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Protective Effects of Dietary Polyphenolic Phytochemicals on Nutrition Transition-Related Cardiovascular Disease

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ABSTRACT: The causal link between massive socio-economic and technological development, nutrition transition, and diet-related chronic disease epidemiology is now well established. Evidence from epidemiological and clinical studies has identified significant reduction in the consumption of fruits, vegetables and other plant-based foods in their traditional, natural forms, as one major consequence of nutrition transition that promotes pathogenesis of diet-related chronic diseases, such as, cardiovascular diseases and their risk factors. Many non-nutrient phytochemicals of plant foods are key mediators of nutritional physiology and health, serving to protect against diet-related CVD and risk factors. This review focuses on the emerging evidence for the protective effects of polyphenolic phytochemicals on risks of diet-related CVD and their possible mechanisms of action. Epidemiological evidence, substantiated by numerous human trials and dietary interventions in humans using polyphenol-rich foods, associates high consumption of certain classes of polyphenols, notably flavonoids (flavones, flavonols, catechins, isoflavones) and lignans, with reduced risk of nutrition–related CVD. The underlying mechanisms of the cardioprotective effects of these polyphenols are just beginning to be unravelled but they involve the fundamentally antioxidant and other biological activities of polyphenols, including their vasoactive, oestrogenic, anti-inflammatory, and anti-hypertensive effects, and entail, at least in part, the inhibition of pathogenesis of atherosclerosis, a precursor of CVD.

Key Words: Polyphenolic phytochemicals; Flavonoids; Cardiovascular diseases; Pharmafoods; Nutraceuticals.

Introduction

It is has been recognised since the agrarian and industrial revolutions in Britain and Europe during the 16^{th} – 18^{th} centuries that a society undergoing a massive socio-economic and technological development may witness equally striking changes not only in lifestyles, food supplies and dietary pattern but also in disease epidemiology. The change in dietary pattern that accompanies rapid socio-economic development and urbanization is termed nutrition transition. Scientific interest in this phenomenon was rekindled towards the end of the 20^{th} century by epidemiological reports linking shifts from the pre-industrial traditional diet to the Western diet with the high incidence of adult-onset diabetes mellitus and other non communicable, chronic diseases among the aboriginal Amerindians (1,2) and Australians (3) who had given up their traditional diets for the Western food culture. More recently, there have been growing reports that nutrition transition related chronic diseases are increasingly becoming a major cause of death in the middle-and lower-income countries as well (4-6) The development was attributed to the attendant changes in the structure and composition of diet, in particular, the relatively higher intake of certain nutrients like fat lacking in unsaturated fatty acids (7,8) the relatively reduced intake of carbohydrates in their natural unrefined forms (9-11) and the reduced consumption of plant-based diets (10) coupled with the shift to sedentary lifestyles(12,13).

The nutrition transition-related diseases arise because in the course of adopting and adapting to a new lifestyle and food culture that is totally different from the traditional diet, there is a progressive reduction in the level of intake of the dietary components with beneficial health effects and an equally progressive increase in the intake of those with adverse health effects. Of recent, there has been an upsurge of scientific interest in the healthful effects of plant based diets (11, 14, 15) generally and of their component non-nutrient phytochemicals(16-19), specifically. This apparently stems from the recognition that a plant- based diet regimen provides the lowest content of promoters and the highest content of inhibitors of metabolic dysregulation that lead to major causes of disease and debility in adults over 40 years of age (5) and that non-nutrient phytochemicals may be one of the major inhibitors of metabolic dysregulation and, consequently important mediator of health (16, 18-20). The positive mediatory effect of dietary fibre (non starch polysaccharides plus lignin) on human health is already well recognized (21-23). This review will focus on the emerging biomedical evidence in support of the reported protective effects of non-nutrient, dietary phytochemicals other than dietary fibre, in particular, the polyphenols, on nutrition-related, non-communicable, chronic diseases, their bioscientific basis, and the underlying biologic mechanisms of their mediation of the identified roles. However, given the multiplicity of non-communicable chronic diseases associated with nutrition transition, detailed review will be limited to cardiovascular disease. The review will be done from the perspective of determinants of nutrition transition and its health outcome.

Determinants of Nutrition Transition and Related Chronic Diseases

Nutrition transition is especially apparent among rapidly developing populations undergoing (demographic) transition from a pre-industrial, traditional society to an industrial, urban society(12, 16) because rapid socio-economic development generally engenders major shifts in food supplies, dietary pattern and lifestyle (especially physical activity in both work and leisure) with concurrent shifts in disease patterns (epidemiological transition) as well (6, 10). A comprehensive study of the situation in Asia and the Pacific, for instance, has demonstrated that nutrition transition is a central part of a sequence of three causally linked transitions, namely demographic, nutrition and epidemiological transitions, in that order (10).

Causally Linked Transitions

Demographic transition is characterized by a shift away from a rural society with a low life expectancy at birth and families with many children, intense physical activity to an urban society with a higher life expectancy at birth with few children and reduced physical activity at work and leisure(24). With respect to physical activity, increased urbanization and technology lead to a shift from physically active to sedentary occupations, less walking and cycling, more use of cars and television, increased use of labour saving devices at work and home, and changes in income profile (10). In the case of nutrition transition, there is typically, a shift away from the mixed plant-and animal-based diet of the hunter-gatherer pre-industrial traditional diet towards a more varied diet that includes pre-processed food, more of food of animal origin (especially those like eggs, milk and dairy products and meat that are major sources of saturated fatty acids), more added sugar and fat, and often more alcohol (16, 25, 26). In other words, there is a trend towards more refined, less bulky more energy dense diets with increased proportion of saturated fat-bearing animal foods. It has been estimated that, for all developing countries combined, the per capita consumption of beef, mutton, goat, pork, poultry, eggs and milk rose by an average of 50% per person between 1973 and 1996 (27). The most immediate result of the combination of transition to a relatively energy dense diet with physically inactive lifestyle is a rapid increase in overweight and obesity (17,28) which are important risk factors of major nutrition-related chronic diseases, such as, adult-onset diabetes mellitus(16,17,29) and cardiovascular disease(6,10,29) Diabetes mellitus and cardiovascular disease (CVD) are among the products of nutrition transition-linked epidemiological transition Epidemiological transition is characterized by a shift from endemic nutrient deficiency and infectious diseases, mostly of early life, to epidemic of nutrition related noncommunicable chronic diseases, generally of later life(30). Among the major noncommunicable chronic diseases which manifest later in life and whose incidence become epidemic during prolonged nutrition transition are adult-onset diabetes mellitus, cardiovascular diseases (i.e. coronary heart disease, ischaemic heart disease or ischaemic stroke and peripheral vascular disease and their precursors like atherosclerosis and hypertension) and certain cancers such as those of the colon, rectum, endometrium, lung and breast (6,10). They manifest later in life presumably as a consequence of increasing access to a modern healthcare system which controls infectious, parasitic, and nutritional diseases and allows most of the population to reach ages in which chronic diseases manifest themselves (17). This range of chronic degenerative diseases were previously regarded as diseases of the affluent industrial society but by mid-1990s had become global public health problems (6,31) and a leading cause of death in developing countries including low income countries of the tropics (5,12,17). A lot of work has been done globally in the past two decades on nutrition and epidemiological transitions and the dominant trends in the emerging data suggest that some of the nutrition related chronic diseases become epidemic at a speed that is a function of the velocity of demographic and nutrition transitions,

and that they may emerge as epidemics in a predictable sequence (10). For example, while adult-onset diabetes emerge at the early stages of dietary changes and economic development and become a public health problem within a generation, cardiovascular diseases and cancers of the colon and rectum emerge at later stages and may take two generations to become epidemic.

The nature of the shift in the structure or pattern of diet during nutrition transition varies from country to country as a result of differences in culture and socioeconomic environment. Consequently, the sequence and rate of emergence of chronic diseases during nutrition transition and the stages at which they become epidemics, vary among populations. Accordingly, a determination and understanding of the characteristic features of nutrition and epidemiological transitions in a given population must precede the design and implementation of any intervention initiative. Such well informed intervention initiatives have led to effective control of nutrition-related chronic disease epidemics in several populations (1, 3, 32-34).

Chronic Disease: A Consequence of Disruption of Homeostatic Balances.

The determinants of nutrition transition and its health consequences have been reviewed from the evolutionary perspectives(13,16). Human nutritional physiology reportedly reflects evolutionary adaptations to patterns of ingestion of both nutrient and nonnutrient components of the diet. Based on available scientific data, it has been postulated that nutrition transition-related chronic diseases emerge basically because of disruption of a number of balances (homeostasis) that had existed prior to the onset of demographic and nutrition transitions: balance between consumption of energy and of micronutrients; between nutrient intake and other environmental factors, including nonnutrient components of the diet; between energy intake and physical activity in work and leisure; and balance between body physiology and certain bioactive nonnutrient components of the diet (nutrition ecology) consumed on a routine basis(16,35). Human interaction with these dietary and nondietary environmental factors is complex and for populations that live in closely defined relationships with the natural environment, such as, those engaged in traditional forms of subsistence agriculture where the ingestion of a range of phytochemicals from plant foods, condiments, beverages and herbal medicine may be part of their normal ecology, the disruption of the inherent homeostasis in their way of life can have negative consequences to body physiology and health (16). Our primate legacy, fossil hominid, and hunter-gatherer lifestyles selected for adaptive metabolically thrifty genotypes and phenotypes have been rendered deleterious through modern lifestyles that increase energy input and reduce output(13) and reduce consumption of nonnutrient phytochemicals (16,35). Many of the essential nonnutrient phytochemical components of plant foods are lost during modern processing of grains or refining of vegetable oils. Apart from disrupting the homeostatic relationships with the natural environment, processing and refining destroy the possible synergistic interactions that may exist among the natural constituents of a foodstuff vis-a-vis the beneficial health effects of the food(36). This can be better appreciated against the background of evidence from several studies(11,14,15,36) indicating that a high consumption of plant-based foods, such as, fruits and vegetables, nuts and whole grains, is associated with a significantly lower risk of coronary heart disease, stroke and hypertension, obesity, and adult-onset diabetes mellitus.

Causes of Variations in Incidence of Chronic Disease

Although all humans are physiologically similar, there are well known genetic differences among populations which reflect adaptations to specific dietary cultures, for example, lactase deficiency among the adult population of ethnic groups with no history of keeping dairy animals (16). Besides, genetic heterozygosity (37,38) and acquired metabolic defects like foetal and infant inadequate nutrition insults (39,41) within populations may affect the manner individuals in a given population may respond to dietary change. A growing body of evidence reviewed by Waterland and Gaza (39) and Popkin and co-workers (10) suggests that early nutrition-related insults may contribute to later diet-related chronic diseases, especially, cardiovascular diseases, obesity, hypertension, and adult-onset diabetes.

Manifestations of foetal and early childhood nutrition insults include low birthweight and stunting. Low birthweight and stunting have both been linked with risks of chronic diseases such as hypertension and CVD later in life (10,42). The biologic mechanisms underlying this phenomenon, otherwise called foetal programming or metabolic imprinting, have been reviewed (39,43) but are, by and large, still unclear. It is believed that early nutrition-related insults may also compromise immune function (44). However, the large variation in risk of chronic diseases within and between populations suggests that behavioral or lifestyle factors, such as, diet and physical inactivity, may play a major role in the aetiology of nutrition transition-related chronic diseases, especially, cardiovascular disease (17). A standardized study of glucose intolerance and high blood pressure in four representative African origin populations as a test of a hypothesis on genetic cause of adult–onset diabetes, for instance, has shown that energy imbalance and intergenerational socioeconomic influences are much more likely causes of diabetes (and probably most other chronic diseases for which diabetes is a major risk factor) than ethnic or genetic variation, which does occur, poorly related to phenotype(45). Non-

genetic intergenerational mechanisms considered in the study include amniotic growth factors and maternal exposure. Evidence from molecular and genetic epidemiological studies indicate that dietary imbalance can alter gene-nutrient interactions in ways that increase the risk of developing chronic diseases(38).

Differences in diet culture have served to explain significant inter-population variations in the incidence of nutrition-related chronic disease, with lower incidence being explained by protective effects of certain components of the diet. Plant foods, as mentioned earlier, account for the bulk of the dietary components associated with protective effects against nutrition-related chronic diseases. The protective plant constituents are mostly those liable to be removed during processing/refining of foodstuffs (e.g. dietary fibre, plant pigments); those found only/more abundant in plant foods and consequently, their consumption is more likely to be adversely affected by the accelerating shift away from the traditional plant-based diet during nutrition transition(e.g dietary fibre, other non-nutrient phytochemicals, unsaturated fatty acids); and those that exert their protective effects only when in their natural forms and consequently, their protective activity is destroyed by processing or refining (e.g. starch polysaccharides). The protective effects of nonnutrient phytochemicals other than dietary fibre, against nutrition-related chronic diseases became increasingly manifest with the observation of a number of cases of low incidence of nutrition-related chronic disease that could neither be explained by the nutrient components of the diet nor by other classical risk factors (i.e incidence of paradoxes) coupled with the emergent scientific evidence of the potential beneficial health effects of certain classes of non-nutrient phytochemicals, in particular, the polyphenols. Incidentially, the best known of these paradoxes in chronic disease incidence are those concerning cardiovascular diseases (CVDs) and which were subsequently explained by the cardioprotective effects of polyphenols in the regular diet.

PHENOLIC PHYTOCHEMICALS AND LOW CVD INCIDENCE PARADOXES

The protective effects of non-nutrient dietary phytochemicals have explained a number of paradoxical inter-population variations in the incidence of CVD, the best known cases being the French(46,47),the Maasai(16,48) and the Mediterranean(49) paradoxes. Emerging evidence indicates that non-nutrient phytochemicals exert their protective effects through mechanisms involving certain novel CVD risk factors rather than the conventional or classical risk factors (i.e., age, sex, genetic, disposition, elevated serum cholesterol and blood pressure, smoking, physical inactivity and obesity). Among the novel risk factors are oxidative stress, endothelial dysfunction, homocysteine concentration, inflammation and thrombosis(17,50). These emerged following a greater understanding of the processes involved in atherosclerosis development and heart disease. Strong epidemiological evidence and a number of clinical trials on intermediary health outcomes have engendered the belief among medical scientists that treating elevated homocysteine (homocysteine lowering) and the other novel risk factors would be important in CVD prevention(50).

The French Paradox

The relatively low rate of atherosclerosis and coronary heart disease among the French population compared to other industrialized European countries with similarly high intakes of saturated fatty acids and comparable serum cholesterol, blood pressure and prevalence of smoking, has been attributed to a lower consumption of whole milk and greater consumption of plant foods and red wine(46) and the French custom of drinking red wine with meals(47). The apparent protective effect of red wine has been attributed largely to polyphenolic compounds contained in it (16,17), based on the generally known antioxidant properties of polyphenolic compounds. They are believed to mediate the observed positive effects of red wine on blood platelet aggregation and lipid metabolism(51). It has been postulated that the polyphenolic compounds contained in red wine retard the progression of atherosclerosis by protecting against lipid peroxidation, particularly of polyunsaturated LDL- cholesterol(16). There is good evidence that oxidative damage to lipids and lipoproteins, particularly LDL oxidation, is linked to development of atherosclerosis(17). However, subsequent studies based on a greater understanding of the processes involved in the development of atherosclerosis, plaque rupture, and thrombosis, suggest that polyphenols may protect against CVD by influencing novel risk factors like vascular endothelial function(17,20). A European Union-funded study on wine and cardiovascular disease recently reviewed by Kelly (17), has provided some insight into how red wine polyphenols may protect against heart disease through modulation of endothelial function.

The pathophysiology of the three main forms of CVD (i.e coronary heart disease, ischaemic stroke, and peripheral vascular disease) is similar and involves atherosclerosis and thrombosis. The development of the former entails impairment of endothelial function. Endothelial dysfunction is considered an early step in the process of atherosclerosis(17,20). The normal healthy endothelium has many anti-atherogenic functions, including, regulation of blood flow, inhibition of blood clotting and prevention of adhesion of inflammatory cells to blood cells lining, which are performed through mediators like nitric oxide, NO and prostacyclin released by the endothelium(17). Endothelial dysfunction is characterized by a decreased bioactivity of NO and impaired flow-mediated vasodilation(20). In that

unhealthy state, adhesion molecules on the surface of activated endothelium mediate the attachment of leukocytes to endothelium. From there, the leukocytes can infiltrate into the artery wall, become resident or macrophages and then, can engulf large amounts of lipid, leading to atherosclerosis. Evidence from the EU-funded study on wine and cardiovascular disease suggest that red wine polyphenols may indeed exert their protective effects by preventing endothelial dysfunction.

The EU-funded study found that red wine polyphenols extract (RWPE) mediated a vasorelaxant effect via the stimulation of NO release from the endothelium in rat aorta, mediated improved vascular tone in rabbits via a reversal of cholesterol-induced endothelial dysfunction, and prevented mechanisms relevant for *in vivo* plaque development, including leukocyte adhesion to endothelial cell and reduction of expression of intercellular adhesion molecules(17). Four polyphenolic components of RWPE were found to induce mechanisms relevant for vasoconstriction; they are two anthocyanins (delphinidin and petunidin), a flavonol (quercetin) and a stilbene (resveratrol). These are among the polyphenolic compounds long suspected to be among the active anti-atherogenic agents in red wine(51).

The Maasai Paradox

The incidence of atherosclerosis and CVD among the Maasai pastoralists of East Africa is low despite their high intakes of fat and cholesterol and low intake of carbohydrates(52,53). The Maasai obtain up to 66% of their energy from fat(52) and subsist on diet high in milk, yogurt and meat(53) , a dietary culture associated with high incidence of CVD (28). Several hypotheses have been propounded to account for the paradoxical situation(51), but none was fully satisfactory until the Maasai ethnobotany factor was brought into the equation, initially by Day and coworkers (48). The subsequent verification of the ethnobotany factor was based on the fact that the Maasai routinely ingest wild plant materials as foods, as ingredients of milk and meat-based soups, as masticants, and as herbal medicines(54).

The Centre for Nutrition and the Environment of Indigenous Peoples of Mc Gill University, Canada has examined plant foods and food additives used by the Maasai of Kenya and Tanzania in relation to their possible role as hypocholesterolaemic agents(16). About 82% of the Maasai food additives screened were found to contain potentially hypocholesterolaemic phytochemicals, including saponins and phenols. On the strength of the findings and known biological activities of the identified phytochemicals(55,56), it was postulated that saponins, polyphenols and phytosterols in foods, medicines and masticants contribute to the phenomenon of low rate of atherosclerosis and coronary heart disease among the Maasai(16). However, there appears to be as yet no epidemiological study to test this hypothesis to confirm the existence of an inverse relationship between the occurrence of these phytochemicals in the Maasai diet and the incidence of cardiovascular diseases. Nonetheless, evidence that plant polyphenols exert positive cardiovascular health benefits and as a result, are serious candidates in explanations of the protective effects of plant foods and additives against CVD as well as other nutrition-related chronic diseases is now well documented(18-20,57,58).

The Mediterranean Paradox

The Mediterranean paradox is the lower incidence of coronary heart disease among the southern European populations inhabiting the Mediterranean region, compared to the rest of Europe, despite comparable blood lipid profiles, blood pressure, saturated fat consumption and smoking. The paradox has been attributed to the protective effects of the traditional Mediterranean diet(49). The Mediterranean diet typically uses olive oil as the major culinary fat and it is to this dietary component that the cardioprotective effects of the Mediterranean diet has been traced.

Unrefined olive oil or extra-virgin olive oil, is rich in monosaturated fatty acids and contains a considerable amount of polyphenolic compounds, such as, hydroxytyrosol and oleuropein(17,59-61). The polyphenolic compounds are responsible for the peculiar, pungent taste and high stability of extra-virgin oil(60). There is good evidence that olive oil polyphenols are powerful antioxidants both *in vitro* and *in vivo* and that they also exert other biological activities that could partially account for the observed healthful effects of the Mediterranean diet (60,61). Evidence from reports of the several human trials reviewed by Patrick and Uzick (59) show that olive oil has a therapeutic effect on myocardial infarction and exerts anti-hypertensive, antioxidant, and anti-inflammatory effects and that olive oil polyphenols rather than the monounsaturated fatty acids (MUFA) may account for the greater part of the observed cardioprotective effects of olive oil.

Among the most celebrated of the early human trials on the cardiovascular health effects of olive oil was the Lyon Diet Heart Study which compared a Mediterranean diet with standard post-infarction “prudent Western diet” in patients who had suffered a first major cardiac infarction. The dramatic improvements recorded early in the trial, with 76% reduction in cardiac events after only 27 months in a 5- year trial, persisted during a mean follow-up of 46 months(49). The study, involving 605 post-infarct patients, achieved a greater reduction in coronary mortality using simple dietary changes than any reported cholesterol-lowering study (62). One of the human trials that provided evidence that the

polyphenols played a more prominent role in the cardioprotective effects of olive oil reported in epidemiological studies and human trials was the study in which the effects of extra-virgin olive oil on blood pressure of mild to moderate hypertension patients were compared to those of sunflower oil (63). Olive oil supplementation produced significantly lower systolic and diastolic blood pressures than sunflower oil supplementation and at the end of the trial, all patients on the sunflower diet required medication while eight patients receiving olive oil diet had no need for drug therapy. Olive oil and sunflower oil are rich in MUFA and polyunsaturated fatty acids (PUFA), respectively. Were unsaturated fatty acid content to be the major cardioprotective principle, sunflower oil diet would have produced the more pronounced effect on hypertension. However, while extra-virgin olive oil is rich in polyphenols which are significantly reduced but not completely absent in refined olive oil(64) polyphenols are completely absent in sunflower oil.

Olive oil polyphenols are believed to exert their protective effects on cardiovascular disease and its risk factors, such as, hypertension and atherosclerosis, through multiple biological mechanisms that largely exploit their antioxidant and anti-inflammatory properties, among others. Several studies with polyphenols extracted from extra-virgin olive oil have shown that they significantly inhibit key elements in the pathogenesis of CVD(17,60,61,65,66). Some of their beneficial activities, such as, inhibition of LDL oxidation(67), reduction of blood pressure(63), and reduction of macrophage uptake of LDL-cholesterol(67) have been confirmed in human trials. The two biomolecular events, LDL oxidation and macrophage uptake of LDL, are key factors initiating intimal cell injury, foam cell formation, and, ultimately, atherosclerosis(68), a precursor of CVD. Nonetheless, a lot more work still need to be done to clearly understand the underlying biologic mechanism whereby olive oil and its polyphenols exert the reported protective effects on cardiovascular disease.

DIETARY POLYPHENOLS ASSOCIATED WITH PREVENTION OF CARDIOVASCULAR DISEASE.

The interest of nutritional science in the healthful effects of plant food ploy phenols has grown beyond resolving the French and other paradoxes. The interest shown in the potential beneficial health effects of polyphenolic compounds in plant foods, beginning from the mid-1990s, has been so phenomenal that before the end of the decade, it culminated in an international conference (The 1st International Conference on Polyphenols and Health, Vichy, France, November 18-21, 2004), one of the main objectives of which was to review the evidence for healthful effects of polyphenols in humans from both clinical trials and epidemiological studies(18).

A critical review of the more than 350 communications presented at the conference (18,19) has led to a number of salient inferences on the beneficial effects of plant polyphenols to human cardiovascular health. These are (a) polyphenols clearly improve the status of different oxidation stress biomarkers; (b) clinical trials in which polyphenols were administered as supplements or with food, have established that some polyphenols do improve cardiovascular health status as indicated by several biomarkers closely associated with risk of cardiovascular disease; (c) epidemiological studies tend to confirm the protective effects of polyphenol consumption against cardiovascular disease; and (d) several types of plant polyphenols have beneficial effects on human cardiovascular disease. More significant progress has been made in the field of beneficial effects of polyphenols on human cardiovascular diseases than on other nutrition-related chronic diseases

Committed biomedical research interest in the health effects of plant polyphenols was stimulated by epidemiological reports suggesting a protective effect of fruits and vegetables on cancer and cardiovascular diseases. Plant polyphenols were considered as serious candidates for the active mediators by the various hypotheses suggested to explain these beneficial effects because they are a large group of natural antioxidants ubiquitous in a diet high in vegetables and fruits(19). A considerable body of literature supports a role for oxidative stress in the pathogenesis of age-related diseases and a contribution of dietary polyphenols to their prevention. In addition to their antioxidant properties, plant polyphenols show several other interesting effects in animal models and *in vitro* systems, which are relevant to the biologic mechanisms that may contribute to their potentially protective roles in CVDs. Specifically, they trap and scavenge free radicals, regulate nitric oxide, decrease leukocyte immobilization, induce apoptosis, inhibit cell proliferation and angiogenesis, and exhibit phytoestrogenic activity(19).They also alter cholesterol homeostasis and increase LDL–receptor activity in human cells *in vitro*(69).

Plant phenols are a very large and diverse group of phytochemicals, comprising about 12 major classes, based on general chemical structure (Table 1); this excludes lignin, a component of dietary fibre. The most ubiquitous of secondary metabolites in plants(70) , all plant phenols are derived from the common intermediate, L-phenylalanine (a protein amino acid), or its close precursor, shikimic acid, through the shikimic acid pathway in plants, and characteristically contain at least one aromatic ring structure with one or more hydroxyl groups. Most of these major classes are, in turn, large and diverse, consisting of several sub-classes. Among the major classes of polyphenols strongly associated with protective effects on cardiovascular disease are flavonoids, stillbenes, lignans and biflavonoids. Those of their members which exert their protective effects via biologic mechanisms involving interaction with oestrogen receptors and/or interference with oestrogen function are classified as phytoestrogens.

Flavonoids

The flavonoids are a very widespread family of structurally related water soluble phenolic compounds largely confined to vascular plants. Over 6000 different flavonoids occurring in plants have been described (19) and they can be grouped into at least nine major classes (see Table 2) based on the oxidation state of the C_3 residue of the phenylpropane unit of the molecule(71). Many flavonoids, such as, anthocyanins (blue to mauve), flavonols (yellow, cream), and flavonones (cream-white), are coloured and serve as major plant colorants. The anthocyanins, for example, are widespread as petal and fruit colorants(72). Consequently, flavonoids are widely found in fruits and vegetables as well as nuts and other plant foods. However, comprehensive data on their contents in foods are only available for flavonols, flavones, and catechins(19).

Like other polyphenols, flavonoids, by virtue of their molecular structures, have antioxidant, free radical-scavenging and electron transport-catalysing properties(73). These chemical properties derive from a hydroxyl group at the C-3 position in ring C, a double bond between C-2 and C-3, a carbonyl group at the C-4 position, and multiple hydroxylation of the A and B aromatic rings. The biological activities of flavonoids, including their health effects, are largely determined by the three major characteristic chemical properties.

A large body of evidence from epidemiological studies and human trials, recently reviewed(19,74), strongly associates high consumption of flavonoids with a reduced risk of cardiovascular disease. Investigations, mostly *in vitro*, designed to elucidate the underlying molecular mechanisms mediating the protective effects of flavonoids against CVD indicate that they exert those effects by reducing oxidative stress, inhibiting low density lipoprotein(LDL) oxidation and platelet aggregation, acting as vasodilators in blood vessels, inhibiting the adherence of monocytes to vascular endothelium, promoting fibrinolysis and acting as immunomodulators and anti-inflammatory agents(74), all of which are relevant events in atherogenesis. They are also believed to affect cellular signalling pathways, modulate cell membrance characteristics and receptor functions and influence gene expression and protein activity(75). Flavonoids' intake reportedly improves insulin resistance and glucose tolerance(19), two biomarkers of adult-onset diabetes mellitus which is a risk factor of CVD.

The epidemiological evidence for an inverse relationship between flavonoid intake and risk of CVD has been confirmed by dietary interventions in humans using flavonoid–rich foods(76). Schroeter and co-workers(20), for example, have shown in healthy male adult that the ingestion of flavonoid-rich cocoa was associated with acute elevation in levels of circulating NO species, an enhanced flow-mediated vasodilation response of conduit arteries, and an augumented microcirculation, which are positive indices of improvement in cardiovascular health. The classes of flavonoids that have been associated with protective effects on cardiovascular health in the epidemiological and human intervention studies include flavonols, flavones, catechins and isoflavones.

Flavonols, Flavones and Catechins.

Twelve prospective, cohort studies on flavonoid intake and risk of coronary artery disease and five prospective, cohort studies on the risk of stroke were published as at the time of the 1st International Conference on Polyphenols and Health(19). Seven of the 12 studies on the risk of coronary artery disease found protective effects of flavonols and flavones or of catechins with respect to fatal and non-fatal coronary artery disease and reductions in mortality were up to 65%. Two of the five studies on stroke risk found an inverse association. In one of the two studies, a protective effect was observed for flavonols and flavones but not for catechins. The nine sets of data suggest protective effects on CVD of high intakes of flavonols and flavones and possibly catechins(19). A greater fraction of studies to date have focused on flavonols and flavones.

Flavonols found to exert protective effects on cardiovascular health include quercetin, kaempferol and myricetin(19,77). Mortality from ischaemic heart disease was found to be lower among Finnish people with high intakes of quercetin(77). Knekt & co-workers also observed an inverse relationship between ingestion of quercetin and myricetin and reduced risk of adult-onset diabetes mellitus, a risk factor of CVD. Quercetin and kaempferol protect against glucose-induced oxidation of human low density lipoproteins (LDL) *in vitro* (78). The catechin (-) – epicatechin and its metabolite epicatechin -7-0- glucuronide have been identified as mediators of the beneficial effects of flavonoid-rich cocoa beverages on vascular functions in human(20). Oral administration of chemically pure (-) – epicatechin to human volunteers closely emulated acute vascular effects of flavonoid–rich cocoa drinks. Several other species of catechins, including (+)-catechin, (+)-gallocatechin, (-)–epigallocatechin, (-)–epicatechin gallate and (-)–epigallocatechin gallate, have been detected in human foods(19) but their individual effects on the vascular system have not been studied. The intake of catechins have been positively correlated with the intake of fruits and vegetables and their antioxidant nutrient constituents(19).

Table 1. The major classes of plant phenols

Class S/No	Class	Basic Carbon Skeleton
1.	Phenols	C_6
2.	Phenolic acids	C_6- C_1
3.	Phenylacetic acids	C_6-C_2
4.	Hydroxycinnamic acids, coumarins, henylpropenes, chromones	C_6-C_3
5.	Quinones	C_6-C_4 (Naphthoquinones) C_6-C_2-C_6 (Anthraquinones)
6.	Xanthones	C_6-C_1-C_6
7.	Stilbenes	C_6-C_2-C_6
8.	Flavonoids	C_6-C_3-C_6
9.	Lignans & neolignans	$[C_6$-$C_3]_2$
10.	Biflavonoids	$[C_6$-C_3-$C_6]_2$
11.	Tannins	$[C_6$-C_3-$C_6]_n$ condensed tannins (flavolans)
12.	Melanins	$[C_6]_n$

Source: Godwin & Mercier (1993)

Table 2: the major types of Flavonoids

S/No	Type	Example*
1.	Flavones	Apigenin(3)
2.	Flavonones	Kaempferol(4)
3.	Catechins (Flavan-3-o1s)	Catechin(5) (-) – Epicatechin(5) (+) – Epicatechin (5)
4.	Flavanones	Naringenin(3)
5.	Dihydroflavonols	Taxifolin(5)
6.	Flavan -3,4-diols (Proanthocyanidins or Leucoanthocyauidins)	Teracacidins(5) Isoteracacidin(5) Leuorobinetinidin(6) Guibaurtacacidin(4)
7.	Anthocyanidins	Pelargonidin(4)
8.	Isoflavones	Genistein(3)
9.	Neoflavones (4-phenylcoumarins)	Dalbergin(1)

Source: Goodwin & Mercier (1993). *Figures in parenthesis represent number of hydroxyl groups per molecule

Isoflavones

Isoflavonoids have been widely associated with reduced risk of cardiovascular diseases and their precursors(17,79,80). The regular consumption of isoflovone-rich foods, such as, soya bean, in China, Japan and other Asian countries is believed to be responsible, at least in part, for the low incidence of CVD in those countries(17). Data from epidemiological reports and laboratories have demonstrated that isoflovones have multi-biological and pharmacological effects in humans and animals, including oestrogenic and anti-oestrogenic effects, cell signalling conduction, and cell growth and death(80). All these mechanisms are relevant to the reported protective role of isoflavonoids against CVD and other nutrition-related chronic diseases like breast and prostate cancers. Thus, it has been postulated that isoflovones protect against CVD not only through their antioxidant and oestrogenic properties but also through their other properties, such as, protein tyrosine kinase inhibitors, regulators of gene transcription and modulators of transcription factors(80).

The range of properties displayed by isoflavones, namely, as an oestrogenic agent, an inhibitor of intestinal glucose uptake and a preventive agent for glucose-induced lipid peroxidation, may also mediate protection against diabetes(78), a risk factor of CVD.

The isoflavones in ingested soyabean, soya products, or other isoflavone-rich foods, are biotransformed by intestinal microflora, the metabolites are absorbed and undergo enterohepatic recycling to reach circulating concentrations that exceed by several orders of magnitude the amounts of endogenous oestrogens (79). Isoflavones and other bioactive phytochemicals with oestrogenic properties are generally classified as phytoestrogens and treated as such.

Phytoestrogens

Data on health benefits of plant products classified as phytoestrogens have been reviewed(81). Evidence from molecular and cellular experiments, animal studies, and to a limited extent, human clinical studies, so reviewed suggests that phytoestrogens may potentially confer beneficial health effects related to cardiovascular diseases and other nutrition-related chronic diseases like cancer. The identified potential health benefits are consistent with the reported epidemiological evidence suggesting that rates of heart disease and other nutrition-related chronic diseases are lower among populations that consume plant-based diets, particularly, among cultures with diets that are traditionally high in phytoestrogen-rich foods. The majority of phytoestrogens found in typical human diets can be categorized into two primary classes: isoflavones and lignans (81), both of which are polyphenols.

Phytoestrogens are found in appreciable amounts in a wide range of commonly consumed foods, with soya and flax products being particularly rich sources of isoflavones and lignans, respectively. Other classes of polyphenol with oestrogenic properties include stilbenes. For example, resveratrol (3, 4', 5-trihydroxystilbene) found in red wine, berries and grapes, among others, has been associated with the low incidence of CVD among the French (16,17).

In terms of molecular structure and activity, phytoestrogens appear to have a number of features in common(18): they have a 2–phenylnaphthalene-type chemical structure similar to those of oestrogens and have been found to bind to oestrogen receptors; they exhibit weak oestrogenic activity of the order of $10^{-2} – 10^{-3}$ that of 17β-oestradiol, but may be present in the body at concentrations 100-fold higher than endogenous oestrogens; and they may exert both oestrogenic and anti-oestrogenic effects on metabolism, depending on several factors such as, their concentration, the concentrations of endogenous oestrogens, and individual characteristics like gender and menopausal status. The anti-oestrogenic activity of phytoestrogens may be partially explained by their competition with endogenous 17 β - oestradiol for oestrogenic receptors. The reported protective effects of phytoestrogens against CVD is consistent with the cardioprotective effects of the endogenous oestrogens which they mimic. For example, morbidity and mortality from CVD reportedly increase sharply in women after menopause, a situation which has been attributed to the loss of the hormone oestrogen (17). However, oestrogenic effects may not be the only biological mechanism through which phytoestrogens exert cardioprotective and other beneficial health effects. Studies have shown that phytoestrogens may also act through influence on growth factor action, vascular smooth muscle cells, lipid oxidation, cell proliferation and differentiation, angiogenesis, enzymes and protein synthesis (80,82,83). In any case, isoflavones, lignans and stilbenes, being polyphenols, are basically antioxidants and it was the antioxidant property of polyphenols that stimulated interest in the health effects of phenolic phytochemicals; antioxidant mechanisms have largely explained protective effects against atherogenesis and CVD. The possible non-oestrogenic mechanisms pertaining to isoflavone were highlighted earlier.

Lignans

Published studies on health effects of lignans were recently reviewed (19). Evidence from several epidemiological studies and human trials reported significant inverse relationships between dietary lignans and risk of CVD. The lignans evaluated in most of the studies were secoisolariciresinol and matairesinol and their enterometabolites. The two and several other lignans are converted by human intestinal bacteria to metabolites collectively called enterolignans, the best known being enterolactone and enterodiol. Significant inverse relationships have been established between plasma concentrations of enterolignans and risk factors of CVD. For example, a cross-sectional study on Finnish male volunteers in the Antioxidant Supplementation in Atherosclerosis Prevention Study (84) established a significant inverse relationship between plasma enterolactone concentrations and the plasma level of F_2- isoprostane, a biomarker of *in vivo* lipid peroxidation.

Other Classes of Polyphenols

Flavonoids, stilbenes and lignans are by no means the only classes of plant polyphenols with bioactivities that may be beneficial to cardiovascular health. They just happen to be the classes of polyphenols on which most studies on health effects of plant polyphenols have been focused. To these classes belong plant polyphenols which, by virtue of their historical antecedents in nutrition transition–related chronic disease epidemiology and chemical structures, are considered more serious candidates for the explanation of the reported protective effects of fruits and vegetables on oxidation-linked chronic diseases such as CVD. A large number of polyphenolic compounds, frequently of diverse chemical structures, is normally found in a given plant food(18) and naturally, only those considered as serious candidates are measured in epidemiological studies and dietary interventions in humans or administered in human trials. However, the beneficial health effects of polyphenols outside these 'elite' classes are now being elucidated largely through animal and *in vitro* cell studies and emerging evidence, in some cases, substantiated by epidemiological studies, has identified other classes of polyphenols with potential beneficial effects on cardiovascular disease. They include phenolic acids like ellagic acid (85) and biflavonoids (86).

Ellagic acid is believed to function either by countering the negative effects of oxidative stress by directly acting as an antioxidant or by activating/inducing cellular antioxidant enzyme systems (85). Biflavonoids have been reported to support arterial wall structural integrity and to interfere with a variety of proatherosclerotic stimuli. Studies on cultured human aortic smooth muscle cells (SMC) suggest that biflavonoids may exert these beneficial effects by, among others, participating in the regulation of SMC–mediated contraction and by countering pathophysiological effects of angiotensin II (86).

Furthermore, ethnopharmacological and ethnobiochemical studies have revealed a wide range of other polyphenols outside the classes discussed above which reportedly exhibited biological activities, *in vitro* and *in vivo*, similar to those observed in studies on polyphenols and the risk of CVD. For example, the aqueous extract of the stem bark of *Sacoglottis gabonensis*, a Nigerian palmwine additive, and its isocoumarin polyphenol isolate, are potent anticoagulants and antioxidants (87). Like the plant polyphenols reported to exert cardioprotective properties, the isocoumarin polyphenol inhibited lipid peroxidation *in vivo*. However, the antioxidant effect was studied in a non-vascular tissue. On the other hand, its anticoagulant action affected two of the clotting factors considered as new risk factors of CVD(59). On the other hand, there are many ethnopharmacological reports of plant food extracts with polyphenol–like cardioprotective effects in which the active principles were not characterized. For example, extracts of a number of spices used by Tibetan highlanders were found to exert strong antioxidant effect on human LDL *in vitro* and to display strong free radical-scavenging activity(88), both of which are typical properties of plant polyphenols.

Conclusion

It is now generally recognized in nutrition science that plant food polyphenols are key mediators of nutritional physiology and health, and that many polyphenols, especially, flavonoids, lignans and stilbenes, exert protective effects on CVD largely by virtue of their biological antioxidant properties which derive from their characteristic chemical structures, and several of their other wide ranging bioactivities, notably, oestrogenic, vasoactive, anti-inflammatory and anti-hypertensive properties. The recognition has been made possible by the large, growing body of convincing evidence from epidemiological studies, human trials and dietary interventions as well as animal and human cell studies. Most studies on the mode of action of polyphenols on health have focused on inhibition of the process of atherosclerosis as the probable principal mechanism through which plant polyphenols protect against CVD and have, indeed, demonstrated that polyphenols associated with protective effects against CVD strongly interfere with various aspects of atherogenesis, including, inhibition of endothelial dysfunction and reduction of macrophage uptake of LDL-cholesterol.

One major indicator of the increasing recognition of the beneficial effects of plant polyphenols on cardiovascular health is the growing literature on dietary intervention initiatives exploiting the growing knowledge of the healthful effects of polyphenolic phytochemicals. The current dietary intervention initiatives range from statutory dietary guidelines in Europe and USA recommending a high consumption of fruits and vegetables on a regular basis(15,36) to the use of plant polyphenol extracts as antioxidant food supplements(89,90). The ongoing efforts at securing statutory standards and approval for the use of plant polyphenol extracts as antioxidant food supplements were preceded by a largely secular initiative, namely, the emergence in the late 1980s of the concept of and advocacy for "functional foods" (i.e foods or food ingredients modified to provide health benefits beyond the traditional nutrients they contain) and its various ramifications like "designer food", "pharmafood" and "nutraceuticals" (91).

The role of plant polyphenols as mediators of nutritional physiology and health is expectedly progressively relegated in nutrition transition since the rate of consumption of plant foods in their natural, unrefined forms is typically reduced in the course of nutrition transition. The characteristic shift from the predominantly plant-based diet of the traditional, rural, pre-industrial society to the Western food culture of the industrial society would lead to a significant reduction in the

level of consumption of plant food polyphenols, with the attendant consequences on cardiovascular health. The rate at which nutrition transition proceeds and the impact it has on diet-related CVD epidemiology vary from population to population and are dependent on the pace of socio-economic and technological development and certain other factors that are unique to each population (e.g; nutritional ecology, traditional food culture). Thus, for an effective intervention policy, the determinants of nutrition transition in a given population, including the polyphenol profiles of the traditional plant foods, condiments and additives and beverages must first be ascertained. This would imply that a lot more work still need to be done in the area of identifying and documenting plant polyphenols that contribute to the reported protective effects of plant-based foods, such as, fruits, vegetables, nuts and whole grains, on the risk of cardiovascular disease as virtually all the reported studies on effects of plant polyphenols on cardiovascular health were done in Europe and America.

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