Bioavailability of Dietary Polyphenols and the Cardiovascular Diseases

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Abstract: Epidemiological studies indicate that the higher intake of fruits and vegetables may reduce the risks of many degenrative diseases like cancer, cardiovascular disease, cataract etc. This is attributed mainly to the intake of dietary polyphenols as seen in Mediterranean diets. However, the bioavailability of polyphenols is reported to be low due to poor absorption in the gut, intestine and colon and depends on the type of compounds, chemical structure, food matrix, extent of conjugation and individual colon microflora. In general, flavonoids, aglycones and pure compounds are absorbed more when compared to the glycosides. Diversity in intestinal mocroflora also contributes to a great extent for the variation in absorption of polyphenols as seen in a few studies for the absorption of isoflavones. Among the polyphenols, isoflavones are known to be more bioavailable followed by phenolic acids, flavanols, flavanones, flavonols and lowest bioavailability was seen for anthocyanins and proanthocyanidins.

Many human and animal studies have shown that dietary polyphenols reduce the cardiovascular diseases by inhibiting LDL oxidation, promoting vasodilation and by antiplatelet properties. However, the relationship between the level of polyphenols in plasma and their *in vivo* cardioprotective effects are poor. It is clear that more studies with improved methods are needed to understand the involvement of polyphenols in reducing the risks of degenerative diseases. In order to translate the *in vitro* results to *in vivo*, bioavailability of dietary polyphenols have to be increased significantly. One way to achieve this would be to study food preparation methods that can increase the bioavailability of these compounds through the use of different additives, cooking methods, enzymes and microorganisms. Microorganisms appear to play an important role in increasing the bioavailability of polyphenols by removing the conjugation and by breaking polyphenols into simpler absorbable phenols. We feel more bacteria and fungi should be used in food preparations such as yeasts for bread and wine making; lactic acid bacteria for fermenting idlies, dosas, curds; and *Bacillus* strains for soy fermentation products to increase bioavalability of polyphenols. Enzyme treated or microbial digested food may become the future of food industry. This article was written with a view to supplement Dr. R. B. Singh's life long ambition to prevent cardiovascular diseases through the use of diet and discuss the above points in greater detail.

Keywords: Dietary polyphenols, bioavailability, cardiovascular diseases, food preparation methods, processing.

INTRODUCTION

Atherosclerosis is the major cause of mortality in the western world, and involves interactions among cells of the arterial wall, blood cells, and plasma lipoproteins [1]. Epidemiological studies have shown the importance of fruit and vegetable enriched diets on the reduced risks of degenerative diseases and the effects have been attributed to presence of antioxidants in the diets [2, 3]. Inverse relationships between plant-derived foods and the risk of coronary heart disease have been reported by many workers [4-8].

Dr. Singh and his co-workers have studied the importance of consumption of fruits and vegetables, whole grains and almonds and walnuts (Indo-mediterrenean diets) [9-12], physical activity [13-15], w-3-fatty acid/w-6 fatty acid ratio [16], antioxidant vitamins [13, 17, 18], magnesium [19] for control of cardiovascular diseases (CVD). Role of nutraceuticals and the expression of genes involved in stress responses [20] in regulating the coronary artery disease have also been studied in detail by this group. Composition of a diet for reducing the risk of CVD in South Asian population has also been recommended [21]. Along with diets rich in nutraceuticals and high in w-3/w-6 fatty acids, the bioavailability of polyphenols are also important for better health benefits from fruits and vegetables.

Dietary antioxidant capacity is related mainly to the total polyphenol content of fruits and vegetables [22-26]. Polyphenols clearly improve the status of different oxidative stress biomarkers [27]. Much uncertainty, however, persists regarding both the relevance of these biomarkers as predictors of disease risk and the appropriateness of the different methods used [28]. It is well established that some polyphenols, administered as supplements with food, do improve health status, and this is indicated by several biomarkers of cardiovascular risk [29-31].

It is also now well established that polyphenols undergo substantial metabolism after ingestion and the plasma concentrations usually ranges from 0 to 4 μmol/L [32, 33] in

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spite of their high concentration in the diet. Therefore, it is essential that we understand the mechanisms involved in the bioavailability of different polyphenols and their relevance for human health. This review will analyse the relationship between plasma concentrations of polyphenols and various factors affecting the atherosclerosis using human or animal intervention studies only rather than *in vitro* experiments.

BIOAVAILABILITY OF POLYPHENOLS

Physiologically active plasma concentration of phenolic compounds is dependent on the bioavailability of polyphenols in humans. Clifford [34] reported that daily intake of total polyphenols and tannins may range from less than 100 mg to in excess of 2 g, with major contribution from coffee and tea. However, only 5% of the dietary polyphenol is absorbed in the duodenum. Over 95% of the intake passes to the colon and is fermented by the gut microflora. A fraction of the microbial metabolites is absorbed and appears in the plasma as mammalian conjugates. Many workers have explained the fate of polyphenols in human digestive system [35-38], a synthesis of this information is presented in pictorial form (Fig. **1**) for easy understanding of the process.

a) Flavonoids

Flavonoid glycosides are absorbed to a limited extent [38] and are cleaved by gut bacterial or human intestinal β-glycosidases [39]. Flavonoid aglycons are conjugated in mucosa and liver by enzymes (UDP-glucuronosyltransferase, sulfotransferase, and catechol-O-methyltransferase) [40]. Bacteria in the lower intestine hydrolyze and metabolise the flavonoid conjugates after biliary excretion, which results in reabsorption of the flavonoid aglycons [40-42].

Quercetin

Quercetin is observed in plasma after the consumption of onions, tea, and apple juice [43-46]. Quercetin and its metabolites retain their antioxidant activity [36] in plasma. Most abundant metabolites of quercetin in rats were the glucuronic and sulfate conjugates of isorhamnetin [36]. Benzoic acid derivatives have also been reported as the major metabolites of quercetin [47] probably due to microbial degradation [48].

Catechins

Epigallocatechin gallate and epicatechin gallate are detected in plasma and urine after tea consumption [49] and o-methylcatechin after red wine consumption [50]. Gallic acid derivatives and 1, 3, 5-trimethoxybenzene were reported to be the major metabolites of epicatechin and epicatechin gallate in healthy humans [47]. Intake of 20 g cocoa significantly increased the metabolites (microbial) and conjugates of (-)-epicatechin, in 24 hr urine and plasma samples of humans [48].

Manach *et al.,* [32] in an exhaustive review of 97 bioavailability studies on humans concluded that the plasma concentrations of total metabolites ranged from 0 to 4 μmol/L with an intake of 50 mg aglycone equivalents, and the relative urinary excretion ranged from 0.3% to 43% of the ingested dose, depending on the polyphenol. Bioavailability of polyphenols have been ranked by some authors, a summary of the ranking is presented in Fig. (**2**).

b) Phenolic Acids

Variation in the absorption rates of phenolic acids in the gastric region was observed in rats. The absorption rate was in the order of p-coumaric acid > ferulic acid > caffeic acid > gallic acid > chlorogenic acid [53].

Ferulic Acid

This compound increased rapidly in plasma after the ingestion of tomatoes and beans [54] or beer [55] and reached peak urinary excretion at 7 or 8 h for tomatoes or beer, respectively and was observed in plasma [56-59]. Free acid are 10 to 17 times more bioavailable than esterified phenolic

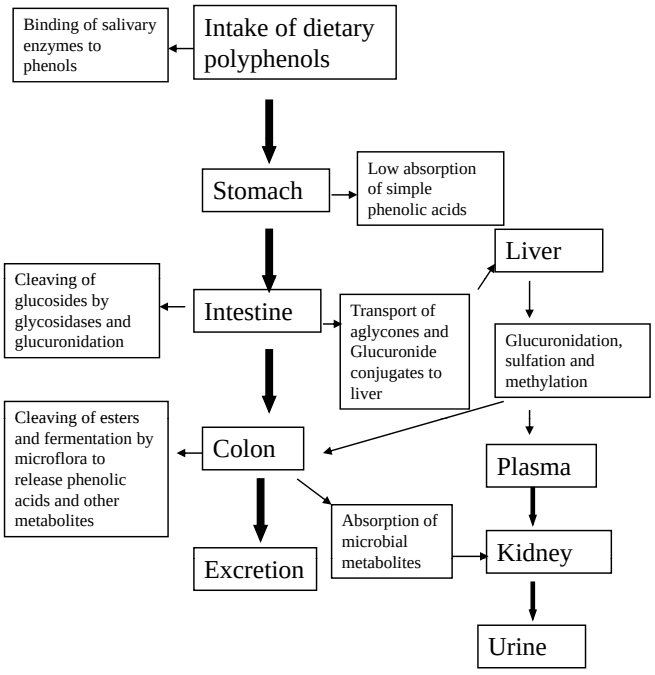

Fig. (1). Metabolism of polyphenols in humans.

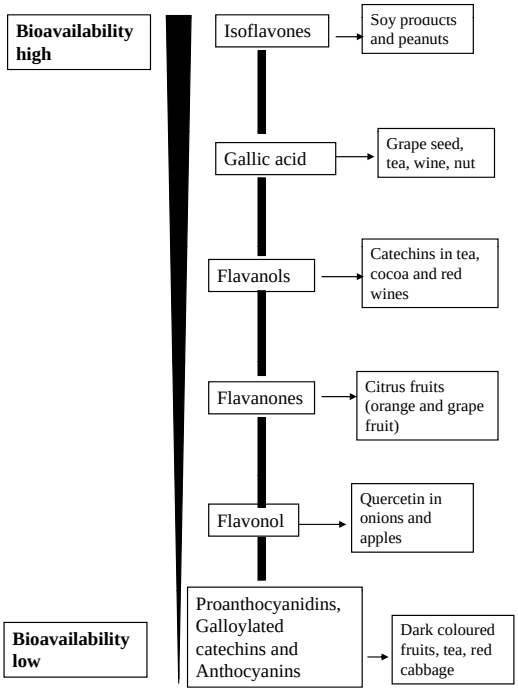

Fig. (2). Bioavailability ranking of polyphenols [Derived from; 32, 51, 52].

acids in humans [60-62]. Phenolic acids could be absorbed in stomach [53] and also intestine and the esterified acids will be metabolised by the colonic microflora.

Chlorogenic Acid

Plasma concentration and the rate of absorption of chlorogenic acid (ester of caffeic and quinic acid) were 100 and 2.4 times less respectively than that of caffeic acid [63, 64]. Urinary excretion of chlorogenic acid was only 0.3% [65]. Bioavailability was not affected by the additions such as milk or sugar in rats [66] and was found in plasma of humans after the consumption of coffee, prune or pure chlorogenic acid [67-69].

Gallic Acid

Plasma concentration of gallic acid reached 4.7 μM after 2 h of Assam tea (200ml) (50 mg of GA) consumption and the urinary excretion was almost 40% [70] and this was similar to the absorption by the ingestion of pure gallic acid [71].

Caffeic Acid

Olthof *et al.* [65] showed in a human study that after ingestion of 2.8 mM of caffeic acid (505 mg), 11% of the ingested dose was excreted in the urine. When volunteers consumed red wine, caffeic acid was detected in plasma [70, 72]. Wide variation among individuals for plasma caffeic acid (58-176 ng/ml) levels was reported when 200 ml of coffee was given [73].

Ellagic Acid

Ellagic acid was found in plasma as ellagic acid and in urine as microbial metabolites in studies using pomegranates, strawberries, raspberries, walnuts or oak-aged wine [74-76].

The above discussion indicates that the phenolic acids are mainly absorbed in the intestine. Identification of microbial metabolites of phenolic acids in urine also indicate the role of colon microflora in the absorption of phenolic acids. Absorption of esterified form of phenolic acids like chlorogenic acid is significantly lower than the free phenolic acids since they have to be metabolised by the gut microflora before absorption.

c) Proanthocyanidins and Anthocyanins

Proanthocyanidins from chocolates are absorbed after they are metabolised by the gut microflora to many aromatic phenolic acids [77]. These phenolic acids have been found to have antioxidant and antilipid peroxidation activities [78]. Anthocyanin recovery in urine within 12 h of wine consumption in humans was to the extent of 1.5 -5.1% only [79]. They are absorbed and transported in human serum and urine primarily as glucuronide and sulfate conjugates [80]. Main metabolites of anthocyanins were glucouronides and sulfates. When pigs were fed marionberry, pelargonidin was relatively more absorbed than cyanidin glucosides [81]. Delphinidn absorption was much less and di and tri saccharides of anthocyanins were found intact in urine. Only 1.5 to 1.8% of pelargonidin metabolites were found in urine after the intake of strawberry puree [82]. Variations in the absorption of different types of anthocyanins indicate that the absorption depends on the chemical structure and the type conjugation of the anthocyanins.

In addition to the plasma concentration or urinary excretion levels, concentration in target tissues is very important for them to be medically beneficial.

d) Uptake of Polyphenols in Tissues

In a study on rats using ferulic acid, hesperetin and genestein Silberberg *et al.* [83] reported that the extent of biliary and intestinal secretion into lumen as conjugates may indi-

cate the lesser availability for tissues. According to them ferulic acid was more available than the other two.

Manach *et al.* [84] in their review have said that polyphenols are seen in various tissues like brain, endothelial cells, kidney, heart, spleen, pancreas, ovary, testis, prostrate, uterus, bone, skin etc, ranging from 3 to 3000 ng/g tissue. They have also cited a few human studies showing the accumulation of polyphenols in prostrate and breast tissues. Epicatechin and catechin were found in brains of rats when repeated feeding of grape seed extract was done instead of 1 acute feeding [85]. Second dosage of pure theaflavin digallte enhanced the tissue uptake in liver and uptake was enhanced when given with black tea extract [86]. Level of absorption of polyphenols and their concentration in target tissues are important for the health benfiting effects and this could be increased by repeated feeding instead of 1 acute intake.

e) Variability among Individuals in Absorption Rates

Interindividual variation in absorption of soy isoflavones has been observed [87]. Microbial degradation rate leads to interindividual differences in the absorption of flavonoids [47, 88] and formation of equol (metabolite with health benefits) from diadzein [89].

French were found to have more equol production followed by Italians and least was in Dutch indicating the variation was due to intestinal microbial activity [90]. Wide variations in plasma antioxidants can be expected between the individuals for the same intake of fruits and vegetables as seen in a human intervention study [91]. It would therefore be important to understand the influence of the diet on the type of gut microflora to maximize health benefits from polyphenols.

PLASMA POLYPHENOL CONCENTRATION AS IT RELATES TO INHIBITION OF LDL OXIDATION

The LDL oxidation plays a pivotal role in early atherogenesis [92, 93]. The oxidative hypothesis of atherosclerosis has stimulated extensive investigation on the role of antioxidants as a possible preventive treatment for atherosclerosis.

Mediterranean diet known to have beneficial effects in reducing the cardiovascular diseases has about 68% of Total Dietary Antioxidant Capacity coming from beverages and 20% from fruits and vegetables, with a very low contribution from cereals. Total phenolics intake was estimated as 1171 mg gallic acid/person/day by the Folin-Ciocalteau method [94].

Fruits and Vegetable Polyphenols and Inhibition of LDL Oxidation

Quercetin metabolite accumulation after onion consumption was not enough to reduce the LDL oxidation in rats [95]. Ingestion of quercetin, kaempferol, myricetin and apigenin through fruits, vegetables and beverages by human subjects did increase plasma flavonoid content significantly but failed to show any direct effect on LDL protection [46]. Dietary supplementation of lycopene significantly increased serum lycopene levels by at least twofold and LDL oxidation was significantly decreased [96, 97].

Short-term supplementation of freeze-dried strawberries reduced the cholesterol levels and decreased lipid peroxidation in women [98]. Consumption of kiwifruit (2 fruits/day) for 8 weeks significantly reduced LDL oxidation [99]. The inhibition of LDL oxidation was shown in healthy as well as carotid artery stenosis (CAS) patients as well as in aged mice using pomegranate juice [100-102]. The protection of LDL was due to enhanced activity of serum paraoxanase-1 (PON-1) [101], reduced NADH-oxidase and increased activity of glutathione reductase by flavonoids [103, 104]. Consumption of cranberry vinegar (200 ml twice every day for 10 weeks) or cranberry juice (7ml/kg/day) or Concord grape juice (10ml/day) reduced LDL oxidation and thiobarbituric acid reactive substances [105-107]. Concord grape juice effect was similar to alpha-Tocopherol (400 IU per day). Almond skin flavonoids (catechin, epicatechin, quercetin, kaempferol, and isorhamnetin) intake reduced plasma LDL oxidation by 18% and was synergistically increased to 52% when vitamin E was added [108]. Ingestion of soy isoflavonoids genistein (12 mg) and daidzein (7 mg) daily for 2 weeks by human subjects reduced the oxidation of LDLs [109].

Cocoa Flavonoids

Cocoa products reduced the LDL oxidation in humans [110]. Consumption of cocoa enriched chocolates or powders significantly reduced the LDL oxidation [111-113]. However in another human intervention study high-flavonoid chocolate consumption did not show significant increase in inhibition of LDL oxidation and total antioxidant capacity [114]. Time of plasma sampling and dosage levels and also interindividual differences in absorption may be responsible for variation in results.

Tea and Coffee Polyphenols and Inhibition of LDL Oxidation

Green tea flavonoids inhibit the LDL oxidation through the protection of LDL-tocopherol and plasma ascorbic acid in ascorbic acid deficient mice [115] and in apoprotein (apo) E-deficient mice [116]. Enriched onion and black tea diet (high flavonoids) failed to reduce the lipid peroxidation marker in humans [117]. Daily consumption of green tea also decreased serum Malondialdehyde-LDL concentrations [118]. The resistance of LDL to oxidative modification increased significantly after coffee drinking (200ml/day) [119]. Drinking 8 cups of coffee per day significantly increased serum concentrations of total cholesterol, HDL cholesterol, and apolipoprotein A-I and decreased the ratios of LDL to HDL cholesterol [120].

Grape Wine Polyphenols and Inhibition of LDL Oxidation

Red wine has been found to be more potent than white wine or pure ethanol in this regard [121]. Grape-derived flavonoids and resveratrol limit *ex vivo* LDL oxidation [122, 123] and reduced atherosclerosis in rats [124]. Reduced atherosclerosis without a reduction in LDL oxidation within the arterial wall was observed following treatment with dealcoholized red wine in mice [125]. One drink of red wine, beer or stout was found to be beneficial but 3 drinks was

pro-oxidant [126] and the pro-oxidant effect was due to ethanol metabolism.

Benito *et al*., [127] concluded that the intake of flavonoids will be beneficial only if there is an oxidative stress. Intake of too much polyphenol without the proportional increase in other vitamins may not be good since phenols at certain concentrations can act as prooxidants and initiate the LDL oxidation as reported for oleuropein and hydroxytyrosol [128]. Phenols may also produce phenoxy radicals and need ascorbic acid to scavenge them. Therefore, polyphenol supplements may not be beneficial as that of dietary polyphenols.

Metabolites of Flavonoids and Inhibition of LDL Oxidation

Conjugates of quercetin like quercetin glucuronides and sulfates have been found to be equally or more active than the glucoside or aglycone in inhibiting the LDL oxidation. The products of small intestine metabolism (quercetin-7-glucuronide, quercetin-3-glucuronide) are more efficient antioxidants than subsequent liver metabolites such as isorhamnetin-3-glucuronide, quercetin-3'-sulfate. Albumin-bound conjugates retained their property of protecting LDL from oxidation [129].

Although there are a few studies showing no effect of some flavonoids on inhibition of LDL oxidation, majority of the studies indicated a positive effect of consumption of fresh fruits and vegetables, as well as beverages such as coffee, tea and juice. Effect of polyphenols on LDL oxidation is summarised in Fig. (**3**).

POLYPHENOLS AND VASODILATION

Endothelial dysfunction is one of the earliest events in atherogenesis. A consequence of endothelial damage is low availability of nitric oxide (NO), the most potent endogenous vasodilator. Polyphenols and their metabolites have positive effects on the vasodilation of arteries.

a) Fruit and Vegetables and Vasodilation

Maize, cranberry and aubergine were found to have higher vasorealxation effect *in vitro* among many fruits, vegetables, nuts, tea and spices [130]. Intake of fruits and vegetables enriched diets and whole grains reduced the markers for endothelial dysfunction but high fat diet increased the marker levels [131, 132]. Consumption of red wine or fruit and vegetables reversed the high fat induced decline in markers [133]. Consumption of orange and blackcurrant juice was better than vitamin E supplementation in human peripheral arterial patients [134]. Dose dependent response of endothelium mediated fore arm blood flow was observed for fruits and vegetables [91]. Flavonoids enhance vasodilation by increasing the NO synthase activity and expression, by scavenging the peroxynitrite-derived radicals, thereby protecting the cofactor tetrahydrobiopterin that is crucial for NO synthase activity and by reducing the activity of stress induced redox genes [135, 136]. Endothelium dependent vasodilation and reduction of NAD(P)H dependent superoxide anions was seen for citrus extract and ferulic acid in rats [137, 138]. Endothelium independent vasodilation was reported for aqueous extract of *Berberis vulgaris* fruits [139]. However, intake of fruit and vegetable did not show any effect on endothelium independent vasodilation in a study on humans [140].

b) Grape Products and Vasodilation

Enhanced vasodilation through Nitric oxide production and NO synthase activity by red wine, grape juice, grape seed extracts and grape specific polyphenols [141-144] may be due to phosphorylation [145]. Polyphenols may increase NO synthesis through increased flux of Ca^{+2} in short term and expression of NO synthase in long term [146]. Red wine polyphenol extract was found to reduce the plasma homocysteine levels and endothelial dysfunction markers in rats [147]. Among the wine polyphenols kaempferol was found to be more effective in endothelium induced vasorelaxation in rats when compared to myricetin and rutin [148]. However, alcohol could cause a reduction in vasodilation when taken in high dosages and the polyphenols present in wine were unable to reverse it in humans [149].

Consumption of grape polyphenols equivalent to 1.25 cups of fresh grapes caused significant improvement in brachial artery flow mediated dilation in a dose dependent manner [150]. A protective effect of resveratrol on portal vein thrombosis was observed in rats [151]. In a recent review Schini-Kerth *et al*. [152] concludes that polyphenols induced endothelium-dependent relaxation also involves endothelium-derived hyperpolarizing factor, besides NO, in several types of arteries.

c) Cocoa Products and Vasodilation

In a human intervention study high-flavonoid chocolate consumption improved endothelium-dependent flow-mediated dilation (FMD) (40%) independent of plasma antioxidant activity [114] and through increased NO production [153]. Many studies have reported significant increase in

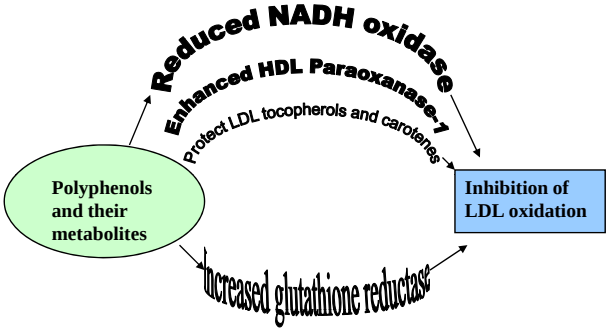

Fig. (3). Mechanism of LDL oxidation inhibition by polyphenols.

flow mediated vasodilation after consumption of cocoa products [154-157]. Corti *et al.* [158] in their review summarise the major effects of cocoa flavonoids as increasing the bioavailability of endothelial NO, improvement in endothelial function, the reduction in platelet function.

d) Tea and Coffee Polyphenols and Vasodialtion

Consumption of black and green teas improved endothelium-dependent arterial vasodilation [159-162] mainly by increasing the NO synthase activity [163]. Attenuation of atherosclerotic lesion in apolipoprotein E $(ApoE)^{-/-}$ gene–knockout mouse was by quercetin and theaflavin (tea polyphenol) indicate the beneficial effect of tea flavonoids [164].

Caffeinated coffee (100 mg of caffeine) was found to reduce the vasodilation in humans indicating the ill effects of caffeine on cardiovascular diseases [165]. On the contrary, caffeine at a dose of 300mg showed a positive effect on fore arm blood flow in young men [166] indicating the nitric oxide mediated vasorelaxation effect of caffeine. However, Riksen *et al.* [167] in a review on coffee and cardiovascular diseases concluded that the detrimental effect of coffee consumption in triggering the coronary events is only in selected patients and not in general population. A dose dependent positive effect of decaffeinated coffee on endothelium mediated vasorelaxation was seen in humans [168]. This indicates the beneficial effect of coffee without caffeine and the results on the effects of caffeine are not conclusive.

e) Metabolites of Polyphenols and Vasodilation

Isorhamnetin and a sulphate metabolite of quercetin were found to be more effective than quercetin [169] on endothelium independent vasorelaxation but quercetin glucouronide metabolite did not show any effect [170].

Dietary polyphenols from fruits, vegetables, tea, wine, juice and cocoa products were found to have significant vasodilation effects both *in vivo* and *in vitro*. Vasodilation effect was mainly brought about by the increased activity of endothelial nitric oxide synthase as well as by endothelial independent mechanisms. Dose dependent effects have been observed for some of the polyphenols indicating the importance of maintaining the plasma levels of polyphenols to get the significant health benefits.

POLYPHENOLS AND PLATELET AGGREGATION

Platelet aggregation is a crucial mechanism in the pathogenesis and clinical expression of coronary acute syndrome. Polyphenol supplementation, either as purified compounds or food extracts, showed some inhibitory effects, both in humans and in animal models. The observed inhibitory effect of polyphenols on platelet aggregation might explain and suggest a role for polyphenols in helping to prevent cardiovascular diseases [119].

Grape Juice and Wine

Grape juice consumption and resveratrol decreased platelet aggregation and superoxide production in healthy volunteers [141, 171]. Grape seed and skin in combination increased the antiplatelet effect [172] mainly due to the synergistic effect of polyphenols [173]. Antiplatelet effect of grape polyphenols was due to Ca^{+2} mobilisation mediated activation of the platelet endothelial cell adhesion molecule-1 [174]. In an experiment on mice with pure compounds of resveratrol, quercetin and gallic acid it was observed that resveratrol and quercetin can inhibit arachidonic acid-induced platelet aggregation but not gallic acid mainly due to the interaction of gallic acid at the level platelet COX-1 enzyme [175]. Dealcoholised red wine completely inhibited the ADP induced platelet aggregation, procyanidins, catechins and monomeric anthocyanidins fraction of red wine also reduced the platelet aggregation whereas, flavonols, resveratrol and polymeric anthocyanidin fraction did not show any effect on platelet aggregation [176].

Fruits

Berry consumption at a dose of 160g/d (total polyphenols 837mg/d) did not show any effect on plasma biomarkers of platelet aggregation however, did show an increased inhibition of platelet aggregation *ex vivo* [177]. Consumption of 2 or 3 kiwi fruits for 28 days reduced platelet aggregation [178]. Antiplatelet effect has been reported for tomato [179] and resvereatrol [180], grape seed extract, chokeberry extracts [181] and mulberry [182]. Pomegranate juice and pomegranate fruit extracts were effective in reducing platelet aggregation, calcium mobilization, thromboxane A_2 production and hydrogen peroxide formation, induced by collagen and arachidonic acid [183]. Other beverages, such as orange juice and grapefruit juice did not show antiplatelet effect [184].

Cocoa

Dietary flavanols from cocoa inhibit the platelet aggregation [155, 185-189] suppressed platelet activation and platelet micro particle formation in humans [190]. Short-term cocoa polyphenol supplementation did not decrease platelet activity in response to exercise in humans [191]. Cocoa consumption had an aspirin-like effect on primary haemostasis and suppressed unstimulated and stimulated platelet activation in whole blood and was found to be better than dealcoholised red wine or pure procyanidins in healthy subjects [187].

Flavonoids like quercetin, apigenin and genistein have been shown to reduce the thrombin responses by interfering in the intracellular signalling and through inhibition of kinases [192]. Polyphenol rich meal every lunch time for 5 days did not show any significant change in *ex vivo* platelet aggregation even though plasma level of flavonoids increased significantly [172] may be due to less than the effective concentration of flavonoids [193, 194].

Olive Oil

Virgin olive oil polyphenol hydroxytyrosol acetate inhibits *in vitro* platelet aggregation in human whole blood when compared to acetyl salicylic acid. This effect involved a decrease in platelet thromboxane synthesis and an increase in leukocyte nitric oxide production [195, 196].

Tea

Tea consumption reduces platelet activation and plasma C-reactive protein in healthy men [197]. Tea polyphenols alleviated the thrombosis in a dose dependent manner in rab-

bits [198]. Hernandez Figueroa *et al.* [199] suggest that a daily intake of 7 cups of green tea is a good measure for coronary heart disease prevention. However, tea consumption could not show any effect after high fat diet intake in humans [200].

Synergistic Effect of Polyphenols

Anthocyanins, metabolites of polyphenols of colonic origin and their mixture showed significant dose dependent activity against platelet aggregation and activation [201] and the mixtures were more active than the individual compounds indicating the synergistic effect. Synergistic effect was also reported for quercetin and catechin in reducing platelet recruitment further proves that the polyphenols are more effective in combination [202].

Results indicate that the polyphenols have a significant antiplatelet effect when tried under *in vitro* conditions. Most of the studies have been done using polyphenol extracts against isolated platelets. However, the *ex vivo* studies using animals and humans have given mixed results. Therefore more animal and human experiments are required to conclusively prove the antiplatelet effects of polyphenols. Use of proper biomarkers for platelet aggregation status of the blood may improve the reliability of results instead of *ex vivo* experiments. Significant synergistic effects of polyphenols have been reported indicating the benefits of having diets with a mixture of polyphenols. Therefore, inclusion of a combination of fruits and vegetables in diet is more beneficial rather than having only a few items.

METHODS OF FOOD PREPARATION AND THE BIOAVAILABILITY OF POLYPHENOLS AND OTHER NUTRIENTS

Diets with high fats and meats increased the inflammation and endothelial dysfunction markers whereas, diets with fruits, nuts and vegetables and whole grains had significantly reduced the markers [131, 132] indicating importance of dietary components as well the food preparation methods of these diets. Food preparation methods may have an influence on the bioavailability of polyphenols and indirectly on human health.

Polyphenol Content and Food Preparation

Changes in polyphenol content of diets with various methods of cooking and food processing is given in Table **1**.

Table 1. Change in Polyphenols Due to Various Cooking and Processing Methods

Methods	Polyphenols	Food	Reference
Cutting	Increase in cyanidin	Red lettuce	[203]
Shredding	Decreased cyanidin	Red onions	[204]
	Decreased cyanidin	Red oak, Lollo Rosso	[205]
Juicing	68% loss in anthocyanins	Berries	[206]
Steaming and juicing	85% loss in quercetin and 70% in myricetin	Billberries	[207]
Cooking in water (Boiling)	Loss in anthocyanins	Berries	[208]
	70-85% loss in quercetin and kaempferol	Broccoli	[209]
	Increased polyphenols	Carrot, Onion and Potato	[210]
	Decreased polyphenols	Broccoli and White cabbage	[210]
Steam cooking	Increased polyphenol, carotenoids and tocopherols	Broccoli	[211]
	No reduction in kaempferol	Red beans	[212]
	Increase in polyphenols	Onions and Broccoli	[210]
	Deceased polyphenols	White cabbage, potato and carrot	[210]
Shallow frying	Antioxidant capacity increased but 20% loss in polyphenols	Vegetables	[213]
Frying	No loss of anthocyanin	Sweet potato	[214]
Microwave heating	Reduce loss of polyphenols	Olive oil	[215]
Mocrowave cooking	Reduced lipid peroxidation	Vegetables	[216]
	Increased polyphenols	Potatoes, onion and carrots	[210]
	Decreased polyphenols	White cabbage	[210]
Boiling and frying	Increased lipid peroxidation	Vegetables	[216]
Blanching	Reduced polyphenols	Fenugreek leaves	[217]
Soaking and dehulling	Reduced polyphenols	Foxtail millet	[218]
Germination	Greater loss of polyphenols		[219]
Fermentation	Increased superoxide scavenging ability	Soy germ	[220]
Osmotic dehydration	25% loss of polyphenol	Apples	[221]

Bioavailability of Polyphenols and Food Preparation

Processing

Lycopene is more readily absorbed from processed tomato paste and puree at 308 fold higher rate than raw tomatoes as it is released from the cellular matrix during processing [222-224]. Higher uptake of lycopene from processed and cooked tomatoes is mainly due to the conversion of *trans* to *cis*-lycopene [222, 225, 226]. The *cis*-Lycopene increased by 5 fold due to heat processing of guava juice [227]. However, processing of blackcurrant had no significant effect on the oral bioavailability of anthocyanins [228].

Food Additives and Bioavailability

Milk, Juice and Fat

Addition of milk to various foods affects the polyphenol absorption, however the results are not uniform [229-234]. Addition of fat increased the absorption of flavonoids in cocoa and strawberries [235, 236]. However, addition of yogurt to orange juice or cream to strawberries did not influence the uptake of flavonoids [237]. Food matrix like wine or juice did not affect the absorption of quercetin or catechin [238]. Addition of 10% NaCl and wheat dietary fibres to soybean resulted in loss of isoflavones and reduced absorption respectively [239, 240]. Isoflavone bioavailability did not vary significantly when soybean isoflavones were ingested by human subjects mixed with juice, cookies and bars [241]. Similar serum isoflavones and equol values were observed in this case. It is indeed worthwhile to study the effect of addition of salt, sugars, acids, oils and spices during food preparation on the bioavailability of polyphenols.

Cooking and Bioavailability of Polyphenols

Cooking increased the bioavailability of kaempferol from seed coats of beans [212]. Extrusion cooking of sorghum (flavonoids), cooking of tomatoes (naringenin and chlorogenic acid), carrots and broccoli (carotenes) increased the bioavailability [242-244].

Fermentation, Enzyme Treatment and Bioavailability of Polyphenols

Bacterial fermentation of soy products increased the bioavailability of isoflavones, saponins, phytosterols, tocopherols and superoxide scavenging ability when compared to seed based products [217, 245-249] mainly due to the formation of isoflavone aglycones.

From the available literature we can conclude that fermentation of foods can increase the bioavailability of isoflavones, addition of little fat, disruption of food matrix and cooking can increase the bioavailability of certain polyphenols, lycopenes and carotenoids. Food additives like salt can reduce the bioavailability of some polyphenols whereas fibres can delay but may improve the absorption.

DIFFERENT METHODS TO INCREASE THE BIOAVAILABILITY AND EFFICIENCY OF PLANT BASED POLYPHENOLS

Only a few methods were reported to increase the bioavailability of polyphenols as listed in Table **2**.

More work needs to be done on increasing the bioavailability of polyphenols using different food preparation methods, instead of increasing the polyphenol content of fruits and vegetables. Many fruit and vegetables already have

Table 2. Bioavailability of Polyphenols from Foods Prepared Using Different Methods

Methods	Food	Absorption	Reference
Fermentation with *Bacillus subtilis* strain or *Aspergillus* fungi	Cooked soybeans and soy flour	Increased isoflavones	[250]
Enzyme digestion with beta-glycosidase and fermentation	Soy milk	Increased isoflavones	[251]
Liquid versus solid form	Soy milk versus textured vegetable protein	Increased absorption from soy milk	[252]
	Soy milk, bar and powdered drink	Increased from soymilk	[253]
Additives			
Raspberry to bread or ice cream	Raspberry phenols	Decreased phenol	[254]
Raspberry with mince	Raspberry anthocyanins	Increased anthocyanin	[254]
Fat salad dressing or addition of fat	Vegetable salads	Increased carotenoid absorption	[255, 256]
Ascorbic acid, Sucrose or soy milk or bovine milk or fruit juice	With Tea	Increased recovery and absorption of catechins	[257-259]
Piperine to curcumin	Curcumin	Increased absorption of curcumin	[260]
Piperine	Epigallocatechin gallate from tea	2 fold increase	[261]
Frequency			
More frequent versus single acute intake	Grape seed polyphenol extract	2-3 fold increase in gallic acid, catechin and epicatechin absorption	[85]

a high content of polyphenols. Polyphenol content is very high in cocoa, tea and coffee and also in red wines but the bioavailability is considered very poor in these plant based products. Therefore one of the challenging areas of work would be to increase the bioavailability of dietary polyphenols using food preparation methods. Available literature indicate that disruption of food matrix, addition of fat, cooking, treatment with enzymes, addition of ascorbic acid, milk and fermentation can increase the bioavailability of dietary polyphenols. We believe a good understanding of polyphenols and increasing their bioavailability will go a long way to prevent cardiovascular diseases, a major area of interest Dr. R. B. Singh has.

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