

REVIEW

How Can Research on Plants Contribute to Promoting Human Health?

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One of the most pressing challenges for the next 50 years is to reduce the impact of chronic disease. Unhealthy eating is an increasing problem and underlies much of the increase in mortality from chronic diseases that is occurring worldwide. Diets rich in plant-based foods are strongly associated with reduced risks of major chronic diseases, but the constituents in plants that promote health have proved difficult to identify with certainty. This, in turn, has confounded the precision of dietary recommendations. Plant biochemistry can make significant contributions to human health through the identification and measurement of the many metabolites in plant-based foods, particularly those known to promote health (phytonutrients). Plant genetics and metabolic engineering can be used to make foods that differ only in their content of specific phytonutrients. Such foods offer research tools that can provide significant insight into which metabolites promote health and how they work. Plant science can reduce some of the complexity of the diet-health relationship, and through building multidisciplinary interactions with researchers in nutrition and the pathology of chronic diseases, plant scientists can contribute novel insight into which foods reduce the risk of chronic disease and how these foods work to impact human health.

INTRODUCTION


A major challenge in human health over the next 50 years will be in the area of chronic, noncommunicable diseases, including heart disease, many cancers, type 2 diabetes, and obesity. In 2005, a chilling report from the World Health Organization highlighted the scale of the challenge: 80% of the mortality from chronic disease occurs in low and middle income countries, 60% of those with chronic conditions are aged between 18 and 64, the poor are most vulnerable to chronic disease because of their increased exposure to risks and lower access to health care, and chronic disease causes poverty through lost capacity and income. Most significantly, the World Health Organization report projected that mortality from chronic disease would increase by 17% worldwide in the decade 2005 to 2015 due to longer average life span, tobacco use, decreasing physical activity, and perhaps most importantly, the increasing consumption of unhealthy foods. Because socio-behavioral risk factors contribute significantly to the incidence of and mortality from chronic disease, 36 million of the 388 million premature deaths predicted for 2005 to 2015 could be avoided if health, science, and public policies were reoriented toward prevention rather than cure. In 2007, the Oxford Health Alliance published a Grand Challenge document (Daar et al., 2007) outlining how such reorientation of policies and priorities might occur. One of the five major objectives identified in this article was to modify the risk factors for chronic disease, and it is in this area, particularly in identifying and understanding the health-promoting components

of food, that plant science could contribute significantly to addressing this Grand Challenge. Research on plants can lead to the identification of those metabolites that promote health and reduce the risk of chronic disease. Research on plants can also lead to the development of tools to assay the health-promoting effects of these metabolites. Greater understanding of the positive contributions that plant-based foods make to human health could contribute significantly to preventing the social and economic burdens of chronic disease globally.

In this article, we will describe some of the ways we believe that plant research can contribute to understanding the relationships between diet and health and to reducing the burden of chronic disease. It is not our intention to provide a comprehensive overview of phytochemicals with proposed or established nutritional benefits; there are several excellent reviews to which the interested reader is referred (Hu and Willett, 2002; Espín et al., 2007; Rao and Rao, 2007; World Cancer Research Fund, 2007). Our aim is to suggest areas where scientific research on plants, including analytical phytochemistry, marker-assisted selection, and metabolic engineering of plants could contribute significantly to advancing our understanding of the complex relationships between diet and health and the mechanisms by which plant-based foods may prevent or ameliorate chronic diseases. We support these suggestions with examples, which are, of necessity, of research closely aligned to our own fields. However, the research strategies illustrated by these specific examples could be applied broadly to promote understanding of the role of diet in chronic diseases and ultimately to promoting human health.

The past 30 years have seen development of an enormous body of evidence on the importance of plant-based foods in

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preventing or reducing the risk of chronic disease. For cardiovascular disease, these claims began with the output of the Lyon-Diet study (Renaud and de Lorgeril, 1989, 1992), which compared mortality from chronic heart disease on a national basis for European communities. Mortality showed a more or less linear relationship to dairy fat consumption, with countries consuming low dairy fat, Mediterranean-style diets (Spain, Portugal, and Italy) having less than half the mortality from heart disease compared with countries with high dairy fat consumption, such as Germany, the United Kingdom, and Scandinavian countries (Figure 1). The exception was France, with an equivalent consumption of dairy fat to the Nordic countries but a mortality rate from heart disease equivalent to those countries on Mediterranean-style diets. This observation became known as the French Paradox and has been attributed to the consumption of moderate amounts of red wine by the French and, particularly, the health-promoting effects of the polyphenols in red wine, based on epidemiological, clinical, preclinical, and cell-based assays (Lippi et al., 2010a, 2010b). Indices of healthy eating that incorporate high consumption of fruit and vegetables, such as the alternative healthy eating index (McCullough et al., 2002), are positively linked with significant reductions in the risk of major chronic diseases, particularly cardiovascular disease (CVD), and in large epidemiological studies adoption of the alternative healthy eating index has been associated with a significantly reduced risk of CVD (25%), of the same order of magnitude as pharmacological interventions, such as statins (20 to 27% reduced risk of CVD; Bernstein et al., 2010). Epidemiological studies also correlate higher consumption of whole-grain cereals, particularly cereal fiber, with lower incidences of CVD, obesity, and type 2 diabetes (Khaw and Barrett-Connor, 1987; Humble et al., 1993; Jacobs et al., 1998; Liu et al., 1999; Meyer et al., 2000; Wang et al., 2007; Larsson et al., 2009).

In 1981, Doll and Peto published their seminal article suggesting that ~80% of the cancers diagnosed in the United States in 1970 might have been avoided by altering lifestyle factors,

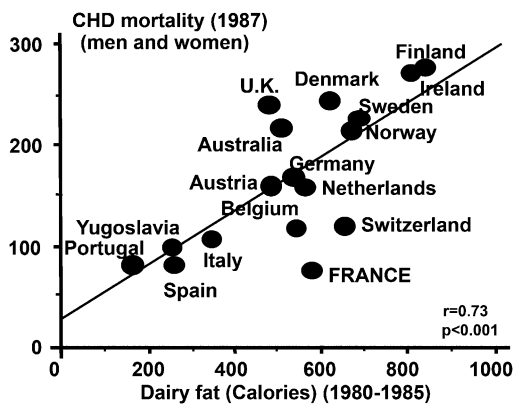


Figure 1. Relation between Age-Standardized Death Rate from Coronary Heart Disease (Mean for Men and Women) and Consumption of Dairy Fat in Countries Reporting Wine Consumption.

Regression equation = $y = 26.3 + 0.27 \text{ dairy fat}$. CHD, coronary heart disease. (Reprinted from Renaud and de Lorgeril [1992], Figure 1, with permission from Elsevier.)

particularly smoking and diet. Since then, many epidemiological studies have linked diets that include abundant consumption of plant-based foods with decreased risk of developing various kinds of cancer (American Institute for Cancer Research, 1997; Gescher et al., 1998). Although a recent meta-analysis of large cohort studies has reported that the inverse association between fruit and vegetable consumption and cancer incidence is weak (Boffetta et al., 2010), the impact of specific fruits or vegetables was not assessed in this study (Willett, 2010). In addition, this meta-analysis was based on relatively low level consumption of fruit and vegetables. If the data for two the highest quintiles of fruit and vegetable consumption are considered (equivalent to the minimum levels of consumption suggested by the 5-a-day program) the inverse relationship to cancer incidence is more striking (Boffetta et al., 2010). The reduced risk of certain cancers associated with diets rich in fruit and vegetables (American Institute for Cancer Research, 1997; Key et al., 1997; Cohen et al., 2000; Kolonel et al., 2000; Knekt, et al., 2002; Thomson et al., 2003; Benetou et al., 2008) and experimental evidence of the chemopreventive effects of metabolites produced by plants has led to the suggestion that such metabolites (phytonutrients) may work by blocking the development of latent precancerous microtumors (Doll and Peto, 1981; Surh, 2003; Dorai and Aggarwal, 2004; Béliveau and Gingras, 2007; Gingras and Béliveau, 2007).

The importance of plant-based foods in the human diet is supported by evolutionary arguments that suggest that the human genome evolved in the context of, and is consequently best adapted to, a hunter-gatherer diet, rich in leafy vegetables and fruits, low in starch and fats (and consequently of low glycemic index), and rich in protein. Some of the increase in chronic disease currently being observed may result from the evolutionary discordance between our genomes that are attuned to extract the maximum calorific content from food (suitable for a hunter gatherer existence) and our diets, which have changed dramatically over the 10,000 years since the start of cereal cultivation and abandonment of hunter-gatherer practices, to become rich in calories from fats and starches and low in fruit and vegetables and the micro- and macronutrients they provide (Figure 2; Eaton et al., 2002a, 2002b; Simopoulos, 2002; O'Keefe and Cordain, 2004; Cordain et al., 2005; Jew et al., 2009). Particularly significant in this respect is the relative decline in consumption of omega-3 (n-3) polyunsaturated fatty acids (PUFAs), which occurred concomitant with increased consumption of cereals (which are low in n-3 PUFAs) and was exacerbated by animal husbandry switching to feed based on cereals. Diets with low n-3:n-6 PUFA ratios have been linked to higher incidences of cancer, CVD, obesity, inflammatory diseases, and, more recently, various mental illnesses, including depression, hyperactivity, and dementia (Bucher et al., 2002; He et al., 2002; Maillard et al., 2002; Erkkilä et al., 2003; Iso et al., 2006; Sontrop and Campbell, 2006; Carpentier et al., 2006; von Schacky and Harris, 2007; Li et al., 2008; Ramel et al., 2009; Riediger et al., 2009). While such evolutionary arguments may appear superficial and designed to appeal to conservatives promoting ethnic dietary habits, studies of present-day hunter-gatherer cultures show members to be almost completely free from chronic degenerative diseases (Eaton et al., 2002b). Such arguments

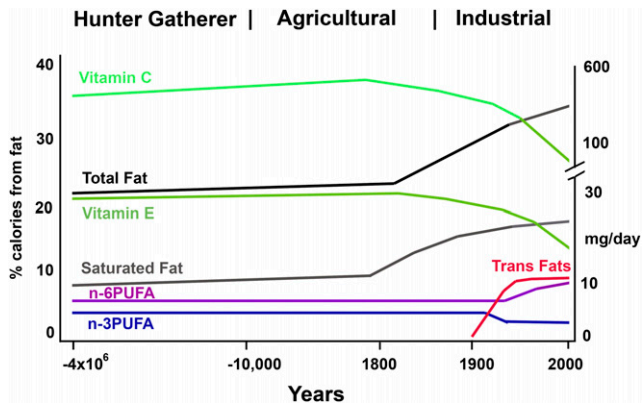


Figure 2. Hypothetical Scheme of Fat, Fatty Acid ($\omega 6$, $\omega 3$, *trans*, and Total) Intake (as Percentage of Calories from Fat) and Intake of Vitamins E and C (mg/day).

Data were extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years. Changes in average consumption of fruit and vegetables mirror changes in the intake of vitamin C ranging from estimates of 550 to 1800 g/day for hunter gatherers (Kuipers et al., 2010) to 77 to 770 g/day for current Europeans (Boffetta et al., 2010). (Adapted from Simopoulos [2002], Figure 1, with permission from Elsevier.)

are also supported by experimental evidence. Feeding human subjects a Simian diet comprised of 63 servings per day of green leafy vegetables, fruits, and nuts resulted in remarkable reductions in total cholesterol and in low density lipid (LDL) cholesterol (25 and 30%, respectively) in just 2 weeks, significantly superior to the Step II diet recommended by the National Cholesterol Education Program (Jenkins et al., 2001). In another study, individuals on a paleolithic diet, high in fruit, vegetables, nuts, lean meat, and fish, presented improved blood pressure and Glc tolerance, increased insulin sensitivity, and improved lipid profiles within the short period of the intervention (Frassetto et al., 2009).

Public bodies have not been slow in attempting to disseminate information on the benefits of fruit and vegetables in the diet. In the United States, the National Research Council and the National Cancer Institute developed the 5-a-day program in the 1980s, and national recommendations to consume at least five servings of fruit or vegetables per day have since been adopted and promoted in many developed countries. However, public information programs are not particularly effective at persuading people to change long-established habits, and most five-a-day websites report lower than 25% adherence to such recommendations (for example, the report of the National Cancer Institute 5-a-day for better health program evaluation report; http://dccps.nci.nih.gov/5ad_3_origins.html; DeBoer et al., 2003; Boffetta et al., 2010). More worryingly, the estimated numbers attaining 5-a-day have decreased over the past 10 years in many countries, including the United States. The reasons for this poor penetration are multifold but include cultural prejudices, difficulties in implementing long-term commitment to dietary changes, cost, poor availability of fruit and vegetables, and, most impor-

tantly, confusion over which fruit and vegetables count toward the 5-a-day tally and which do not qualify. It is in the area of identifying plant-based foods that promote health by reducing the risk of chronic disease and the comparative evaluation of different foods that plant sciences can make the biggest contribution to meeting the Grand Challenge of chronic disease and contribute significantly to promoting human health.

IDENTIFICATION OF NUTRITIONAL FACTORS IN PLANT-BASED FOODS

While most foods are now labeled with nutritional information detailing their content of protein, carbohydrates (sugars), fats, and additives, many health-promoting factors in foods are not measured or listed. Information on fiber content of processed foods is usually provided, although it would be useful for consumers also to know the fiber content of the fresh fruit and vegetables they consume. In addition, although viscous fiber (the sticky type of soluble fiber found in oats, barley, and beans, and certain vegetables, such as okra and aubergine) has been shown experimentally to lower the glycemic index of foods and to have a beneficial impact on obesity, type 2 diabetes, and risk factors for CVD (Khaw and Barrett-Connor, 1987; Humble et al., 1993; Jacobs et al., 1998; Liu et al., 1999; Meyer et al., 2000; Wang et al., 2007; Larsson et al., 2009), the relative content of viscous fiber compared with particulate cereal fiber in foods is usually not provided. This may be because the viscous fiber content of most foods in Western diets is very low (Kendall et al., 2010). However, the content of cereal fiber (which in epidemiological studies has been correlated to reduced risk of CVD, type 2 diabetes, and obesity, but for which no metabolic experiments have shown impacts on glycemic index), particularly whole grains, is advertised widely for foods together with claims for their health-promoting properties. With greater qualitative as well as quantitative information on the fiber content of different plant-based foods and information on the relative impact of that fiber on glycemic index, many processed foods in Western diets could be reformulated to include higher levels of plant fiber, especially viscous fiber and unprocessed grains, to improve glycemic control and body weight management.

Knowledge of vitamins and their presence in certain foods is long standing (Asensi-Fabado and Munné-Bosch, 2010). Vitamins are groups of compounds not synthesized by humans but necessary for human life. They are therefore essential in the diet, and humans must consume vitamins periodically to avoid deficiency. The human body can store different vitamins to differing extents: vitamins A, D, and B₁₂ are stored in significant amounts, and an adult's diet may be deficient in vitamins A and D for many months and B₁₂ in some cases for years before developing a deficiency condition. However, vitamin B₃ (niacin and niacinamide) is not stored in significant amounts; reserves may last only ~10 to 14 d, and disease from deficiency is more acute than for other vitamin deficiencies. The first symptoms of vitamin C deficiency (scurvy) vary from a month to more than 6 months, depending on previous dietary history.

Restrictive diets based on staple crops like maize, rice, or cassava have the potential to cause prolonged vitamin deficits,

which result in painful and potentially deadly diseases. Well-known human vitamin deficiencies involve vitamin B1 (thiamine), which causes beriberi; vitamin B3, (niacin) which causes pellagra; vitamin C (ascorbic acid), which causes scurvy; and vitamin D, which causes rickets. The first symptom of vitamin A deficiency is night blindness, which can lead to complete blindness in children. Vitamin A deficiency also results in reduced resistance to infectious diseases. Between 250,000 and 500,000 children are estimated to suffer from blindness due to vitamin A deficiency each year, and an estimated 650,000 children under the age of 5 years old die from this deficiency each year. Vitamin A deficiency is particularly prevalent in rural populations in developing countries where diets may be based almost exclusively on a single starch-based crop (so-called staple crops, such as rice, maize, or cassava), and supplements may not reach those needing them due to limitations in distribution (Mayer et al., 2008). Many staple crops are deficient in provitamin A carotenoids, such as β -carotene.

The classic example of the contribution of plant science to biofortification for the elimination of vitamin A deficiency is Golden Rice (Ye et al., 2000). β -Carotene is a particularly good source of provitamin A because each molecule of β -carotene can be cleaved and converted to two molecules of retinoic acid. Rice, particularly milled rice, does not provide any dietary β -carotene, and there is no natural variation for β -carotene content in endosperm that would allow biofortification through breeding. Using four genes encoding the biosynthetic enzymes for β -carotene synthesis regulated so that they are expressed in rice endosperm, Golden Rice lines accumulating β -carotene were first reported in 2000 (Ye et al., 2000). Since then, significant effort has been invested in enhancing the levels of β -carotene that accumulate in the endosperm to ensure that a single serving of Golden Rice can supply the daily recommended intake of provitamin A to avoid deficiency and the accompanying disease symptoms. Enhanced levels have been achieved using genes encoding enzymes that are highly efficient in producing β -carotene (Paine et al., 2005). Golden Rice-2 accumulates up to 37 μ g β -carotene per gram of rice (some 23-fold more than the original Golden Rice). Recently, this β -carotene has been shown to be bioavailable in amounts sufficient that 100 to 200 g Golden Rice per day can provide adequate provitamin A to children to avoid deficiency (Tang et al., 2009). Since the reporting of Golden Rice, other staple crops on which many depend almost exclusively for their nutrition have been produced enriched in β -carotene, including maize and cassava (Harjes et al., 2008; Welsch et al., 2010; Yan et al., 2010), which have been produced by selective breeding as well as by genetic engineering (Naqvi et al., 2009). Golden phenotypes resulting from enhanced β -carotene content have also been achieved in other crops, such as potato (Diretto et al., 2010), although since these are no longer staple crops providing the bulk or sole source of nutrients to population groups, it is less likely that these will be useful in terms of alleviating vitamin A deficiency.

Enhancement of other vitamins (for example, folate) in staple crops such as rice and maize have been reported (Storozhenko et al., 2007; Bekaert et al., 2008; Naqvi et al., 2009), which may provide useful biofortified crops for populations (particularly rural communities) reliant on vitamin-deficient staple crops (Table 1).

Other biofortification programs include enrichment of micronutrients, such as iron in rice, wheat, and beans, achieved through conventional breeding (White and Broadley, 2005) and in rice through overexpression of ferritin (Goto et al., 1999; Lucca et al., 2001) (Table 1).

Vitamins do not include the large number of other nutrients in plant-based foods that promote health. These other nutrients are increasingly known as phytonutrients and are compounds in plant-based foods that play a potentially beneficial role in the prevention and treatment of disease. They include polyphenols (flavonoids and stilbenes), carotenoids, plant sterols, and PUFAs. All have been shown to promote health when consumed as part of the diet. The carotenoids and polyphenols are often grouped together as dietary antioxidants, although this is an intellectual shortcut that has resulted in considerable confusion and consequent debate concerning the relative contributions of such dietary components in promoting health. The danger in phytonutrients being assessed collectively as antioxidants is that these compounds do not act directly as antioxidants once absorbed but rather have specific and likely different effects on animal cell signaling and physiology.

While the antioxidant capacities of many phytonutrients have been offered to explain their health-promoting properties (Rice-Evans et al., 1997), such claims have been challenged because the low bioavailability of many dietary phytonutrients makes it highly unlikely that, once absorbed, they operate directly as antioxidants to promote health. It is important to distinguish the site of action of dietary phytonutrients in this context. Flavonoids have been shown to have protective activity in the gastrointestinal (GI) tract, having antispasmodic, antisecretory, antidiarrhoeal, antiulcer, and bactericidal properties (Di Carlo et al., 1993; Sunairi et al., 1994; La Casa et al., 2000; Isomoto et al., 2005; Lima et al., 2005). Within the GI tract, the strong antioxidant capacities of flavonoids (Rice-Evans et al., 1997) are likely to play direct roles in their gastroprotective effects, although the metabolism of flavonoids by the gut microbionota is also likely to impact their efficacy and modes of action in suppressing or modulating gastric pathologies, such as ulcers and inflammatory bowel diseases.

Given that reactive oxygen and nitrogen species (RONS) underpin many degenerative pathologies, including CVD, neurodegenerative diseases, chronic inflammation, certain cancers, and tissue aging (Wickens, 2001; Koutsilieri et al., 2002; Closa and Folch-Puy, 2004; Valko et al., 2006), it is possible that phytonutrients, even those with low bioavailabilities (such as anthocyanins, which have <1% bioavailability), contribute directly to suppression of some signaling pathways activated by generation of RONS (Virgili and Marino, 2008). However, the view that such phytonutrients also act on the signaling pathways that respond to RONS independently of their antioxidant activities, and in this way impact inflammation and the inception of chronic disease, is gaining considerable ground (Figure 3; Virgili and Marino, 2008). Other examples of specific effects of phytonutrients are the interactions of isoflavonoids with the estrogen receptor, which may explain the significantly lower incidence of steroid hormone-responsive cancers (25% lower incidence of prostate cancer and 10% lower incidence of breast cancer) in Asian communities consuming a high soy diet (rich in genistein

and daidzein isoflavones). Other examples include the cardioprotective effects of the phytonutrients in red wine. Red wine contains varying levels of resveratrol, flavonols, anthocyanins, and catechins (epigallocatechin gallate), which are all effective antioxidants. However, the cardioprotection afforded by these polyphenols is likely the result of multiple biological activities independent of their antioxidant activities, including the improvement of endothelial function, the reduction of LDL uptake, the reduction of LDL oxidation and aggregation, the reduction of blood pressure, and the inhibition of platelet aggregation (Miura et al., 2001a, 2001b; Wang et al., 2002; Jalili et al., 2005; Machha and Mustafa, 2005; Bose et al., 2008). In addition, carotenoids are lipophilic antioxidants, whereas the polyphenols are predominantly water soluble. Generally, foods rich in both soluble and membrane-associated antioxidants are considered to offer the best protection against disease (Yeum et al., 2004). Consequently, chemical analysis of plant-based foods could contribute significantly to the evaluation of their health-promoting potential by listing their contents of lipophilic and hydrophilic antioxidants separately.

MEASUREMENT OF PHYTONUTRIENTS IN PLANT-BASED FOODS

The growing realization that phytonutrients may have many specific effects on animal physiology and disease means that

the careful analytical assessment of the phytonutrient content of different plant foods is essential for their reliable evaluation as components in recommended health-promoting diets. In addition, the bioavailability of different phytonutrients needs to be determined, plus their metabolism within the human GI tract. For example, much of the confusion over the relative impact on isoflavonoids in the diet may have resulted from the fact that gut microbionota are responsible for metabolizing isoflavonoids to a significantly more bioactive form, equol (Setchell et al., 2002). While most animal models used for dietary studies (rat, mouse, and pig) produce equol, in human only 30% of the population produce equol (Setchell et al., 2002). The ability to produce equol is developed by individuals and is carried with them despite aging and changes in diet. Whether or not individuals are equol producers is not usually recorded in epidemiological studies, a confounding factor that may well have influenced the interpretation of the health-promoting effects of isoflavonoids in many epidemiological studies (Lampe, 2009).

The need for quantitative and qualitative information on the phytonutrient content of different plant-based foods extends beyond the measurement of generalized groups of metabolites. For example, health-promoting properties have been proposed for anthocyanins, the blue or red pigments present in many berries and some vegetables. However, anthocyanins vary considerably from species to species due to differences in the number of hydroxyl groups in the molecule, the degree of methylation of these hydroxyl groups, the nature and number of

Table 1. Summary of Biofortification of Essential Vitamins and Micronutrients Achieved in Staple Crops

| Target Micronutrient/Vitamin | Crop | Base Level (ppm) | Biofortification Level (ppm) | | References |
|-----------------------------------------------|------------------------|-------------------------------------------------------|------------------------------|--------------------------|------------------------------------------------------------------------------------------------------------------------|
| | | | Breeding | GM | |
| β-Carotene (β-C) or total carotenoids (TC) | Rice | 0 | | 2–37 | Ye et al. (2000) Paine et al. (2005) |
| | Maize | ~0 ^a | 14 (β-C); 60 (TC) | 10–20 | Meenakshi et al. (2007) Naqvi et al. (2009) Harjes et al. (2008) Yan et al. (2010) |
| | Cassava | 0.4 (β-C) ^a ; 0.6–1.5 (TC) ^a | 1.5 (β-C); 6–11.5 (TC) | 21.5 (TC); 7 (β-C) | Welsch et al. (2010) Rojas et al. (2009) |
| | Sweet potato Potato | 0 2–5 ^a | 32 | 40 (β-C) 100–200 (TC) | Meenakshi et al. (2007) Diretto et al. (2010) |
| Folate | Rice Maize | <1 100 | | 17 200 | Storozhenko et al. (2007) Naqvi et al. (2009) |
| Vitamin C | Maize | 18 | | 107 | Naqvi et al. (2009) |
| Iron | Beans Rice | 40 3 | 80–100 6–8 | | Meenakshi et al. (2007) Meenakshi et al. (2007) Wirth et al. (2009) Goto et al. (1999) Lucca et al. (2001) |
| | Wheat | 38 | 46–61 | | Meenakshi et al. (2007) |
| Zinc | Beans Rice | 30 13–20 | 40–50 24–35 | | Meenakshi et al. (2007) Meenakshi et al. (2007) Wirth et al. (2009) |
| | Wheat | 30 | 30 | 37–55 | Meenakshi et al. (2007) |

GM, produced by genetic engineering techniques; Breeding, produced using non-GM variation.

^aDepending on variety.

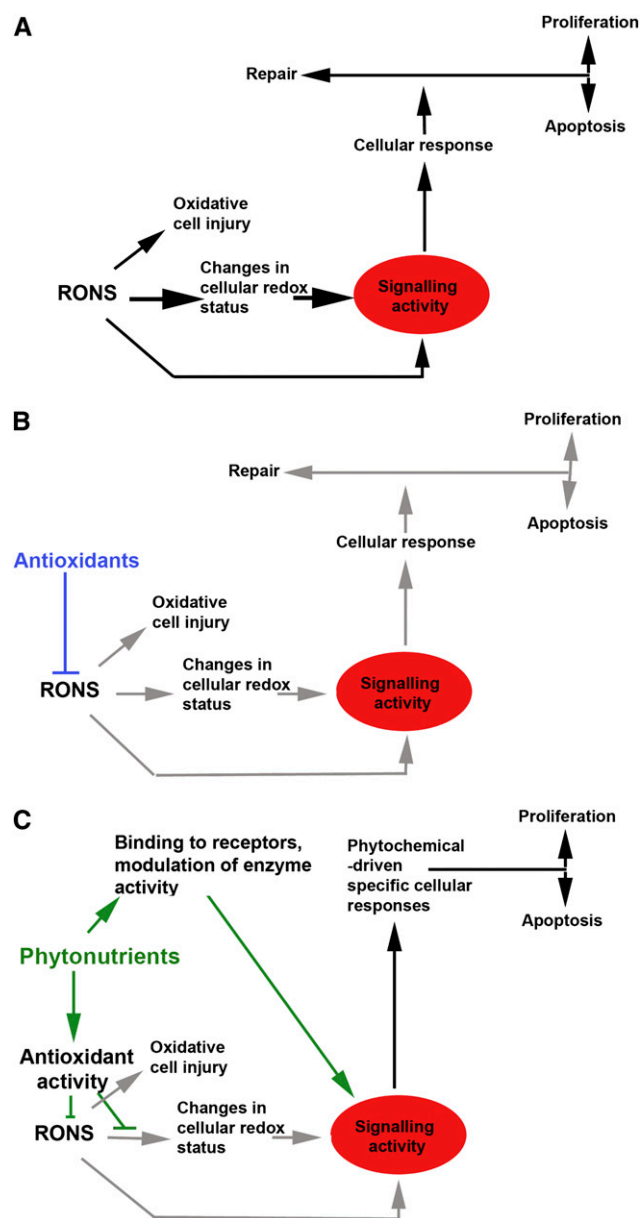


Figure 3. Classical View of How Antioxidants Influence Cell Signaling and Summary of the Current View of How Phytonutrients Impact Cell Signaling.

(A) The classical view of oxidant–antioxidant effects on animal cell signaling and response. RONS can act on cell signaling, either directly or indirectly, through changes in the redox cellular equilibrium (e.g., a decrease in the reduced-to-oxidized glutathione ratio).

(B) Antioxidants modulate RONS-mediated cell responses by shielding their reactivity or reducing their availability, at both the extracellular and the intracellular levels.

(C) The current view of how phytonutrients impact cell signaling. Phytonutrients interact with cell signaling thanks to mechanisms independent of their antioxidant properties, by directly affecting the activities of a wide spectrum of cellular targets, including key enzymes and membrane and nuclear receptors. In the presence of perturbations in the cellular redox status, a combination of the two paths occurs. (Adapted from Virgili and Marino [2008], Figure 1, with permission from Elsevier.)

the sugar moieties attached to the anthocyanidin molecule and the position of the attachment, and the nature and number of aliphatic or aromatic acids attached to the sugars. Some differences in the efficacy of different anthocyanins as antioxidants in cellular assays have been demonstrated (Kong et al., 2003), but relative differences in the ability of different anthocyanins to protect against chronic diseases have not yet been investigated even in preclinical animal studies. However, there are significant differences in the bioavailability of different anthocyanin species (Prior and Wu, 2006; McGhie and Walton, 2007), and these differences alone could cause significant variations in the efficacy in promoting health of different anthocyanin species in foods. Absorption of anthocyanins is influenced by glycosylation and acylation as well as by the physicochemical environment of the food matrix and by food processing (McGhie and Walton, 2007; Capanoglu et al., 2008). All of these variables make prediction of the likely beneficial properties of any one phytonutrient in any one food extremely difficult. Dietary recommendations would benefit greatly from precise identification and quantification of different metabolites in plant-based foods and their processed products, combined wherever possible with information on bioavailability and efficacy. Food composition databases are being established (Toledo and Burlingame, 2006; Burlingame et al., 2009) that are valuable for nutrition education, community nutrition, nutritional labeling, dietary recommendations, and efforts to improve crops through breeding, since these provide repositories of information on the natural variation in nutrients available in cultivars and varieties of different food crops.

ASSESSMENT OF THE IMPACT OF INDIVIDUAL PHYTONUTRIENTS ON DISEASE

For the general scientist reading the literature on the impact of diet on health, the overriding impression is one of complexity almost refractive to simplification. Our food consists of complicated mixtures of proteins, carbohydrates, fats, and both micro- and macronutrients, making it extremely difficult to identify and dissect out the contributions of any single component to nutrition and health. All nutrients are subject to metabolism by the enzymes of the GI tract and by the gut microbionota, and the gut microflora may be highly personalized for individuals and yet change with time. In addition, phytonutrients may impact the composition of the gut microflora, which can, in turn, impact the risk of chronic disease, as has been shown for the role of the microflora of the GI tract in relation to obesity (Gill et al., 2006; Ley et al., 2006; Turnbaugh et al., 2006). The sites of absorption of different nutrients within the GI tract vary, and the degree of absorption (bioavailability) may vary significantly for slightly different chemical species. Phytonutrients are usually further metabolized once absorbed. Finally, the efficacy of different phytonutrients in promoting health likely varies significantly as a result of the specificity with which such compounds and/or their metabolites impact different animal signaling pathways, although any such differences in efficacy currently remain ill defined.

Our advances in plant science provide us with the tools to reduce some of the complexity in the diet–health relationship

particularly through the development of near-isogenic foods that differ only in the content of specific phytonutrients under investigation. An elegant demonstration of such an approach was provided by experiments establishing that anthocyanins in maize could offer cardioprotection against ischemia-reperfusion injury in rats (Toufektsian et al., 2008). In this case, use of maize lines carrying *R* and *C1* alleles that regulate anthocyanin accumulation in kernels allowed the production of isogenic material either containing or not containing anthocyanins in the kernels (Procissi et al., 1997). The resulting purple and yellow maize was used to supplement rodent diets at 20%. Rats were fed for 8 weeks on diets with anthocyanins (supplied at 0.08 mg/kCal/day) or without anthocyanins and then the resistance of their hearts to ischemia reperfusion injury was tested using the Langendorff heart model. Those animals fed the purple maize-supplemented diet showed significantly smaller areas of damaged tissue (infarct size) compared with those on the yellow maize-supplemented diet in both ex vivo and in vivo assays, demonstrating that an anthocyanin-rich diet can offer significant cardioprotection in rats, albeit at an estimated 13-fold higher levels of anthocyanin consumption than in the average Western diet (Figure 4).

A similar approach using tomatoes genetically engineered to produce high levels of delphinidin and petunidin anthocyanins demonstrated that dietary consumption of high levels of anthocyanins can extend the life span of *Trp53*^{-/-} (p53 knockout) cancer-prone mice by as much as 30% (Figure 5; Butelli et al., 2008). This type of approach, using either selected isogenic food material or food material produced by genetic engineering, provides powerful tools for assigning health-promoting properties to specific phytonutrients within a whole-food context, independent of the complexities of bioavailability. Our understanding of plant metabolism and the genetics of its regulation are advancing to the point where foods differing in significant

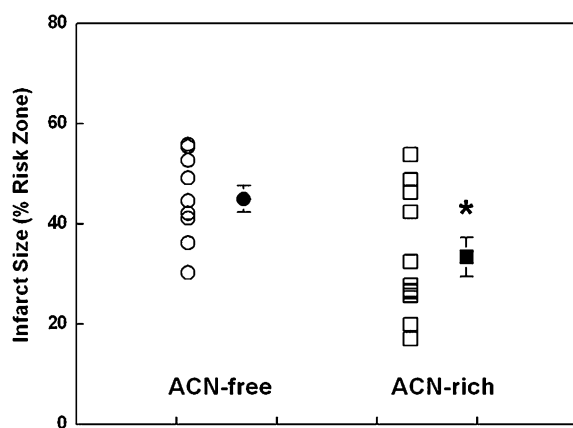


Figure 4. Effect of Chronic Consumption of Anthocyanins on the Sensitivity to ex Vivo Ischemia-Reperfusion Injury as Assessed by Infarct Size in Rats.

Open symbols represent individual values, and closed symbols are means \pm 6 SE, $n = 10$ /group. *Different from anthocyanin (ACN)-free; $P < 0.05$. (Reprinted from Toufektsian et al. [2008], Figure 2, with permission from the American Society for Nutrition.)

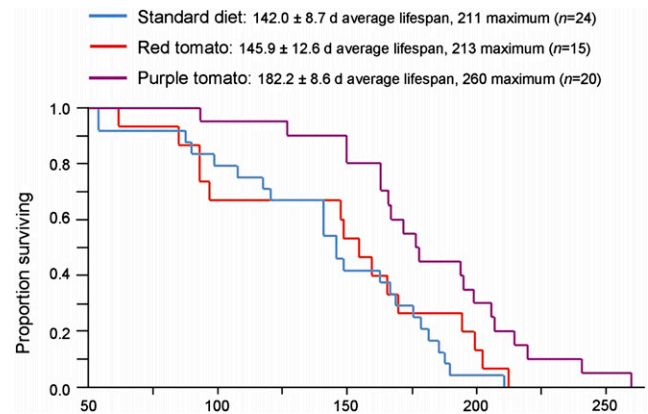


Figure 5. Life Expectancy of *Trp53*^{-/-} Mice Fed the Standard Diet or Diets Supplemented with 10% Red or Purple Tomato Powder.

Kaplan-Meier survival plot; errors given in \pm SE. (Reprinted from Butelli et al. [2008], Figure 4, with permission from Nature Biotechnology.)

levels of many phytonutrients could be developed and assayed for their effects in whole animal models of disease. For example, there is persuasive evidence from epidemiological studies that tomato consumption is inversely related to the risk of developing prostate cancer (Mills et al., 1989; Tzonou et al., 1999; Giovannucci, 2005). Some epidemiological studies have suggested lycopene to be the active component in this effect (Giovannucci, 2005). Intervention studies with lycopene extracts have shown even short-term lycopene consumption to be associated with reduced levels of high grade prostatic intraepithelial neoplasia, with smaller tumors and lower levels of the prostate-specific antigen (Kucuk et al., 2001). Tomato represents an excellent vehicle for providing dietary lycopene, which avoids many of the problems of insolubility associated with the purified compound. More robust data on the relative impact of dietary lycopene on health, particularly its impact on prostate cancer, could be derived by comparing diets supplemented with high lycopene tomatoes or regular tomatoes on animal models of disease. There are several well-characterized high lycopene varieties of tomato that have up to 6 times higher lycopene levels than regular varieties. Mutants of tomato also exist that fail to produce lycopene (Torres and Andrews, 2006; Nashilevitz et al., 2010). Diets supplemented with equivalent amounts of these different tomato lines could be used to test the efficacy of lycopene in reducing the inception or progression of prostate cancer in TRAMP or PTEN mouse models of the disease (Lamb and Zhang, 2005).

ESTABLISHMENT OF TOXICITY AND DOSE-RESPONSE CURVES FOR PHYTONUTRIENTS ON A FOOD-BASED SCALE

Isogenic food materials could be used to establish dose-response curves for specific phytonutrients and to test for their toxicity at the highest levels of consumption. The advantages of these materials for such assays are that they maintain the phytonutrients in their specific food matrix, which can have a large impact on phytonutrient bioavailability, and they permit assessment of

phytonutrients in the specific chemical forms that they are most commonly consumed. Generally, these types of dose–response experiments have not yet been done but are important to underpin reliable dietary recommendations on consumption and to avoid diets with very high levels of phytonutrients that could involve toxicity or reduced effectiveness.

For example, in the experiments that demonstrated cardioprotection by consumption of maize anthocyanins, the level of dietary anthocyanins in the rodent feed was estimated to be 13 times higher than levels present in an average Western diet (Toufektsian et al., 2008). Despite this, the anthocyanin dose tested represented a physiological dose that would correspond to an intake of 156 mg anthocyanins daily in humans, levels similar to those consumed in a typical Mediterranean diet. It remains to be determined whether higher levels would offer more protection or, indeed, whether lower levels of dietary anthocyanins would offer the same or lower degrees of cardioprotection.

Similarly in experiments demonstrating high anthocyanin consumption to extend the life span of cancer-prone mice, the tomato lines with the highest levels of anthocyanins were used to supplement the rodent diets (Butelli et al., 2008). Relative to an average human diet (0.2 mg anthocyanin/kg/day), the levels of consumption of anthocyanins in these experiments were also high (80 mg/kg/day), but not unachievable, since the tomato lines used accumulated anthocyanins in fruit equivalent to the levels in many berries (Paredes-López et al., 2010). The high levels of anthocyanins did not have any toxic effects in either the cardioprotection or cancer assays, and wild-type mice showed normal growth and development on the high anthocyanin tomato-supplemented diet compared with the tomato-supplemented diet and the standard diet (Butelli et al., 2008). Generally, it is believed that because many phytonutrients constitute components of the normal human diet and have done so since the time humans were hunter-gatherers, they are unlikely to have previously unrecognized, harmful secondary effects, a view supported by the limited assessments of toxicity that have been undertaken.

Phytonutrients that are produced specifically by certain plant species may be toxic at high levels of consumption or in combination with specific drug therapies. Resveratrol, the stilbene most widely available in wine and grape, has recognized properties in cardioprotection, neuroprotection, chemoprevention, anticancer, and anti-aging (Albani et al., 2010; Hsieh and Wu, 2010; Mouchiroud et al., 2010; Yap et al., 2010). Tests on resveratrol fed at 1000-fold excess of its average daily (human) consumption failed to show any effects on feeding efficiency, hematological variables, clinical markers, or pathology of rats over 28 d (Paredes-López et al., 2010). Subsequent human trials with similar levels of SRT501, a resveratrol formulation with greater bioavailability than natural *trans*-resveratrol, showed lower glycemic index and improved Glc control in patients diagnosed with a precondition for type 2 diabetes. However, subsequent trials with 5 g per day SRT501 fed to patients with multiple myeloma resulted in 20% of those on SRT501 developing kidney failure. While this negative outcome could have resulted from other components of the SRT501 formulation or resulted from specific interactions between SRT501 and the drugs used to treat multiple myeloma, these data suggested that,

at very high concentrations and with increased bioavailability (fivefold), resveratrol may show some toxicity, at least to patients with multiple myeloma (The Myeloma Beacon, 2010). As a result of these effects, further development of SRT501 was halted in November, 2010.

Other flavonoids may have specific toxic effects. Silybin, a flavonoid from milk thistle, has been shown to inactivate some P450 enzymes and hepatic glucuronosyltransferases, offering the possibility that it could interfere with the action of other drugs at high doses. Some isoflavonoids have proestrogenic effects (for example, genistein in rodents; Allred et al., 2001a, 2001b), and consumption of foods such as soy (containing high levels of isoflavonoids) by postmenopausal women with a family history of breast cancer needs to be considered carefully.

COMPARATIVE ASSAYS OF HEALTH-PROMOTING EFFECTS OF DIFFERENT PHYTONUTRIENTS

Breeding strategies exploiting existing natural variation in some crops can be used to produce comparable foods enhanced with different phytonutrients. Using geographic accessions of maize with different alleles of the *MYB* and *bHLH* regulatory gene families (*C1/P1* and *B1/R1*, respectively) that control activation of the anthocyanin biosynthetic pathway, isogenic lines accumulating different levels of anthocyanins in different tissues can be obtained. For example, by selecting specific alleles of different *bHLH* genes, anthocyanin biosynthesis can be directed to different tissues of the seed, such as pericarp (*Sn1*; Tonelli et al., 1991), aleurone (*R1*; Procissi et al., 1997), or scutellum (*Hopi1*; Petroni et al., 2000). Using different geographic alleles of the *MYB* regulatory genes (e.g., *pl1-W22*, *pl1-bo13*, and *P11-Rhoades*), the extent of anthocyanin accumulation can be modulated (Pilu et al., 2003). Maize with one of these allelic combinations (*R1 C1*) was used to demonstrate the cardioprotective effect of dietary anthocyanins in rats (Toufektsian et al., 2008), and other combinations have been generated to provide isogenic lines with increasing levels of cyanidin-based anthocyanins in kernels (K. Petroni, R. Pilu, and C. Tonelli, unpublished data).

High levels of other classes of flavonoids can be obtained by selecting branch-specific regulatory genes, such as the *P1 MYB* regulatory gene that activates the synthesis of phlobaphenes (3'-deoxyflavonoids) in maize kernels (Grotewold et al., 1994). Furthermore, combining the anthocyanin regulatory genes (e.g., *R1 C1*) with mutations in some biosynthetic genes, kernels with high levels of metabolic intermediates can be obtained. For example, a mutation in the *Pr* gene, encoding flavonoid 3'-hydroxylase, can be used to obtain kernels enriched in pelargonidin-based anthocyanins, and high levels of flavonols (kaempferol and quercetin) can be obtained by selecting for mutations in the *A1* gene, encoding dihydroflavonol reductase. Combination of *pr* and *a1* mutations provides maize containing high levels of the flavonol, kaempferol (K. Petroni, R. Pilu, and C. Tonelli, unpublished data).

Metabolic engineering of plants will also allow the development of foods enhanced with equivalent levels of different phytonutrients. This has been demonstrated for tomato where

lines enhanced in flavonols (at equivalent levels to the anthocyanins in the purple tomatoes) have been developed by engineering fruit-specific expression of a transcription factor that activates flavonol biosynthesis (Butelli et al., 2008; Luo et al., 2008). Comparison of the effects of diets supplemented with tomatoes enriched in either anthocyanins or flavonols on inception and progression of disease in cellular assays or animal disease models will allow the quantitative assessment of the efficacy of these different flavonoids within a common food matrix. The novelty of this approach is that the health-promoting effects of individual food bioactives within a whole food matrix can be comparatively assessed relative to a true matched matrix control. Tomato lines enriched in other flavonoids could be generated by combining the fruit-specific expression of transcription factors that induce anthocyanin production with mutations affecting activity of enzymes in the biosynthetic pathway. In this way, tomato fruit enriched in dihydroflavonols have been developed by combining the fruit-specific expression of *Delila* and *Rosea1* transcription factors (Butelli et al., 2008) with the *aw* mutation that blocks dihydroflavonol 4-reductase activity in tomato (Bongue-Bartelsman, et al., 1994; E. Butelli and C. Martin, unpublished data).

Similar strategies could be adopted to generate tomato fruit enriched in other flavonoids (Xie et al., 2006; Shih et al., 2008), which could be used for comparative evaluation of these flavonoid species in reducing the risk of chronic disease. Tomatoes also can be used to express specific genes for the synthesis of polyphenolic phytonutrients that are made only by select species, such as resveratrol (Giovinazzo et al., 2005) and genistein (Shih et al., 2008). Tomato lines producing different levels of carotenoids could be generated, as described earlier, for assessment of the health-promoting properties of these phytonutrients, relative to different polyphenols.

ASSAYS OF THE IMPACT OF THE FOOD MATRIX ON THE ABILITY OF PHYTONUTRIENTS TO PROMOTE HEALTH

One of the most intriguing results to emerge recently is that purified polyphenols supplied as dietary supplements do not have the same effects in promoting health as the same phytonutrients in a food context (Prior et al., 2008; Titta et al., 2010). For example, anthocyanins from several food sources have been reported to reduce weight gain and adipocyte development in C57BL/6J mice fed a high-fat diet (Tsuda et al., 2004; Jayaprakasam et al., 2006; Titta et al., 2010), and diets supplemented with anthocyanin-rich foods, such as blood orange juice and berry extracts, have been shown to reduce insulin resistance and blood Glc levels (Guo et al., 2007; Prior et al., 2008). However, when purified anthocyanins are used instead of anthocyanins in their native nutritional context, the same impact on weight gain is not observed (Prior et al., 2008; Titta et al., 2010). There are numerous reports of the efficacy of phytonutrients being heavily influenced by their dietary context (Eberhardt et al., 2000; Liu, 2003, 2004; de Kok et al., 2008), a fact that may well explain much of the conflicting evidence in the literature on the roles and effectiveness of dietary phytonutrients in promoting

health. Clearly, the nutritional context is very important in determining the efficacy of a phytonutrient at promoting health, and this fact needs to be taken into consideration in both experimental design and the subsequent formulation of dietary recommendations. Nutritional context could influence the effects of polyphenols by affecting their bioavailability or be the result of different dietary phytonutrients acting synergistically (Liu, 2003, 2004; de Kok et al., 2008), once absorbed. In the example where anthocyanins in blood orange juice limited the weight gain of mice on a high-fat diet, whereas purified cyanidin 3-glucoside had no significant impact on weight gain (Titta et al., 2010), the most likely explanation is that the food matrix impacts bioavailability, since cyanidin 3-glucoside is relatively unstable in solution and interaction with other compounds in orange juice may improve its stability. Alternatively, an explanation for the differing efficacy between anthocyanins in orange juice and dissolved in acidified water may lie in synergistic interactions of the anthocyanins with other metabolites in the juice (Liu, 2003, 2004).

The impact of the food matrix on the efficacy of phytonutrients in promoting health could be assayed by comparing the effects of phytonutrients accumulated to equivalent extents in different foods on inception and progression of disease in animal models. The anthocyanins accumulated in some varieties of potato, such as Purple Majesty, are the same chemical species as those that accumulate in tomato. Consequently, comparison of the health-promoting effects of anthocyanins in tomato and potato could be made to gauge the impact of large amounts of starch (in potato) on the efficacy of anthocyanins in protecting against disease. Differences in bioavailability determined by differences in the food matrix would be reflected by different levels of the phytonutrients or their metabolites in plasma or urine of animals. Differences in bioavailability potentially determined by the food matrix could be investigated further using model gut technology or cell-based systems that report adsorption such as CaCo2 cells (Mandalari et al., 2008a, 2008b, 2010; Cencic and Langerholc, 2010).

IDENTIFICATION OF THE MECHANISMS OF ACTION OF PHYTONUTRIENTS IN REDUCING CHRONIC DISEASE

Because isogenic foods provide true matched matrix controls to gauge the effects of dietary phytonutrients, perhaps the greatest contribution they can make to the field is identification of the mechanisms of action of phytonutrients in counteracting disease. An indication of the insight that can be provided by such studies is given by the experiments investigating the mechanisms of cardioprotection by anthocyanin-rich diets in the rat (Toufektsian et al., 2011). The levels of plasma lipids in rats fed for 8 weeks on diets supplemented at 20% with either purple maize (providing 0.24 mg anthocyanin per g food pellet) or yellow maize (no anthocyanins) were measured. No differences were found in total levels of saturated and monounsaturated fatty acids nor in total n-6 PUFAs between the two dietary groups, but there were significantly higher levels of n-3 PUFAs in the plasma of animals fed the high anthocyanin diet and consequently enhanced n-3:n-6 PUFA ratios in the plasma of these animals. These enhanced n-3:n-6 PUFA ratios were also observed in plasma of animals

fed anthocyanin-rich diets supplemented with palm oil (rich in monounsaturated fat, with no n-3 PUFAs) and in those fed anthocyanin-rich diets supplemented with fish oil (rich in n-3 PUFAs). These analyses demonstrated that high levels of dietary anthocyanins can give rise to alterations in endogenous PUFA metabolism that favor the formation of anti-inflammatory n-3 PUFAs. Such effects had been suggested from epidemiological studies of red wine drinkers (de Lorgeril et al., 2008; di Giuseppe et al., 2009), but the complexity of the foods involved and the absence of appropriate controls did not allow the mechanistic interpretation of the role of dietary flavonoids. By contrast, the isogenic maize lines allowed for relatively highly controlled studies of the mechanism of action of dietary anthocyanins, which are supported by the data from the human epidemiological studies. The beneficial impact of high n-3:n-6 PUFA ratios on CVD is well recognized (Bucher et al., 2002; He et al., 2002; Maillard et al., 2002; Erkkilä et al., 2003; Iso et al., 2006; Sontrop and Campbell 2006; von Schacky and Harris, 2007; Ramel et al., 2009; Riediger et al., 2009), and it may be that the primary mechanism of action of dietary anthocyanins is through their modulation of plasma n-3 PUFA levels. n-3 PUFAs are believed to promote health in various ways: through altering membrane fluidity, beneficially altering the binding of inflammatory cytokines to their receptors, and promoting the production of less inflammatory (anti-inflammatory) eicosanoids compared with n-6 PUFAs. It is possible that the diverse effects of anthocyanins on different chronic diseases (Butelli et al., 2008; Toufektsian et al., 2008; Vauzour et al., 2008; Spencer, 2009; Titta et al., 2010) reflect their primary mechanism of action, which is to modulate endogenous n-3 PUFA metabolism and enhance n-3:n-6 PUFA ratios. Alternatively, it may be that the impact of anthocyanins on n-3 PUFA metabolism is just a part of their physiological mode of action; these mechanistic questions are now amenable to investigation through the use of tools such as those developed by plant breeding and plant metabolic engineering.

INVESTIGATION OF THE EFFECTS OF COMBINATIONS OF PHYTONUTRIENTS ON HEALTH

The contribution that plant science can make to investigation of the impact of plant-based foods on human health is essentially reductionist. It can normalize much of the complexity of foods allowing the stepwise investigation of the effects of phytonutrients on chronic disease inception and progression and allow the investigation of the impact of other food components on phytonutrient efficacy, as described above. Consequently, these systems can also be used to investigate the effects of combinations of phytonutrients and to observe whether they work additively or synergistically against the inception and/or progression of chronic diseases. This potential is exemplified by the experiments establishing cardioprotection by anthocyanin-rich diets in rats (Toufektsian et al., 2008) and equivalent experiments demonstrating protective effects of dietary n-3 PUFAs on myocardial resistance to ischemia-reperfusion injury (Zeghichi-Hamri et al., 2010). Investigation of the degree of cardioprotection offered by anthocyanins on the fish oil-supplemented diets described by Toufektsian et al. (2011) would allow the establishment of

whether anthocyanins and n-3 PUFAs work additively or synergistically to promote cardioprotection and would provide additional insight into the mechanisms of action of dietary flavonoids.

INVESTIGATIONS OF THE EFFECTS OF PHYTONUTRIENTS ON DIFFERENT DISEASES

Once isogenic food materials have been prepared, the impact of target phytonutrients on a range of different chronic diseases can be assessed. Thus, the rodent pellets supplemented with purple or yellow maize that were developed to test whether dietary anthocyanins can give cardioprotection (Toufektsian et al., 2008) could also be used in the obesity model to assess the impact of maize anthocyanins on weight gain by mice on a high-fat diet and many other disease models. An efficient strategy for testing different phytonutrients is to undertake preliminary investigations with cell-based models of different chronic diseases to determine whether preclinical feeding studies with disease models are worth undertaking. Other advantages of isogenic foods are that they can be used for producing extracts for cell-based assays, used as supplements for whole animal studies, and used to make foods for human intervention studies. In this way, the studies on phytonutrients can progress from models to humans using exactly the same experimental food material. Where health-promoting properties have been reported, as, for example, for blood orange juice (Titta et al., 2010), this scientific information may then be used to drive development of new, improved food products and inclusion of these food products in dietary recommendations, both general recommendations and personalized ones, for example, such as might be developed for subjects at risk of complications of the metabolic syndromes CVD, type 2 diabetes, and obesity.

CONCLUSIONS

Although there are fairly obvious ways that research on plants can contribute to human health, generally the discipline of nutrition has avoided study or understanding of plants beyond basic analytical measurements of foods. Progress in understanding how dietary plant material can promote human health could be made by encouraging much closer multidisciplinary collaborations between plant breeders, plant metabolic engineers, chemists, nutritionists, experimental medics, clinicians, and epidemiologists. Such collaborations are difficult because researchers in these different fields speak different scientific languages and often have different expectations of their research. Such interdisciplinary research could provide the foundations of a new discipline investigating the potential for health promotion through crop/food improvement. Importantly, this research will require public funding to promote public trust and to avoid promotion of single proprietary foods, which has so often happened with industry-sponsored research in the past (Rowe et al., 2009). There also needs to be a conceptual shift away from the view that chronic disease is the realm of pharmaceutical treatments only. The potential impacts of such shifts in scientific philosophy and policy are enormous; they could impact the health of all people and particularly act against rising obesity,

which is a major contributor to the global burden of chronic disease and disability. Recent increases in consumption of more energy-dense, nutrient-poor foods with high levels of sugar and saturated fats, combined with reduced physical activity, have led to obesity rates that have risen threefold or more since 1980 in some areas of North America and Europe. Obesity poses a major risk for serious diet-related chronic diseases, including type 2 diabetes, CVD, hypertension, stroke, and certain forms of cancer. The health consequences of obesity range from increased risk of premature death to serious chronic conditions that reduce the overall quality of life. Of special concern is the increasing incidence of childhood obesity. Understanding which dietary phytonutrients and plant fibers can limit weight gain in obesity models and reduce the risk of associated chronic diseases could contribute significantly to reducing the incidence of this problem.

There will also be a direct impact of this type of research on dietary recommendations. The message that individuals should try to consume a variety of food products that are rich in phytonutrients on a daily basis is generally accepted. However, the scientific basis for this strategy of improving health through diet is largely missing because of the imprecision with which the contribution of individual phytonutrients is understood. What is needed are precise dietary recommendations based on understanding the scientific basis of the link between dietary phytonutrients and health, the levels at which they offer the best protection, the phytonutrients that are best, whether they are best consumed as supplements or in foods, and whether one phytonutrient-rich food is equivalent to another.

One of the most alarming facts to emerge from the 5-a-day campaign is that despite putting out dietary recommendations to consume at least five portions of fruit and vegetables a day for the past 25 years, still <25% of Americans achieve this level of intake. Similar figures apply in European countries (Boffetta et al., 2010; <http://www.guardian.co.uk/uk/2007/dec/30/schools.schoolmeals>; <http://www.telegraph.co.uk/health/healthnews/7572108/Britons-eating-fewer-vegetables-despite-5-a-day-campaign.html>; http://www.who.int/dietphysicalactivity/media/en/g_s_fv_ppt_mmeyer.pdf). This means that despite broadly disseminated public information programs on how to eat healthily, people are eating less well than they did in the past. It is extremely difficult to get people to change their diets; therefore, it is vital to target dietary improvement to the young. It may also help to add back the health-promoting phytonutrients that are missing from many convenience foods. This can be done only on the basis of understanding the mechanisms of action of dietary bioactives and the importance of food context on their efficacy. In some crops, this can be done by breeding, but for others, the genetic variation for phytonutrient enrichment either does not exist or is not adequate to provide health-promoting phytonutrients at sufficient levels. In tomato, which is consumed widely in pizza, ketchup, and other fast foods, high levels of anthocyanins (3 mg per g fresh weight) can be achieved by genetic engineering, (Butelli et al., 2008). The high anthocyanin, purple tomatoes had demonstrable, health-promoting effects on the life span of cancer-prone mice. This research raised awareness of the importance of anthocyanins in the diet. Growers of introgression varieties of tomatoes, containing anthocyanins, albeit at significantly lower levels than in the lines created by Butelli et al.

(2008), started to advertise these varieties as being health promoting (http://fbae.org/2009/FBAE/website/news_08_09_sun-black.html), and recently new varieties of anthocyanin-rich potatoes have been bred and marketed with similar claims for their health-promoting properties (for example, <http://www.bbc.co.uk/news/uk-scotland-11477327>). Research in this area will have a significant impact on fresh fruit and vegetable businesses. It may also advance the possibilities for health-promoting genetically modified foods with significant consumer advantages.

Food security, as defined by the Food and Agriculture Organization of the United Nations, exists when all people, at all times, have physical and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life. Through the development of plant research to address how plant-based foods contribute to human health, the broader objectives of food security for the 21st century will be addressed for worldwide improvements in our quality of life.

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