intake and all-cause, cancer, and cardiovascular disease mortality in a community-dwelling population in Washington County, Maryland. *Am J Epidemiol*. 2004;160(12):1223-33.

- Gillman M W, Cupples L A, Gagnon D et al. Protective effect of fruits and vegetables on development of stroke in men. *JAMA* 1995; 273: 1113–17
- Greenberg E R, Sporn M B. Antioxidant vitamins, cancer, and cardiovascular disease. *N Engl J Med* 1996; 334: 1189–90.
- Hall JN, Moore S, Harper SB et al. Global variability in fruit and vegetable consumption. *Am J Prev Med* 2009;36:402–409.e5.
- He FJ, Nowson CA, MacGregor GA. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet*. 2006;367:320–6.
- He, F. J., Nowson, C. A., Lucas, M., MacGregor, G. A. increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. *J. Hum. Hypertens.* 2007;21(9):717-28
- Heidemann C, Schulze MB, Franco OH, et al. Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all-causes in a prospective cohort of women. *Circulation* 2008;118(3):230–7.
- Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with β -carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 1996;334:1145–9.
- Henry RR, Crapo PA, Thorburn AW. Current issues in fructose metabolism. *Annu Rev Nutr* 1991;11:21-39.
- Hertog M G L, Feskens E J M, Hollman P C H, et al. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993; 342: 1007–11
- Hirvonen T, Pietinen P, Virtanen M, et al. Intake of flavonols and flavones and risk of coronary heart disease in male smokers. *Epidemiology*. 2001;12:62–7
- Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr* 2009;90:1–10
- Horner, N. K., Patterson, R. E., Neuhouser, M. L., et al. Participant characteristics associated with errors in self-reported energy intake from the Women’s Health Initiative food frequency questionnaire. *Am. J. Clin. Nutr.* 2002;76:766–773.
- Hornero-Me’ndez D, Mi’nguez-Mosquera MI. Bioaccessibility of carotenes from carrots: Effect of cooking and addition of oil. *Innovat Food Sci Emerg Tech* 2007;8:407–412
- Hornick BA, Weiss L. Comparative nutrient analysis of commonly consumed vegetables. *Nutr Today*. 2011;46:130–7
- Howard BV, Van Horn L, Hsia J, et al. Low-fat dietary pattern and risk of cardiovascular disease: the women’s Health initiative randomized Controlled Dietary Modification Trial. *JAMA* 2006a;295:655–666.

Howard BV, Manson JE, Stefanick ML, et al. Low-fat dietary pattern and weight change over 7 years: the women's Health initiative Dietary Modification Trial. *JAMA*. 2006b;295:39–49.

Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002;13:3-9

Hu F, Rimm E, Stampfer M, et al. prospective study of major dietary patterns and risk of coronary heart disease in men. *Am J Clin Nutr* 2000;72:912-21

Huang, H. Y. Caballero B, Chang S, et al. The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a National institutes of Health state-of-the-science conference. *Ann. Intern. Med.* 2006;145:372–385.

Hung HC, Joshipura KJ, Jiang R, et al. Fruit and vegetable intake and risk of major chronic disease. *J Natl Cancer Inst* 2004;96(21):1577–84.

Ingester LM, Feinleib M. Could salicylates in food have contributed to the decline in cardiovascular disease mortality? A new hypothesis. *Am JPublic Health*. 1997;87: 1554-1557.

Janssen PLTMK, Hollman PCH, Reichman E, et al. Urinary salicylate excretion in subjects eating a variety of diets shows that amounts of bioavailable salicylates in food are low. *Am J Clin Nutr*. 1996;64:743-747.

Jenkins DJ, Kendall CW, Faulkner D, et al. A dietary portfolio approach to cholesterol reduction: combined effects of plant sterols, vegetable proteins, and viscous fibers in hypercholesterolemia. *Metabolism* 2002;51:1596–1604

John, J. H., Ziebland, S., Yudkin, P., et al. Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. *Lancet* 2002;359:1969–1974 .

Joshipura KJ, Hu FB, Manson JE, et al. The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann Intern Med*. 2001;134:1106–14

Kelly JH Jr, Sabate J. Nuts and coronary heart disease: an epidemiological perspective. *Br J Nutr* 2006;96(suppl):S61–7.

Key TJ, Thorogood M, Appleby PN, et al. Dietary habits and mortality in 11,000 vegetarians and health conscious people: results of a 17 year follow up. *BMJ* 1996;313:775–9

Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ*. 1996;312:478-81.

Knekt P, Reunanen A, Järvinen R, et al. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 1994;139:1180–9.

Knekt, P. Ritz J, Pereira MA, et al. Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am. J. Clin. Nutr.* 2004;80:1508–1520.

- Kok F, de Bruijn A, Vermeeren R, et al. Serum selenium, vitamin antioxidants, and cardiovascular mortality: a 9-year follow-up study in the Netherlands. *Am J Clin Nutr* 1987;45:462–8.
- Kopsell, D. A. and Lefsrud, M. G. Spinach cultivar variation for tissue carotenoid concentrations influences human serum carotenoid levels and macular pigment optical density following a 12-week dietary intervention. *J. Agric. Food Chem.* 2006;54:7998–8005.
- Krauss RM, Eckel RH, Howard B, et al. AHA Dietary Guidelines, revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation.* 2000;102(18):2284-99.
- Kromhout D. ‘Protective nutrients’ and up-to-date dietary recommendations. *Eur Heart J.* 2001;Suppl 3:D33-6.
- Kurilich, A. C., and Juvik, J. A. Quantification of carotenoids and tocopherol antioxidants in *Zea mays*. *J. Agric. Food Chem.* 1999;47:1948–1955.
- Kushi L H, Folsom A R, Prinaes R J, et al. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 1996; 334: 1156–62
- Lawlor, D. A., Davey, S. G., Kundu, D., et al. Those confounded vitamins: what can we learn from the differences between observational versus randomised trial evidence? *Lancet* 2004;363:1724–1727.
- Lee, R. D., and Nieman, D. C. *Nutritional Assessments*, 3rd Edition. McGraw-Hill, New York, 2003.
- Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135–43.
- Lidder S & Webb AJ. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. *Br J Clin Pharmacol* 2013;75:677–696.
- Liu S, Lee IM, Ajani U, et al. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: The Physicians’ Health Study. *Int J Epidemiol.* 2001;30(1):130-5.
- Liu S, Manson JE, Lee IM, et al. Fruit and vegetable intake and risk of cardiovascular disease: the women’s health study. *Am J Clin Nutr.* 2000a;72:922–8.
- Liu S, Willett WC, Manson JE, et al. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am J Clin Nutr.* 2003;78(5):920-7.

- Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake and risk of coronary heart disease in US women. *Am J Clin Nutr* 2000b;71:1455–1461.
- Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. *Am J Clin Nutr* 1996;64:190–6.
- Lunn J, Buttriss JL. Carbohydrates and dietary fibre. *Nutr Bull* 2007;32:21–64.
- Mann JI, Appleby PN, Key TJ, Thorogood M. Dietary determinants of ischaemic heart disease in health conscious individuals. *Heart* 1997; 78: 450–455.
- Marchioli, R., schweiger, C., Levantesi, G., et al. Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. *Lipids*. 2001;36(Suppl.):s53–s63.
- McCall DO, McGartland CP, McKinley MC, et al. Dietary intake of fruits and vegetables improves microvascular function in hypertensive subjects in a dosed dependent manner. *Circulation* 2009;119:2153–2160.
- Mellen PB, Walsh TF, Herrington DM. Whole grain intake and cardiovascular disease: a meta-analysis. *Nutr Metab Cardiovasc Dis* 2008;18:283–90.
- Melse-Boonstra A, West CE, Katan MB, et al. Bioavailability of heptaglutamyl relative to monoglutamyl folic acid in healthy adults. *Am J Clin Nutr* 2004;79: 424–429
- Meyer KA, Kushi LH, Jacobs DR Jr, et al. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. *Am J Clin Nutr* 2000;71:921–930.
- Milenkovic D, Deval C, Dubray C, et al. Hesperidin displays relevant role in the nutrigenomic effect of orange juice on blood leukocytes in human volunteers: a randomized controlled cross-over study. *PLoS One* 2011;6(11):e26669
- Moyer V. Vitamin, Mineral, and Multivitamin Supplements for the Primary Prevention of Cardiovascular Disease and Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med*. 2014;160:558-564
- Ness AR, Ashfield-Watt PA, Whiting JM, et al. The long-term effect of dietary advice on the diet of men with angina: the diet and angina randomized trial. *J. Hum. Nutr. Diet* 2004;17:117–119
- Ness AR & Powles, JW. Fruit and vegetables, and cardiovascular disease: a review. *Int. J. Epidemiol.* 1997;26:1–13.
- Ommen GS, Goodman GE, Thomquist MD, et al. Effects of a combination of β -carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 1996;334:1150–5.
- Padayatty SJ, Katz A, Wang Y, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J Am Coll Nutr.* 2003;22(1):18-35.

- Pan XR, Li GW, Hu YH, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing iGT and Diabetes study. *Diabetes Care* 1997;20:537–544.
- Pandey D K, Shekelle R, Selwyn B J, et al. Dietary vitamin C and b-carotene and risk of death in middle-aged men: The Western Electric study. *Am J Epidemiol* 1995; 142: 1269–78
- Pereira MA, Ludwig DS. Dietary fiber and body-weight regulation. Observations and mechanisms. *Pediatr Clin North Am* 2001;48:969–980
- Pereira MA, O'Reilly E, Augustsson K, et al. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Arch Intern Med.* 2004;164:370–6.
- Rao AV, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. *Nutr Res* 1999;19:305–323.
- Rimm EB, Ascherio A, Giovannucci E, et al. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *JAMA.* 1996;275:447-51.
- Rimm E B, Stampfer M J, Ascherio A, et al. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; 328:1450–56.
- Rowe P M. Beta-carotene takes a collective beating. *Lancet* 1996; 347: 249.
- Ruiz-Rodriguez A, Mari'n FR, Ocaña A, Soler-Rivas C. Effect of domestic processing on bioactive compounds. *Phytochemistry Rev* 2008;7:345–384.
- Ruxton CH, Gardner EJ, Walker D. Can pure fruit and vegetable juices protect against cancer and cardiovascular disease too? A review of the evidence. *Int J Food Sci Nutr* 2006;57: 249–272
- Sacks FM, Svetkey LP, Vollmer WM, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. *N Engl J Med* 2001;344:3–10.
- Samman S, Sivaraman G, Man JC, et al. A mixed fruit and vegetable concentrate increases plasma antioxidant vitamins and folate and lowers plasma homocysteine in men. *J Nutr.* 2003;133(7):2188-93.
- Sanchez-Moreno C, Jimenez-Escrig A, Saura-Calixto F. Study of low-density lipoprotein oxidizability indexes to measure the antioxidant activity of dietary polyphenols. *Nutr Res* 2000;20(7):941–53
- Sargeant LA, Khaw KT, Bingham S, et al. Fruit and vegetable intake and population glycosylated haemoglobin levels: the EPIC-Norfolk Study. *Eur J Clin Nutr.* 2001;55(5):342-8.
- Satija A & Hu F. Cardiovascular Benefits of Dietary Fiber. *Curr Atheroscler Rep* 2012;14:505–514

- Sesso HD, Buring JE, Christen WG, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health study ii randomized controlled trial. *JAMA* 2008;300:2123–2133.
- Singh R, Rastogi S, Verman R, et al. Randomized controlled trial of cardioprotective diet in patients with recent acute myocardial infarction: results of one year follow up. *BMJ* 1992;304:1015-9
- Slavin JL, Martini MC, Jacobs DR Jr, Marquart L. Plausible mechanisms for the protectiveness of whole grains. *Am J Clin Nutr* 1999;70(Suppl 3):459-63S.
- Smith CE & Tucker KL. Health benefits of cereal fibre: a review of clinical trials. *Nutr Res Rev* 2011;15:1–14.
- Song W, Derito CM, Liu KM, et al. Cellular antioxidant activity of common vegetables. *J Agric Food Chem* 2010;58:6621–9.
- Steffen LM, Jacobs DR Jr, Stevens J, et al. Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr*. 2003;78(3):383-90.
- Steinmetz K A, Potter J D. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control* 1991; 2: 325–57.
- Stephens NG, Parsons A, Schofield PM, et al. Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996;347:154–60.
- Stephoe A, Perkins-Porras L, McKay C, et al. Behavioural counselling to increase consumption of fruit and vegetables in low income adults: randomised trial. *BMJ*. 2003;326(7394):855.
- Swanson JE, Laine DC, Thomas W, Bantle JP. Metabolic effects of dietary fructose in healthy subjects. *Am J Clin Nutr* 1992;55:851–856
- Timpson NJ, Forouhi NG, Brion MJ, et al. Genetic variation at the SLC23A1 locus is associated with circulating concentrations of L-ascorbic acid (vitamin C): evidence from 5 independent studies with >15 000 participants. *Am J Clin Nutr* 2010;92:375–382.
- Thompson, GR & Grundy, SM. History and development of plant sterol and stanol esters for cholesterol-lowering purposes. *Am. J. Cardiol*. 2005;96:3D–9D.
- Tohill, B. C. Dietary intake of fruit and vegetables and management of body weight. World Health Organization, 2005 [online],
http://www.who.int/dietphysicalactivity/publications/f&v_weight_management.pdf
- Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 2003;348:2599-608.

- Tucker KL, Hallfrisch J, Qiao N, et al. The combination of high fruit and vegetable and low saturated fat intakes is more protective against mortality in aging men than is either alone: the Baltimore longitudinal study of aging. *J Nutr.* 2005;135:556–61.
- Tuomilehto, J. Lindström J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.* 2001;344:1343–1350.
- Van Duyn MA, Pivonka E. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *J Am Diet Assoc.* 2000;100:1511–21.
- Vinson JA, Liang X, Proch J, et al. Polyphenol antioxidants in citrus juices: in vitro and in vivo studies relevant to heart disease. *Adv Exp Med Biol.* 2002;505:113-22.
- Wang X, Ouyang Y, Liu J, et al. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* 2014;29(349):g4490
- Wannamethee SG, Lowe GD, Rumley A, et al. Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. *Am J Clin Nutr.* 2006;83(3):567-74.
- Willett W. *Nutritional Epidemiology.* New York: Oxford University Press, 1990
- WHO MONICA project. Ecological analysis of the association between mortality and major risk factors of cardiovascular disease. *Int J Epidemiol* 1994; 23: 505–16.
- Witztum J L. The oxidation hypothesis of atherosclerosis. *Lancet* 1994; 344: 793–95.
- Wolfe KL, Kang XM, He XJ, et al. Cellular antioxidant activity of common fruits. *J Agric Food Chem* 2008;56:8418–26.
- World Health Organization. Report of a joint WHO/FAO Expert Consultation. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser* 2003;916:1-149.
- United States Department of Agriculture and United States Department of Health and Human Services. *Dietary Guidelines for Americans 2010.* 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010. <http://www.dietaryguidelines.gov>.
- Villegas R, Shu X, Gao Y, et al. vegetable but not fruit consumption reduces the risk of type 2 diabetes in Chinese women. *J. Nutr.* 2008;138:574–580.
- Vinson, J.A., Dabbagh, Y.A., Serry, M.M., Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *J. Agric. Food Chem.* 1995;43: 2800–2802.
- Vivekananthan, D. P., Penn, M. S., Sapp, S. K., et al. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 2003;36:2017–2023.

Yamada T, Hayasaka S, Shibata Y, et al. Frequency of citrus fruit intake is associated with the incidence of cardiovascular disease: the Jichi Medical School cohort study. *J Epidemiol.* 2011;21(3):169-75.

Ye, Z. & Song, H. Antioxidant vitamins intake and the risk of coronary heart disease: meta-analysis of cohort studies. *Eur. J. Cardiovasc. Prev. Rehabil.* 2008;15:26–34.

Ylonen K, Alfthan G, Groop L, et al. Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. *Am J Clin Nutr* 2003;77:1434–1441

Yochum LA, Folsom AR, Kushi LH. Intake of antioxidant vitamins and risk of death from stroke in postmenopausal women. *Am J Clin Nutr* 2000;72:476–83

Yusuf S, Dagenais G, Pogue J, et al. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 2000;342:154–60.

Zhang W, Iso H, Ohira T, et al. Associations of dietary magnesium intake with mortality from cardiovascular disease: the JACC study. *Atherosclerosis* 2012;221:587-95.

Table (1): Summary of prospective studies on the association between fruits and vegetables consumption and CVD

Study	Study cohort	Exposure measure	Follow-up period	Main findings	Reference
The Adventist Health Study	Prospective cohort investigation of 31,208 non-Hispanic white California Seventh-Day Adventists	65 item FFQ	6years	No associations between CVD risk with fruit index or legumes	Fraser et al.,1992
The Caerphilly Study	Prospective Ischaemic Heart Disease Study of a sample of 2512 men aged 45-59 years	FFQ	5years	There was a trend of increasing IHD risk with decreasing vitamin C intake, the relative odds of an IHD event being 1.6 among men in the lowest one-fifth of the vitamin C distribution, but this was not statistically significant	Fehily et al.,1993
Health Professional Follow Up Study	39,910 U.S. male health professionals 40 to 75 years of age	131 item FFQ	4years	Evidence of an association between a high intake of vitamin E and a lower risk of coronary heart disease in men	Rimm et al.,1993
The Zutphen Elderly Study	805 men aged 65-84 years	cross-check dietary history	5years	<ul style="list-style-type: none"> • Flavonoids in regularly consumed foods may reduce the risk of death from CHD in elderly men • Intakes of tea, onions, and apples were also inversely related to coronary heart disease mortality, but these associations were weaker 	Hertog et al.,1993
Longitudinal population study in Finland	A cohort of 5,133 Finnish men and women aged 30-69 years and free from heart disease at baseline	dietary history	14years	Antioxidant vitamins protect against CHD, but it could not exclude possibility that foods rich in these micronutrients also contain other constituents that provide the protection	Knekt et al.,1994
The Massachusetts	1299 elderly living in	43category FFQ	4.75year	Increased dietary intake of carotenoids decreases the	Gaziano et al.,1995

Health Care Panel Study	Massachusetts residents			risks of CVD mortality; however, confounding cannot be ruled out	
The Department of Health and Social Security nutritional survey	Elderly people living in the community who had taken part in 8 areas in Britain	7day weighed record	20year	Vitamin C concentration in elderly people is strongly related to subsequent risk of death from stroke but not from CHD	Gale et al.,1995
Framingham Study	832 men, aged 45 through 65 years, who were free of cardiovascular disease at baseline	24hr recall	20year	Intake of fruits and vegetables may protect against development of stroke in men	Gillman et al.,1995
The Western Electric study	1,556 employed, middle-aged men, aged 40-55 years	Diet history	24year	Consumption of foods rich in vitamin C and beta-carotene reduces risk of death in middle-aged men	Pandey et al.,1995
Postmenopausal women	34,486 postmenopausal women with no cardiovascular disease	127item FFQ	7year	<ul style="list-style-type: none"> • In postmenopausal women the intake of vitamin E from food is inversely associated with CHD mortality and that such women can lower their risk without using vitamin supplements • The intake of vitamins A and C was not associated with lower risks of CHD mortality 	Kushi et al.,1996
Longitudinal population study in Finland	A cohort of 5,133 Finnish men and women aged 30-69 years and free from heart disease at baseline	dietary history	14years	People with very low intakes of flavonoids have higher risks of CHD	Knekt et al.,1996
Prospective observation of vegetarians, semi-vegetarians, and meat eaters in UK	10,802 men and women in the UK aged between 16 and 79		13.3year	<ul style="list-style-type: none"> • Deleterious effects of saturated animal fat and dietary cholesterol are more important in the aetiology of IHD than the protective effect of dietary fibre. • Reduced intakes of saturated animal fat and cholesterol may explain lower rates of IHD 	Mann et al.,1997

				among vegetarians compared with meat eaters	
The Women's Health Study	39876 female health professionals with no previous history of CVD or cancer	FFQ	5year	Higher intake of fruit and vegetables may be protective against CVD and support current dietary guidelines to increase fruit and vegetable intake.	Liu et al.,2000a
A combined analysis of the Nurses' Health Study and the Health Professionals' Follow-up Study	<ul style="list-style-type: none"> • Nurses' Health Study: 84 251 women 34 to 59 years of age. All were free of diagnosed CVD, cancer, and diabetes at baseline. • Health Professionals' Follow-Up Study: 42 148 men 40 to 75 years. All were free of diagnosed CVD, cancer, and diabetes at baseline. 	FFQ FFQ	NHS: 14year HPFS: 8year	Consumption of fruits and vegetables, particularly green leafy vegetables and vitamin C-rich fruits and vegetables, appears to have a protective effect against CHD	Joshiyura et al.,2001
The Physicians' Health Study	22 071 US male physicians aged 40-84 years	FFQ	12year	An inverse association between vegetable intake and risk of CHD	Liu et al.,2001
Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study in Finland	27,110 male smokers, aged 50-69 years, without history of cancer	FFQ	6.1-year	Intake of flavonols and flavones seemed to be inversely associated with the risk of lung cancer, but not with that of other cancers	Hirvonen et al.,2001
The first national health and nutrition examination survey (NHANES-I) follow-up study	9608 adults aged 25-74 years participating free of CVD at the time of their baseline	FFQ	19year	An inverse association of fruit and vegetable intake with the risk of CVD and all-cause mortality in the general US population	Bazzano et al.,2002
The atherosclerosis risk in	15,792 adults aged 45-64 years participating free	FFQ	11year	A beneficial effect of whole-grain and fruit and vegetable consumption on	Steffen et al.,2003

communities (ARIC) study	of CAD at the time of their baseline			the risks of total mortality and incident CAD was suggested but not on the risk of ischemic stroke	
The Prospective Epidemiological Study of Myocardial Infarction (PRIME) study	men aged 50-59 years, free of CHD, who were recruited in France (n 5982) and Northern Ireland (n 2105)	FFQ	5year	Frequency of citrus fruit, but not other fruits, intake is associated with lower rates of acute coronary events in both France and Northern Ireland, suggesting that geographical or related factors might affect the relationship between fruit consumption and CHD risk	Dauchet et al.,2004
The Odyssey Cohort	8,394 residents in Washington County, Maryland	61item FFQ	15year	Participants with the highest quintile of fruit and vegetable intake had a lower risk of all-cause mortality and CVD mortality than those in the lowest quintile	Genkinger et al.,2004
A combined analysis of the Nurses' Health Study and the Health Professionals' Follow-up Study	Over 100000 participants in the Nurses' Health Study and the Health Professionals' Follow-up Study	FFQ		Fruit and vegetable intake was inversely associated with risk of CVD, with relative risk for an increment of 5 servings daily of 0.88 (95% CI = 0.81 to 0.95), and green leafy vegetables showed the strongest inverse association	Hung et al.,2004
The Baltimore longitudinal study of aging	501 healthy men at baseline	7day diet records	18year	<ul style="list-style-type: none"> • Protective effects of low saturated fat and high fruits and vegetables intake against CHD mortality. • The combination of both behaviors is more protective than either alone, suggesting that their beneficial effects are mediated by different mechanisms. 	Tucker et al.,2005
Nurses' Health Study	72 113 women who were free of CVD and cancer at baseline	FFQ	18years	A more prudent diet containing high intakes of fruits and vegetables was associated with a reduced risk of	Heidemann et al.,2008

				cardiovascular mortality and a reduced risk of all-cause mortality when comparing individuals in the highest to the lowest quintile of diet prudency.	
The Jichi Medical School cohort study	10,623 participants (4147 men, 6476 women) who had no history of CVD or carcinoma at baseline	5 category FFQ	10.7 years	Frequent intake of citrus fruit may reduce the incidence of CVD, especially cerebral infarction, in men and women	Yamada et al.,2011

CAD: coronary artery disease, CHD: coronary heart disease, IHD: ischemic heart disease, CVD: cardiovascular disease, FFQ: food frequency questionnaire

Table (2): Shortcomings of epidemiological studies

Study design	Description	Limitation
Ecological studies	To explore relationships between environmental factors and diseases at a population level	<ul style="list-style-type: none"> • Do not provide precise information about individuals in a population • Subject to many uncontrolled biases
Case-control studies	Individuals diagnosed with disease are compared with disease-free individuals	<ul style="list-style-type: none"> • Rely on retrospective assessment of dietary intake by questionnaire • Subject to selection and recall bias and potential bias due to the changes in diet and lifestyle following CVD events
Cohort studies	The exposure of people who are initially assumed to be healthy are assessed and the group is followed over a period of time. During the follow-up period, some individuals will develop disease	<ul style="list-style-type: none"> • Measurements are made at baseline before disease is diagnosed, but diet may change with time • Cohort studies are less subject to recall bias
Randomized controlled trials	The participants are allocated to treatment/ intervention or control/ placebo groups using a random mechanism. Best for studying the effect of an intervention	<ul style="list-style-type: none"> • Expensive in terms of time and money • Often short duration with less power to detect clinical outcomes • Subject to volunteer bias • Ethically problematic