

Micronutrients: dietary intake *v.* supplement use

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Whilst clinical deficiency of micronutrients is uncommon in the developed world, a suboptimal intake of certain micronutrients has been linked with an increased risk of chronic diseases such as CVD and cancer. Attention has therefore focused on increasing micronutrient status in order to theoretically reduce chronic disease risk. Increasing micronutrient status can involve a number of approaches: increasing dietary intake of micronutrient-rich foods; food fortification; use of supplements. Observational cohort studies have demonstrated an association between high intakes of micronutrients such as vitamin E, vitamin C, folic acid and β -carotene, and lower risk of CHD, stroke and cancer at various sites. However, randomised intervention trials of micronutrient supplements have, to date, largely failed to show an improvement in clinical end points. The discordance between data from cohort studies and the results so far available from clinical trials remains to be explained. One reason may be that the complex mixture of micronutrients found, for example, in a diet high in fruit and vegetables may be more effective than large doses of a small number of micronutrients, and therefore that intervention studies that use single micronutrient supplements are unlikely to produce a lowering of disease risk. Studies concentrating on whole foods (e.g. fruit and vegetables) or diet pattern (e.g. Mediterranean diet pattern) may be more effective in demonstrating an effect on clinical end points. The present review will consider the clinical trial evidence for a beneficial effect of micronutrient supplements on health, and review the alternative approaches to the study of dietary intake of micronutrients.

Micronutrients: Disease: Supplementation: Dietary intake

Clinical deficiency *v.* suboptimal status of micronutrients

A micronutrient is defined as a substance needed only in small amounts for normal body function. Micronutrient deficiencies are an important cause of malnutrition and associated ill health in the developing world. Adding single or multiple nutrients to the food chain has been shown to be of great benefit in terms of combating clinical deficiency (Caballero, 2003). For example, vitamin A supplementation and fortification of table salt with I have both shown enormous health impact in several populations (Sommer *et al.* 1986), while a recent trial in Tanzania suggests that a multivitamin supplement (thiamin, riboflavin, pyridoxine, niacin, cobalamin, folate and vitamins C and E) may delay the progression of HIV disease (Fawzi *et al.* 2004; Lanzillotti & Tang, 2005). Clinical deficiency of micronutrients is uncommon in the developed world, but interest has increasingly focused on non-clinical

deficiencies, or suboptimal status of micronutrients and the effect such deficiencies may have on risk of chronic disease. Suboptimal status of micronutrients such as vitamins C and E and folate has been proposed to play a role in the development of CVD, cancer at various sites, chronic renal failure and age-related macular degeneration (Fairfield & Fletcher, 2002).

Increased dietary intake *v.* fortification *v.* supplementation

Correcting micronutrient deficiencies can be approached in a number of ways. Programmes can be implemented that are designed to encourage individuals to consume more micronutrient-rich foods, commonly-eaten foods can be fortified with micronutrients or the use of micronutrient supplements can be encouraged (Caballero, 2003). Each of these approaches has advantages and disadvantages, and

Abbreviation: Hcy, homocysteine.

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their success depends on the specific population, the deficient micronutrient and the extent of deficiency. For the purposes of the present review the focus will be on the developed world, on the general population and the evidence linking suboptimal micronutrient status and chronic disease risk.

Epidemiological evidence linking micronutrient status with disease

A wealth of observational studies has examined the link between micronutrient status and risk of chronic disease (for review, see Fairfield & Fletcher, 2002).

For example, two large longitudinal studies in the USA (Rimm *et al.* 1993; Stampfer *et al.* 1993) have examined the association between antioxidant intake and risk of CHD. In a group of 39 910 male health professionals those who took vitamin E supplements in doses of ≥ 100 mg/d for >2 years had a 37% lower relative risk of CHD compared with those who did not take vitamin E supplements, after adjustment for age, coronary risk factors and intake of vitamin C and β -carotene (Rimm *et al.* 1993). In the Nurses' Health Study of 87 245 female nurses participants who took vitamin E supplements for >2 years had a 41% lower relative risk of major coronary disease (Stampfer *et al.* 1993). This effect persisted after adjustment for age, coronary risk factors and use of hormone-replacement therapy, aspirin, vitamin C and β -carotene. High vitamin E intakes from dietary sources were not associated with a marked decrease in risk; even the highest dietary vitamin E intakes were far lower than intakes among supplement users.

Similarly, high β -carotene intake has been associated with a reduced risk of lung cancer (Peto *et al.* 1981), while a high intake of antioxidant vitamins (C and E) has consistently been shown to be associated with a reduced risk of age-related macular degeneration in case-control studies (Hogg & Chakravarthy, 2004).

Finally, numerous studies of folate, homocysteine (Hcy) and CVD have associated an increased intake of folate, and a reduced concentration of Hcy, with a reduced risk of CVD (Rimm *et al.* 1998; Voutilainen *et al.* 2001), and this association has been confirmed by meta-analysis (Boushey *et al.* 1995; Homocysteine Studies Collaboration, 2002; Wald *et al.* 2002).

Clinical trial evidence linking micronutrient status with disease

Clinical trials to assess the effect of micronutrients and disease have largely assessed the effect of micronutrient supplements. The clinical trial evidence for the beneficial effects of supplemental intake in healthy populations, with the purpose of preventing future diseases, is still limited. With the exception of folic acid to prevent neural-tube defects (MRC Vitamin Study Research Group, 1991), the numerous supplementation trials have yielded rather disappointing or conflicting results for most of the nutrients evaluated. The clinical trial evidence will be summarised.

In the last decade the results have been published of a number of prospective randomised placebo-controlled 3–6 year clinical trials that have tested the effect of vitamin E and other antioxidant vitamins or their combinations on the clinical end points of CVD and cancer. These trials have consistently shown that commonly-used antioxidant regimens (vitamins E and C and β -carotene, singly or in combination) do not markedly reduce overall cardiovascular events or cancer (Brown & Crowley, 2005). For example, one of the largest intervention trials, the Heart Protection Study (Heart Protection Study Collaborative Group, 2002), has shown no effect of antioxidant vitamin supplementation (600 mg vitamin E, 250 mg vitamin C and 20 mg β -carotene daily) on any vascular or non-vascular end point in $>20\,000$ subjects. Similarly, a recent study, the SUPplémentation en Vitamines et en Minéraux AntioXydants Study (Zureik *et al.* 2004), has found no effect of low-dose antioxidant supplementation on carotid atherosclerosis and arterial stiffness as assessed by pulse wave velocity. This lack of effect of vitamin E supplementation has been confirmed by meta-analysis (Vivekananthan *et al.* 2003). The recent Heart Outcomes Prevention Evaluation – The Ongoing Outcomes extended follow-up of the Heart Outcomes Prevention Evaluation trial has even suggested an increased risk of heart failure in the vitamin E-supplemented group (Lonn *et al.* 2005). Indeed, a recent meta-analysis has found a significant dose-dependent adverse mortality effect of vitamin E when taken at ≥ 400 mg daily (relative risk 1.04, $P = 0.04$; Miller *et al.* 2005).

The only trial of vitamin E showing unequivocally positive results is the Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease Trial in haemodialysis patients with pre-existing CVD (Boaz *et al.* 2000). This small study (n 196) found that patients in the supplemented group (800 mg vitamin E/d) had a relative risk of 0.46 (95% CI, 0.27–0.78) for a composite end point consisting of myocardial infarction (fatal and non-fatal), ischaemic stroke, peripheral vascular disease (excluding the arterio-venous fistula), and unstable angina. Median follow-up was 519 d. Thus, a beneficial effect of vitamin E supplementation in subjects with end-stage renal failure cannot be excluded.

β -Carotene supplementation has been evaluated in a number of trials examining both cancer and CVD end points. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Trial (Alpha-Tocopherol, Beta Carotene Cancer Prevention Trial Study Group, 1994), conducted among 29 133 male heavy smokers in Finland, found no reduction in CHD morbidity or mortality during 5–8 years of treatment with vitamin E (50 mg daily) or β -carotene (20 mg daily). Those subjects assigned to β -carotene experienced an 11% increase in IHD deaths. Subjects who received β -carotene had a marked increase in lung cancer incidence compared with subjects who received placebo. In a further analysis (Rapola *et al.* 1997) a subgroup of subjects with a history of previous myocardial infarction were considered. The end point of this substudy was the first major coronary event after randomisation. The proportion of major coronary events was not decreased with either α -tocopherol or β -carotene supplements. In fact, β -carotene conferred an

excess of fatal IHD (75% increase in risk). The Beta-Carotene and Retinol Efficacy Trial (Omenn *et al.* 1996), designed to test the effects of a combined supplement of 30 mg β -carotene and 7.5 mg retinol daily among 18 314 cigarette smokers and individuals with occupational asbestos exposure, was also ended early when researchers recognised an elevated risk of death from lung cancer in those receiving β -carotene and, again, no beneficial effect on CVD was found. Both trials have since reported longer-term follow-up of the study participants (Goodman *et al.* 2004; Alpha-Tocopherol, Beta Carotene Cancer Prevention Trial Study Group, 2004), and both cases confirm that high-dose β -carotene supplementation increases the incidence of lung cancer and the incidence of all-cause mortality in smokers.

A recent Cochrane systematic review has shown no effect of vitamin supplementation on gastrointestinal cancer (Bjelakovic *et al.* 2004). The researchers looked at fourteen trials with >170 000 randomised participants. There were ten different interventions and 2100 cancer end points over five sites (oesophagus, stomach, large bowel, pancreas and liver), with interventions and cancer sites considered individually and in combination. The most-highly-aggregated meta-analysis, the effect of all supplement interventions *v.* placebo on all cancers, resulted in a pooled relative risk of 0.96 (95% CI 0.88, 1.04). The only micronutrient for which a protective effect was observed was Se (Bjelakovic *et al.* 2004).

Another specific disorder that may benefit from supplemental antioxidant vitamins is age-related macular degeneration. In a placebo-controlled factorial design trial comparing an antioxidant regimen (400 mg vitamin E, 500 mg vitamin C, 15 mg β -carotene), with Zn (80 mg) and placebo (Age-Related Eye Disease Study Research Group, 2001), subjects with moderate or severe age-related macular degeneration were found to experience reduced risk of progression of the disease and loss of visual acuity at 5 years follow-up in the combined antioxidants and Zn group. Groups with initially mild age-related macular degeneration did not benefit, and the supplements also had no effect on cataract development.

In terms of the effect of antioxidant supplementation on diabetes, several of the clinical trials of vitamin E and CVD have included subjects with diabetes. Subgroup analysis has shown no effect of antioxidant vitamin supplementation in subjects with diabetes (Johansen *et al.* 2005).

Despite the epidemiological evidence linking folate and CVD, as yet there are few intervention studies reporting a reduction in cardiovascular morbidity or mortality with B-vitamin supplementation. Vermeulen *et al.* (2000) have reported a randomised placebo-controlled trial of folic acid and vitamin B₆ among 158 healthy siblings of 167 patients with premature atherothrombotic disease. The primary end point was the development or progression of sub-clinical atherosclerosis as estimated from exercise electrocardiography, the ankle-brachial pressure index and carotid and femoral ultrasonography. B-vitamin supplementation reduces total plasma Hcy and is associated with a marked reduction in abnormalities on exercise electrocardiography, with no effect on other end points. An

editorial accompanying the article (Bostom & Garber, 2000) has queried the use of these surrogate end points, pointing to a lack of sensitivity and specificity. Another study (Schnyder *et al.* 2001) has shown an effect of a combination of folic acid (1 mg), cobalamin (400 mg) and pyridoxine (10 mg) *v.* placebo on restenosis after 6 months in 205 patients after successful coronary angioplasty. Only three published studies (Baker *et al.* 2002; Liem *et al.* 2004; Toole *et al.* 2004) have to date examined the effect of B-vitamin supplementation on hard clinical end points. The Vitamin Intervention for Stroke Prevention trial (Toole *et al.* 2004), which randomised 3680 adults with non-disabling cerebral infarction to daily high-dose B-vitamins (25 mg pyridoxine, 0.4 mg cobalamin, 2.5 mg folic acid) or low-dose B-vitamins (200 mg pyridoxine, 6 mg cobalamin, 20 mg folic acid) for 2 years with good compliance with the intervention, found no difference between the groups in terms of stroke, coronary event or death. Similarly, the Cambridge Heart Antioxidant Study 2 (Baker *et al.* 2002), which examined high-dose folic acid (5 mg/d) in 2000 patients with IHD, with a median follow-up 1.7 years, showed a reduction in non-fatal myocardial infarction, but no reduction in total deaths. The final study (Liem *et al.* 2004) was a pilot study that examined the effect of 5 mg folic acid/d when added to statin therapy on the incidence of recurrent major clinical events up to 1 year post-myocardial infarction. No beneficial additive effects on cardiovascular mortality or morbidity were seen.

Finally, a recent meta-analysis has shown little evidence of a beneficial effect of multivitamin supplementation in preventing infections in the elderly (El-Kadiki & Sutton, 2005).

Contrast between epidemiological and clinical trial evidence

The discordance between data from cohort studies and that from clinical trials is difficult to interpret. Various aspects of the trial design need to be considered. It may be that the duration of clinical trials is too short to show a benefit, and that micronutrient intake over many years is required to prevent disease. Careful thought needs to be given to the design of clinical trials, with dose, duration of treatment and follow-up period, the population's initial dietary micronutrient intake and status, and the extent and distribution of existing disease being taken into consideration. For example, positive animal models have nearly always tested the effects of antioxidants on the early stages of CHD. Whether or not antioxidants have inhibitory effects on the later stages of CHD, which is what has actually been tested in human randomised controlled trials, remains to be seen.

In addition to trial design issues outlined earlier, the biological plausibility of the trials also requires consideration. The significant results linking micronutrient intake with disease risk observed in cohort studies may be related to confounding with other diet and lifestyle behaviours. Slattery *et al.* (1995), examining dietary antioxidants and plasma lipids in the Coronary Artery Risk Development in Young Adults Study, have found that intake of

antioxidants is associated with other lifestyle factors such as physical activity and non-smoking. Serdula *et al.* (1996) have shown that frequency of fruit and vegetable intake increases as physical activity level increases, and that consumption of fruit and vegetables is lower in those who reported being sedentary, heavy smokers or heavy drinkers. Antioxidant intakes are linked with social class, being higher in more affluent groups (Brown, 1963). While these variables can be individually controlled for in analyses, it may be that a complex lifelong behaviour pattern needs to be studied before conclusions are made regarding micronutrients and disease.

In addition, the complex mixture of micronutrients found in a diet high in fruit and vegetables may be more effective than large supplemental doses of a small number of micronutrients. It could be that several of these compounds work together but have no effect individually, or that other dietary components (e.g. trace elements) may be effectors of micronutrient action.

With these criticisms of the randomised controlled trials of micronutrient supplements in mind, a number of alternative approaches to the study of micronutrients and disease have been considered.

Dietary patterns and disease

The series of clinical trials reported earlier have led some researchers to question the wisdom of testing single preventive agents for disease. It has been suggested that, instead of chasing a magic single dietary bullet, it is necessary to show a beneficial effect of the complete dietary pattern before attempting to learn what might have possible causative roles (Leaf, 1999). For example, observational studies of vegetarians support the hypothesis that such a diet might lower the risk of diseases such as obesity, diabetes mellitus, hyperlipidaemia, hypertension, CVD and cancer (Segasothy & Phillips, 1999). Key *et al.* (1999) have combined data from five prospective studies to compare the disease rates of vegetarians with those of non-vegetarians with similar lifestyles. A total of 76 172 men and women (27 808 vegetarians) were included in the analysis (with correction for age, gender and smoking). Mortality from IHD was found to be 24% lower in vegetarians than in non-vegetarians (death rate ratio 0.76 (95% CI 0.62, 0.94); $P < 0.01$). The reduction in mortality was found to be greater at young ages and restricted to those who had followed their current diet for >5 years. No significant differences were observed between vegetarians and non-vegetarians in mortality from cerebrovascular disease, stomach cancer, colo-rectal cancer, lung cancer, breast cancer, prostate cancer or all other cancers combined. The difficulty with studies of vegetarians is that they tend to differ from the rest of the population, i.e. they tend to smoke less, have a lower BMI and a lower alcohol intake and come predominantly from higher social classes, all of which are known to confer a health advantage (Segasothy & Phillips, 1999).

Dietary pattern analysis has recently emerged as an alternative approach in the examination of diet and the risk of chronic disease. Several studies have suggested that

dietary patterns derived from factor or cluster analysis predict disease risk or mortality (Hu, 2002). The technique is based on using information from food-frequency questionnaires or dietary records to identify common underlying patterns of food consumption. It aggregates specific food items or food groups on the basis of the extent to which those items or groups correlate with each other. A summary score for each pattern is then derived, which can be used in correlation or regression analysis with disease outcomes.

An analysis of the Health Professionals Follow-Up Study has looked at overall dietary patterns and their relationship with CHD risk (Hu *et al.* 2000). The authors have identified two major dietary patterns. They call these patterns the 'prudent pattern', which is characterised by higher intake of vegetables, fruit, legumes, whole grains, fish and poultry, and the 'Western pattern', characterised by higher intake of red meat, processed meat, refined grains, sweets and desserts, French fries and high-fat dairy products. In a later publication the same group (Fung *et al.* 2001) have shown that the Western pattern is positively correlated with insulin, C-peptide, leptin and Hcy concentrations, and inversely correlated with plasma folate. The prudent pattern is positively correlated with plasma folate and inversely correlated with insulin and Hcy. After adjustment for age and CHD risk factors, the relative risk for highest quintile of the prudent pattern score was reported to be 0.70 (95% CI 0.56, 0.86), while that for the Western pattern score was 1.64 (95% CI 1.24, 2.17). These associations persisted in subgroup analyses corrected for cigarette smoking, BMI and parental history of myocardial infarction.

Other studies have related dietary patterns to overall mortality (Osler *et al.* 2001), markers of inflammation and endothelial dysfunction (Lopez-Garcia *et al.* 2004), risk factors for CVD (Kerver *et al.* 2003) and bone density (Tucker *et al.* 2002). Studies examining derived dietary patterns and cancer end points have, until recently, been rarer, but a number of recent studies have shown associations between dietary patterns and oral and pharyngeal (de Stefani *et al.* 2005), prostate (Walker *et al.* 2005), colo-rectal (Kim *et al.* 2005) and lung cancers (Balder *et al.* 2005), but no association between dietary patterns and breast (Fung *et al.* 2005) and pancreatic cancers (Michaud *et al.* 2005).

Intervention trials with clinical end points have tended to focus on two factors, fruit and vegetables and the Mediterranean diet. The evidence linking each of these factors with chronic disease will now be examined.

Fruit and vegetables and chronic disease risk

Despite widespread acceptance that the consumption of fruit and vegetables is good for health (Ness *et al.* 1999), formal research on the intake of these foods in relation to disease is surprisingly sparse. The effect of fruit and vegetable intake on CHD has been examined as part of the Nurses' Health Study and the Health Professionals' Follow-Up Study. After adjustment for standard cardiovascular risk factors, individuals in the highest quintile of

fruit and vegetable intake were found to have a relative risk for CHD of 0.80 (95% CI 0.69, 0.93) compared with the lowest intake quintile. Green leafy vegetables and vitamin C-rich fruits and vegetables have been shown to contribute most to the apparent protective effect of total fruit and vegetable intake (Joshiyura *et al.* 2001). The same research group have examined the link between fruit and vegetable intake and ischaemic stroke (Joshiyura *et al.* 1999) in similar cohorts. In this analysis individuals in the highest quintile of fruit and vegetable intake were found to have a relative risk of 0.69 (95% CI 0.52, 0.92) compared with the lowest quintile of intake; moreover, a one serving per d increase in fruit or vegetable intake was shown to be associated with a 6% lower risk for ischaemic stroke, after controlling for standard cardiovascular risk factors. Cruciferous vegetables, green leafy vegetables, citrus fruit including juice and citrus fruit juice were found to contribute most to the apparent protective effect of total fruits and vegetables. Further analysis examined the effect of fruit and vegetable consumption on the incidence of chronic disease (including cancer and CVD; Hung *et al.* 2004). Total fruit and vegetable intake was found to be inversely associated with risk of CVD but not overall cancer incidence, with a relative risk for an increment of five servings daily of 0.88 (95% CI 0.81, 0.95) for CVD and 1.00 (95% CI 0.95, 1.05) for cancer.

Similarly, Liu *et al.* (2000) have shown, using data from the Women's Health Study (39 876 female health professionals), that CVD risk is about 30% lower in women who eat five to ten servings of fruit and vegetables per d than in those who eat 2.5 servings per d. Women with a high fruit and vegetable intake were found to be older, smoke less, drink and exercise more and to be more likely to use postmenopausal hormones or multivitamin supplements. After adjustment for these and other CVD risk factors the risk reduction was reported to be attenuated to approximately 15% and was not statistically significant. In contrast, using National Health and Nutrition Examination Survey data Bazzano *et al.* (2002) have shown a marked reduction in stroke, IHD, CVD and all-cause mortality in those participants who consumed fruit and vegetables at least three times daily as compared with less than once daily, after adjustment for established CVD risk factors.

Lock *et al.* (2005) have estimated the global burden of disease attributable to low consumption of fruit and vegetables. The associations, stratified by fourteen geographical regions, gender and age, were estimated using information on fruit and vegetable consumption in the population and six health outcomes (IHD, stroke, stomach, oesophageal, colo-rectal and lung cancer). The analysis has suggested that 2.6×10^6 deaths worldwide and 31% of the CVD (and between 2 and 19% of the cancers) may be attributed to inadequate consumption of fruit and vegetables. About 1.8% of the total burden of disease worldwide was estimated to be attributable to inadequate fruit and vegetable consumption, compared with 1.3% for physical activity, 2.3% for overweight and obesity, 2.8% for high cholesterol and 4.1% for tobacco (Lock *et al.* 2005).

Only one randomised intervention study has examined the effects of increased fruit and vegetable consumption on a confirmed classical cardiovascular risk factor. John *et al.*

(2002) have examined the effect of advice to encourage an increase in consumption of fruit and vegetables to at least five daily portions on blood pressure after 6 months. Self-reported fruit and vegetable intake was found to have increased by a mean of 1.4 portions in the intervention group and by 0.1 portions in the control group, producing an increase in plasma vitamin C, lutein, β -cryptoxanthin, α - and β -carotene (John *et al.* 2002) and flavonol concentrations (Huxley *et al.* 2004). Systolic and diastolic blood pressure was reported to have fallen more in the intervention group than in controls (differences 4.0 ($P < 0.0001$) and 1.5 ($P = 0.02$) mmHg respectively), a fall which would be expected to reduce CVD in the general population.

A recent study that examined the effect of fruit and vegetables on endothelial function, has shown that adding antioxidant-rich foods (tomatoes, carrots and peppers providing 184 mg vitamin C, 19.65 mg vitamin E, 15 mg β -carotene and 9.2 g fibre) to a high-fat meal partially reduces the endothelial dysfunction induced by the high-fat meal alone (Esposito *et al.* 2003).

A number of studies have looked at the biochemical effects of increasing fruit and vegetable intake, linking the biochemical changes with CVD risk. One study asked subjects with normal lipid concentrations who ate three or fewer servings of fruit and vegetables daily to consume eight servings per d. Plasma vitamin C, α -carotene and β -carotene concentrations were found to have increased after 8 weeks, but concentrations of retinol, α -tocopherol, lipids and lipoproteins remained unchanged, despite some increase in dietary vitamin E and a small reduction in saturated fat intake (Zino *et al.* 1997). By contrast, Singh *et al.* (1992a) have found that the administration of fruit and vegetables to subjects at high risk of CVD for 12 weeks lowers total cholesterol and LDL-cholesterol and triacylglycerols, and increases HDL-cholesterol. Wise *et al.* (1996) gave dehydrated fruit and vegetable extracts to fifteen healthy adults aged 18–53 years for 28 d, which was shown to produce 50–2000-fold increases in plasma carotenoid and tocopherol levels, with plasma lipid peroxides being reduced 4-fold. Chopra *et al.* (2000) have examined the effect of an increased intake of vegetables (by 300–400 g daily) on LDL oxidation in smokers and non-smokers. Supplementation with green vegetables (rich in the carotenoids β -carotene and lutein) was found to have no effect on the resistance of LDL to oxidation in either smokers or non-smokers. After supplementation with red vegetables (rich in lycopene) only non-smokers were found to show an increase in the lag time to LDL oxidation. Dragsted *et al.* (2004) have also shown an increase in plasma lipid oxidation lag times during fruit and vegetable intervention, and an increase in erythrocyte glutathione peroxidase activity, while Cao *et al.* (1998) have shown that an increased consumption of fruit and vegetables (ten servings per d for 15 d) increases plasma antioxidant capacity, as measured by the oxygen radical absorbance-capacity assay.

Fruits and vegetables are complex foods and contain many bioactive components in addition to the antioxidants vitamin C and carotenoids. Flavonoids are plant-derived compounds that can inhibit LDL oxidation both *in vitro*

and *in vivo* (Aviram & Fuhrman, 1998; Nigdikar *et al.* 1998). Many fruits and vegetables, in particular leafy green vegetables, are also good sources of folate. A study by Brouwer *et al.* (1999) has examined the effect of a diet high in citrus fruit and vegetables on folate status and total plasma Hcy levels. Folate levels were found to be increased and total plasma Hcy decreased in those subjects receiving this diet compared with a low-folate diet.

Fruit and vegetable intake combined with a low-fat diet

A number of studies have combined a low-fat diet with increased fruit, vegetable and whole-grain consumption. Recent observational evidence from the Baltimore Longitudinal Study of Ageing (Tucker *et al.* 2005) indicates that, over an average 18 years follow-up, the men who consumed the combination of five or more servings of fruit and vegetables per d and $\leq 12\%$ energy from saturated fat are 31% less likely to die from any cause ($P < 0.05$) and 76% less likely to die from CHD ($P < 0.001$). This combination of high fruit and vegetable and low saturated fat intakes was found to be more protective than either of the interventions alone. This work is backed up by a controlled feeding study in healthy volunteers (Gardner *et al.* 2005) that has shown that a 4-week low-fat diet intervention that also incorporates markedly more vegetables, legumes and whole grains (therefore likely to increase micronutrient status), enhances the lipid-lowering effect of a typical low-fat diet.

Similarly, a clinical trial by Singh *et al.* (1992b) has examined the effect of a low-fat diet, or a low-fat diet with extra fruit, vegetables, nuts and grain products on cardiovascular complications and mortality after acute myocardial infarction. Cardiac events were found to be significantly reduced in the group advised to eat extra fruit, vegetables, nuts and grains after 1-year follow-up ($P < 0.001$).

The Dietary Approaches to Stop Hypertension Study (Appel *et al.* 1997) has also assessed the effect of a fruit and vegetable-rich intervention *v.* a combination diet that is rich in fruit and vegetables and low-fat dairy products, with reduced saturated and total fat, on blood pressure. Whilst the fruit and vegetable-rich intervention was found to lower both systolic and diastolic blood pressure, the effect was smaller than for the combination diet (Appel *et al.* 1997). This effect was found to be improved further with the accompanying restriction of Na intake (Sacks *et al.* 2001).

Mediterranean diet and disease

It has been recognised for some time that there is a lower mortality from CHD in countries bordering the Mediterranean compared with those in Northern Europe (Tunstall-Pedoe *et al.* 1999). Defining a Mediterranean-style diet is challenging, given the broad geographical region and the major cultural, ethnic, religious, economic and agricultural production differences, which result in different dietary practices in these areas. Nevertheless, there is a dietary pattern that is characteristic of Mediterranean-style diets.

This pattern emphasises a diet that is high in fruits, vegetables, bread and other forms of cereals, potatoes, beans, nuts and seeds, and includes: olive oil as an important fat source; dairy products; fish and poultry consumed in low to moderate amounts; eggs consumed zero to four times weekly; little red meat. In addition, wine is consumed in low to moderate amounts with meals. At the core there are many similarities between the American Heart Association Step I and II diets and the Mediterranean-style diet; however, the Mediterranean diet adds specificity in relation to the form and types of fat-containing foods and oils (de Lorgeril *et al.* 1999; Kris-Etherton *et al.* 2001).

The effect of adherence to a Mediterranean diet on longevity has been evaluated in a number of large epidemiological studies. In the Healthy Ageing: a Longitudinal Study in Europe project, adherence to the Mediterranean diet was found to be associated with a lower risk of all-cause mortality (n 2339; hazard ratio 0.77 (95% CI 0.68, 0.88); controlled for age, gender, years of education, BMI and other factors; Knoops *et al.* 2004). Similarly, in the European Prospective Investigation of Cancer study, among 74 607 subjects, a two-unit increase in the modified Mediterranean diet score was shown to be associated with an 8% reduction in mortality (Trichopoulou *et al.* 2005). A similar effect of the Mediterranean diet has been observed in a smaller Greek population (Trichopoulou *et al.* 2003).

Several studies have examined the effects of a Mediterranean-type dietary intervention on intermediate end points such as endothelial function (Fuentes *et al.* 2001; Vogel *et al.* 2000; Sondergaard *et al.* 2003; Ambring *et al.* 2004; Ros *et al.* 2004). Fuentes *et al.* (2001) have studied twenty-two hypercholesterolaemic men using a randomised cross-over design with the subjects following an initial diet high in saturated fat, followed by assignment to either the National Cholesterol Education Program stage 1 diet (low in fat and saturated fat), or a Mediterranean diet. While plasma total cholesterol and LDL-cholesterol, apoB and P-selectin levels were shown to decrease during both the National Cholesterol Education Program stage 1 and Mediterranean diets, the Mediterranean diet was also found to produce an increase in flow-mediated dilatation, suggesting an improvement in endothelial function. It is likely that this effect could have been the result of increased antioxidant intake, based on the suggestion by Vogel *et al.* (2000) that, in terms of their postprandial effect on endothelial function, the beneficial components of the Mediterranean diet appear to be antioxidant-rich foods. Their study has shown that a meal containing olive oil as a fat source reduces brachial artery flow-mediated vasodilation by 31%, but this decrease is reduced by the concomitant administration of vitamins C and E (by 71%) or balsamic vinegar and salad (by 65%).

A recent study has assessed the effect of a Mediterranean-style diet on endothelial function and vascular inflammatory markers in patients with the metabolic syndrome (Esposito *et al.* 2004). After 2 years patients following the Mediterranean-style diet were found to have significantly reduced serum concentrations of high-sensitivity C-reactive protein ($P = 0.01$), IL-6 ($P = 0.04$), IL-7 ($P = 0.04$) and IL-18 ($P = 0.03$), as well as decreased insulin resistance ($P < 0.001$). Endothelial function was also

found to be improved in the intervention group ($P < 0.001$). At the end of the study forty patients in the intervention group still had features of the metabolic syndrome, compared with seventy-eight patients in the control group ($P < 0.001$).

Only two studies to date have examined the effect of a Mediterranean-type diet on clinical end points. In the Lyon Diet Heart Study (de Lorgeril *et al.* 1994, 1998, 1999), a randomised controlled trial with free-living subjects, those in the intervention group had a 50–70% reduction of cardiac end points. Patients in this study were recruited between 1988 and 1992 and less than one-third were on lipid-lowering drugs. Dietary compliance with the experimental diet at the end of the Lyon Diet Heart Study (mean follow-up 4 years) was reported to be good. In the final report of this study de Lorgeril *et al.* (1999) reported marked reductions in three composite outcomes (1, cardiac death and non-fatal myocardial infarction; 2, composite outcome 1 plus unstable angina, stroke, heart failure and pulmonary or peripheral embolism; 3, composite outcome 2 plus minor events requiring hospital admission); adjusted risk ratios ranged from 0.28 to 0.53. The reduction in risk in the Lyon study was not found to be associated with differences in blood total cholesterol between the control and experimental groups and, interestingly, the survival curves showed a very early separation quite unlike that seen in the majority of the statin trials (Leaf, 1999). Cancer-related end points were also improved in the intervention group (de Lorgeril *et al.* 1998).

In terms of dietary change, it was found that subjects in the control group averaged (% energy): 34 from fat; 12 from saturated fat; 11 from monounsaturated fat; 6 from polyunsaturated fat; 312 mg cholesterol/d. In contrast, subjects on the Mediterranean-style diet averaged (% energy): 30 from fat; 8 from saturated fat; 13 from monounsaturated fat; 5 from polyunsaturated fat; 203 mg cholesterol/d. Those subjects on the Mediterranean diet consumed less linoleic acid (3.6% energy *v.* 5.3% energy), and more oleic acid (12.9% energy *v.* 10.8% energy), α -linolenic acid (18:3n-3; 0.84% energy *v.* 0.29% energy) and dietary fibre. Plasma fatty acid analysis conducted after 52 weeks of follow-up confirmed the dietary fatty acid data (de Lorgeril *et al.* 1994). The plasma levels of the fatty acid 18:3n-3 were shown to be inversely associated with composite outcome 1. There are theoretical and also outcome data to support the hypothesis that n-3 fatty acids modulate the risk of myocardial ischaemia and, in particular, sudden death (Davignus *et al.* 1997; Albert *et al.* 1998; GISSI-Prevenzione Trial Group, 1999; Leaf, 1999).

It is important to note that the Mediterranean dietary pattern is not simply characterised by an alteration in the composition of fat intake. A diet rich in whole grains, fruits and vegetables will also be rich in fibre and also micronutrients, including folate, carotenoids and the antioxidant vitamins. In the Lyon Diet Heart Study (de Lorgeril *et al.* 1999) a marked increase in both vitamin E and vitamin C status was demonstrated. Interestingly, when Trichopoulou *et al.* (2003) were assessing the effects of Mediterranean diet score on survival it was found that there were no strong associations for any of the individual

dietary components of the Mediterranean diet score, suggesting that it is the overall pattern that is protective.

Although the results of the Lyon Diet Heart Study (de Lorgeril *et al.* 1994, 1998, 1999) were impressive, there were methodological limitations. The study was stopped early, because of marked beneficial effects noted in the original cohort, and this finding may have led to an over-estimation of risk reduction. The baseline diet was assessed only in the experimental group, and the diet of the control group was assumed to be comparable. Thus, it is unclear whether any dietary changes were made by the control group. In addition, dietary data are reported for only eighty-three (of 303 randomised into the study) and 144 (of 302 randomised into the study) subjects in the control and experimental groups respectively. With only 30% of the total control cohort and <50% of the total experimental group providing dietary data at the conclusion of the study, the diet of the other subjects who completed the study is not known. This situation raises questions about the role of diet in accounting for the results reported for recurrent coronary events, and emphasises the need for other trials of similar, but improved, design in different populations.

In the second randomised controlled trial with a clinical end point, Singh *et al.* (2002) have tested the effects of an Indo-Mediterranean diet rich in α -linolenic acid on progression of coronary artery disease in high-risk patients. Consumption of this diet (rich in whole grains, fruits, vegetables, walnuts (*Juglans regia*) and almonds (*Amygdalus communis* L.) was found to result in fewer total cardiac end points, sudden cardiac deaths and non-fatal myocardial infarctions than in the control group, who consumed a local diet similar to the National Cholesterol Education Program stage 1 diet.

Micronutrient status and other lifestyle factors

A recent study (Napoli *et al.* 2004) has highlighted the possibility that dietary factors may interact with lifestyle factors to influence disease risk. The study examined the effect of combining physical activity and antioxidant interventions in hypercholesterolaemic mice. In male mice on an atherogenic diet it was found that graduated and moderate exercise decreases atherosclerotic lesions, and the addition of antioxidants to this intervention (vitamins C and E in drinking water) further reduces atherosclerosis. This study suggests that physical activity may interact with micronutrient status in the prevention of disease.

Conclusions

Classic micronutrient-supplementation randomised controlled intervention trials have largely failed to show an effect on chronic disease risk. Alternative approaches to the study of micronutrients and disease are to not examine micronutrients in isolation but to look at whole foods that are rich in micronutrients, e.g. fruit and vegetables, or to look at micronutrients as part of a complete dietary pattern, e.g. the Mediterranean dietary pattern. There is strong scientific evidence that an increase in fruit and

vegetable intake reduces disease risk, while the Mediterranean diet has been associated with increased survival and reductions in CVD and cancer end points. The huge public health implications of these studies (the Lyon Diet Heart Study (de Lorgeril *et al.* 1994, 1998, 1999) has produced greater reductions in CVD mortality in a secondary prevention trial than any of the cholesterol-lowering studies to date) demand continued research.

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