Hypertension, Nitric Oxide, Oxidants, and Dietary Plant Polyphenols

Monica Galleano[a], Olga Pechanova[b] and Cesar G. Fraga[a,c,*]

[a]*Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, University of Buenos Aires-CONICET, Junin 956 C1113AAD, Buenos Aires, Argentina;* [b]*Institute of Normal and Pathological Physiology and Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovak Republic;* [c]*Department of Nutrition, University of California, One Shields Ave., Davis, CA 95616, USA*

Abstract: Fruits and vegetables are key foods whose high ingestion is associated with the improvement of numerous pathological conditions, including hypertension. Such health promoting actions have been increasingly ascribed to the antioxidant characteristics of different polyphenols in fruits and vegetables. Consequently, based on this assumption, many beverages and foods rich in polyphenols, grape, tea, cocoa, and soy products and many of their chemical constituents purified, are being studied both, as antioxidants and antihypertensive agents. This paper reviews the current evidence linking high polyphenol consumption with reductions in blood pressure. Basic chemical aspects of flavanols, flavonols, isoflavones and stilbenes, as possible responsible for the observed effects of those foods on blood pressure are included. Human intervention studies by using grapes and wine, cocoa and chocolate, black and green tea, soy products, and purified compounds ((+)-catequin, quercetin, (-)-epigallocatechin gallate) are summarized. The discussed hypothesis, strongly supported by experimental data in animals, is that by regulating nitric oxide bioavailability, polyphenols present in fruits and vegetables affect endothelial function and as a consequence, blood pressure. Even when data are not definitive and many questions remain open, the whole evidence is encouraging to start considering diets that can provide benefits to hypertensive subjects, and those benefits will be more significant in people that do not have controlled his/her elevated blood pressure.

Keywords: Antioxidants, hypertension, nitric oxide, NOS, NOX, renin-angiotensin, polyphenols.

1. INTRODUCTION

Cardiovascular disease, including stroke, is the leading cause of death and disability in developed countries, and hypertension is a key modifiable risk factor for these pathologies. Hypertension is a highly prevalent cardiovascular risk factor in the USA with nearly 50 million people having elevated blood pressure (BP) (30% of the adults according to the US National Health and Nutrition Examination Survey, NHANES), and this figure is similar in practically any adult population group in the world. Untreated hypertension is important, counting for about 30-40% of hypertensive people in the USA, and 60-75% in Canada and European countries [1]. An elevation in the systolic or diastolic BP is a risk factor associated with heart and kidney disease, atherosclerosis, diabetes, eye damage, and stroke. These complications of hypertension are normally the result of high BP during years. In addition, hypertension is closely associated to obesity, and is a risk factor defining metabolic syndrome. Thus, the diagnosis of hypertension in an individual is important so are the efforts to normalize BP and, thereby, prevent complications. A variety of antihypertensive drugs are commonly used to reduce BP in hypertensive people but lifestyle, including diet, is a relevant strategy to maintain BP levels. As a consequence, specific modification of dietary habits in a population can have a major impact on BP and cardiovascular diseases [2]. To set new goals to improve diets, it is important to understand how macro and micronutrients can interact with biological systems to enhance health.

Fruits and vegetables are key foods whose high ingestion is associated with the improvement of numerous pathological conditions, including hypertension. Such health promoting actions have been increasingly ascribed to the antioxid-ant characteristics of different polyphenols in fruits and vegetables [3]. Consequently, based on this assumption, many beverages and foods rich in polyphenols, as wine, tea, various fruits and berries, cocoa and soy-based products, as well as some of their purified chemical constituents, are being studied both, as antioxidants and antihypertensive agents.

In this paper we will review the current evidence linking high polyphenol consumption with reductions in BP. The hypothesis is that by regulating nitric oxide (NO) and oxidant (reactive oxygen species, ROS) bioavailability, polyphenols present in fruits and veget-ables affect endothelial function and, as a consequence, BP.

2. PLANT POLYPHENOLS: DIETARY SOURCES AND CHEMISTRY

Polyphenols are secondary metabolites of plants and include thousands of structural variants, from simple molecules (such as phenolic acids) to highly polymerized compounds (such as condensed tannins). Considering this heterogeneity, we have attempted to focus this work on the most abundant polyphenols presented in beverages and foods with docu-

*Address correspondence to this author at the Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956 C1113AAD, Buenos Aires, Argentina; Tel:/Fax: ++54-11-4964-8244; E-mail: cfraga@ffyb.uba.ar

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mented effects on BP: flavonoids (flavanols or flavan-3-ols, flavonols, isoflavones), and stilbenes (resveratrol).

Flavonoids are a chemically defined family of polyphenols that have a basic structure of two aromatic rings (A and B) linked through three carbons that usually form an oxygennated heterocycle (ring C) (Fig. (**1**)) [4]. Flavanols, flavonols, isoflavones, flavanones, flavones, and anthocyanidins are subclasses of flavonoids. These subclasses of chemicals are characterized by specific substitutions in the B- and C-rings: flavan-3-ols present a hydroxyl group at position 3 (ring C), flavonols have a double bond between C-2 and C-3, a hydr-oxyl group at position 3 and a keto group at position 4 (ring C), and in isoflavones one phenolic ring has migrated from C-3 to C-2 and a keto group is present at position 4 (ring C) (Fig. (**1**)). Stilbenes, on the other hand, constitute a phenolic family bearing a basic structure of two aromatic rings linked through two carbons with a double bond (Fig. (**1**)).

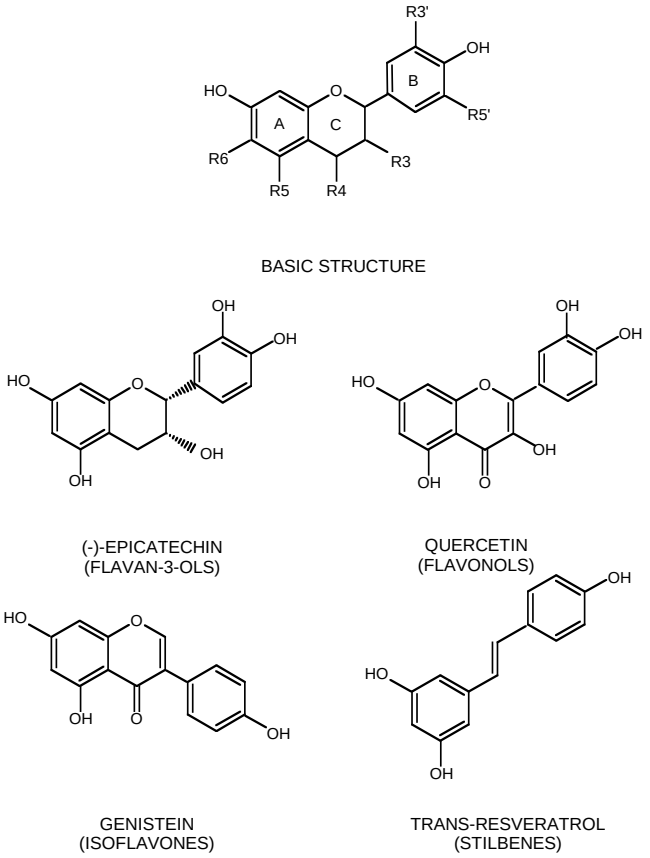

Fig. (1). Chemical structures of polyphenolic compounds.

2.1. Flavanols

Flavanols (flavan-3-ols) are present in high concentrations in grapes, cocoa, apples, nuts, and pomegranate, among other widely used nutrients. Common flavanols abundant in human diet are (-)-epicatechin (EC), (+)-catechin, and gallate modified (-)-epicatechin. Flavan-3-ols could be present as monomeric units (in the aglycone form or as gallate derivatives) or as oligomers. Tannins are classified in two major groups: the condensed tannins and the hydrolysable tannins. Condensed tannins (or proanthocyanidins, or procyanidins) are oligomers of flavan-3-ols and their chemical structure is defined not only by the kind of monomer, but the way that monomers are linked. There are several oligomerization patterns and different plants present a particular one, e.g. cocoa procyanidins are mostly B-type dimers (the monomeric units are linked through 4→8 carbon-carbon bonds) (Fig. (**1**)).

2.2. Flavonols

Flavonols, e.g. quercetin, kaempferol and myricetin, are present in several plants used as foods. Quercetin is especially high in onions, spinach, cauliflower and strawberries. Flavonols in plant tissues are almost always in the form of glycosylated (e.g. quercetin- 4'-O-glucoside) co-njugates, i.e., quercetin-3,4'-O-diglucoside and isorhamnetin-4'-O-glucoside. A whole range of other quercetin conjugates such as quercetin-3-O-galactoside, quercetin-3-O-rhamnos-ide, quercetin-3-O-xyloside, quercetin-3-O-rutinoside, quercetin-3-O-arabinopyranoside, and quercetin-3-O-arabinofuranoside are found in apples (Malus x domestica) [5]. Quercetin-3-O-rutinoside, on the other hand is the main flavonol in tomatoes (Lycopersicon esculentum), asparagus (Asparagus officinalis), peaches (Prunus persica) and nectarines (Prunus persica var. nectarina) [6, 7]. Quercetin-3-O-glycoside, quercetin-3-galactoside and quercetin arabinoside have also been detected in mangoes (Mangifera indica). Other flavonols in the diet include kaempferol-3-O-rutinoside in kiwi fruit (Actinidia deliciosa) and conjugates of myricetin in berries [8]. Grapes of Vitis vinifera, grape products and wines contain a wide range of flavonols such as quercetin, myricetin, kaempferol, isorhamnetin, quercetin-3-O-glucoside, quercetin-3-O-glucuronide, quercetin-3-O-glucoside, quercetin-3-O-galactoside, kaempferol-3-O-glucoside, and kaempferol-3-O-galactoside [9]. Tea (Camellia sinensis) infusions also contain a diverse spectrum of flavonols linked to mono-, di- and tri-saccharides [10].

2.3. Isoflavones

Isoflavones occur mainly in legumes, being soy and soy-based foods the major sources of these compounds in the human diet [11]. Isoflavones in soybeans are found in different chemical forms: aglycones, glucosides, acetylglucosides, and malonylglucosides [12], and they do not form oligomers [13]. The major isoflavones in soybeans are daidzin (7,4'-dihydroxyisoflavone 7-glucoside) and genistin (4',5,7-trihydroxyisoflavone 7-glucoside) as glucosides and their corresponding aglycone forms: daidzein (4',7-dihydroxyisoflavone) and genistein (4',5,7-trihydroxyisoflavone) (Fig. (**1**)).

2.4. Stilbenes

Grapes, peanuts and a group of berries (blueberries, cranberries, and bilberries) are the most important stilbene sources in the human diet [14, 15]. The parent compound of this family of molecules is resveratrol (3, 5, 4' trihydroxystilbene) (Fig. (**1**)). Resveratrol is a fat-soluble compound that oc-curs i) in *trans* and *cis* configurations, ii) in free forms (aglycones) and as glucosides, and iii) as monomers, oligomers and polymers (viniferins) [16]. As it is abundantly available in grape skins (predominantly as resveratrol-3-*O*-beta-gluco-side) and conserved after wine elaboration, red wine represents its main source in the human diet [16].

3. BLOOD PRESSURE, NITRIC OXIDE AND REACTIVE OXYGEN SPECIES (ROS)

BP is influenced and regulated by a wide variety of conditions and chemical entities that interact among them in very complex ways. These entities include from atoms, e.g. sodium, to complicated systems, e.g. the renin-angiotensin system. In this complex scheme, NO appears to play a pivotal role in the regulation of vascular homeostasis, and then BP. We will center the discussion on the effects of dietary polyphenols on NO bioavailability as a result of diminishing oxidant (reactive oxygen species, ROS) steady-state levels.

NO production in biological systems is mainly associated to the oxidation of the non-essential aminoacid (+)-arginine. In mammalian cells, this oxidation is catalyzed by nitric oxide synthase (NOS) activity associated to different NOS isoforms: endothelial (eNOS) expressed both in endothelial cells and cardiomyocytes, being the main source of NO in the vasculature; neuronal (nNOS) present mainly in neurons; inducible (iNOS) expressed in response to different stimulus (for example bacterial endotoxin and a variety of proinflammatory cytokines) [17]; and mitochondrial (mtNOS) present in the inner membrane of the mitochondrion [18, 19].

NO main function in the vasculature is associated to the reaction with the heme group of the soluble guanylate cyclase wich activates this enzyme increasing the formation of cGMP, and consequently the activation of cGMP-dependent pathways. NO also reacts with several metal centers, molecular oxygen, and ROS. Relevant is that NO reacts with superoxide radical (O_2^-), the one-electron reduction product of oxygen in a near diffusion controlled reaction to form peroxynitrite ($ONOO^-$) which is a potent oxidant (Fig. (**2**)) [20]. As a result, the quantitative relationship between superoxide and NO is crucial in defining a beneficial or detrimental action of NO.

The most important sources of O_2^- in the vascular environment are: i) non phagocytic NAD(P)H oxidase (NOX); and ii) uncoupled eNOS [21]. In addition, as in any other mammalian cells, mitochondria respiratory chain [22, 23]; xanthine oxidase, and cytochrome P450 [24, 25] are also sources of O_2^-. NOX utilizes NADH/NADPH as the electron donor to reduce molecular oxygen to O_2^- .This enzyme is regulated by multiple effectors: mechanical forces, growth factors, cytokines, and vasoactive agents ([26]. Angiotensin II (Ang-II) is one of the NOX effectors and the major component of the renin-angiotensin system (RAS), and consequently a major player in BP regulation. Ang-II physiological action is to bind the AT1 receptor and stimulate sodium retention and vasoconstriction [27]. Additionally, this binding activates NOX [28] (Fig. (**2**)) with the consequent increase in O_2^- generation. Experimental evidence is clearly demonstrating that NOX is up-regulated in hypertension [27, 29] and also that the activation of NOX involves protein kinase C, phospolipase D, c-Src (protein tyrosine kinases that associate with AT1), and receptor tyrosine kinases [30]. Ang-II binding to AT1 also mediates the uncoupling of eNOS to generate O_2^- with the consequent loss of NO formation [31].

Based on the mechanisms and evidence discussed above, it was suggested that ROS and oxidative stress are involved in the pathogenesis and/or maintenance of hypertension [25, 26]. On this basis, antioxidant doses of vitamin C and vitamin E, alone or in combination, have been extensively studied in hypertension to reduce both, oxidative stress and high BP [32, 33]. Data are inconclusive because while many stud-

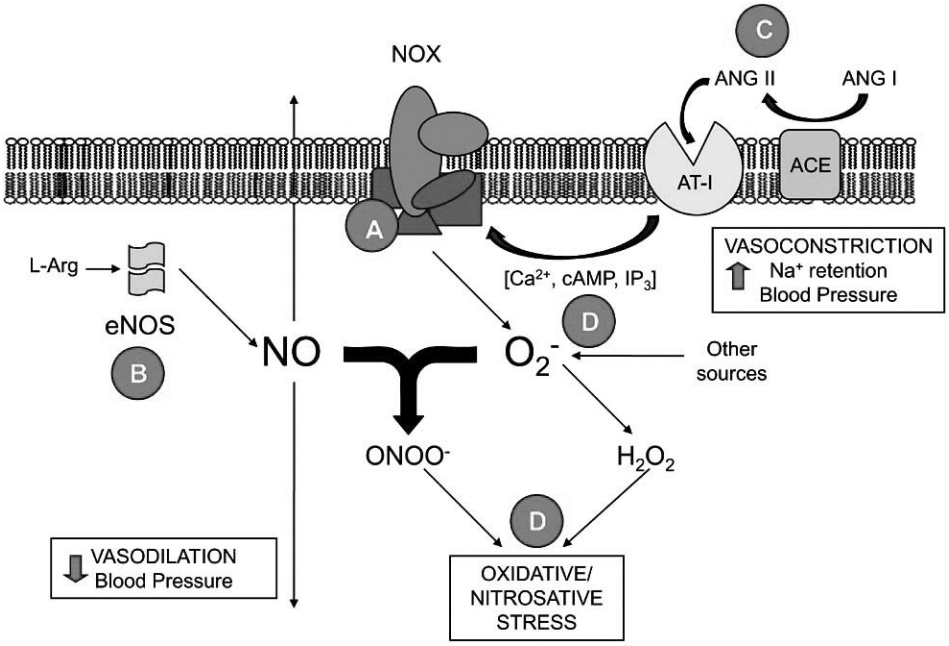

Fig. (2). Nitric oxide and reactive oxygen species in the regulation of blood pressure: Scheme relating polyphenols (circles) with NO and superoxide (O_2^-). Polyphenols could act by (**A**) decreasing expression and/or activiy of NOX, (**B**) increasing expression and/or activity of eNOS, (**C**) modifying membrane-related events, leading to changes in NO and superoxide production. Additionally polyphenols could act as scavenging O_2^-, H_2O_2, and other oxidants that mediate damage to cell components (**D**). NO generated in endothelial cells will diffuse outside the cell and reach smooth muscle cells where it will induce vascular relaxation.

ies have showed significant reductions in BP associated to the consumption of these antioxidants [34-36], others failed in demonstrating effects [37-41]. However, in the last years a number of studies in humans, animals and in ex-vivo models have focused on the effects of plant polyphenols on hypertension based on the antioxidant effects of these compounds, as will be described in the following sections.

4. HYPERTENSION AND POLYPHENOLS

4.1. Polyphenol-Rich Beverages and Foods: Clinical Studies and Epidemiological Trials

4.1.1. Flavanol-Contaning Beverages and Foods

For years, moderate red wine consumption was empirically associated with beneficial effects on cardiovascular health. The epidemiological explanation of the French Paradox (cardiovascular mortality lower than the expected as result of the high consumption of fats in the French population) because of the high wine consumption [42], triggered an enormous amount of research on wine and health. However, intervention studies with wine are not abundant, and the few available, are of difficult interpretation due to the wide differences in the used wines. Such differences define very diverse concentrations of the main compounds associated with the health effects of wine, i.e. flavonoids and resveratrol. Also, in most of the studies the effect of the whole wine is not separated from the potential effects of ethanol [43]. A small group of studies have been carried out by using grape juice or grape extracts in humans to evaluate the effect of these products on BP with divergent results (Table **1**).

Cocoa and cocoa products have been thoroughly studied and gained attention because their antihypertensive and vasculature-associated effects. A sub-study of the Zutphen population showed that cocoa (chocolate) consumption was associated with a decrease in BP and cardiovascular mortality [44]. The Kuna Indians of Panama have a very low incidence of hypertension and cardiovascular disease, but when members of this tribe moved to urban places, their BP increased. The move leads to cultural changes including a decrease in cocoa consumption, making cocoa the potential responsible of the observed changes in BP [45]. Intervention trials with cocoa and cocoa products have included different groups of subjects: normotensive (young, old, overweight, hypercholesterolemic), pre-hypertensive, hypertensive stage 1, and hypertensive with impaired glucose tolerance. As showed in Table **1**, most of the studies showed that cocoa consumption was associated with a decrease in BP. A particular aspect relevant in cocoa derived product, as is the presence of sugar, was analyzed in one of the studies, concluding that sugar attenuated the BP-lowering effect [58].

The results of the studies analyzing the effect of tea (green or black) on BP are more controversial. The relationships of tea consumption with blood cholesterol, systolic BP, coronary heart disease mortality, and all causes mortality were studied in Norwegian men and women without history of cardiovascular disease or diabetes. Subjects were divided into different groups according to tea consumption. Systolic BP was inversely related to tea consumption [85]. In another study were examined the effects of tea drinking on the risk of newly diagnosed hypertension in more than 1500 Taiwanese detecting that the risk of developing hypertension was significantly lower in those subjects who drank more than 120 ml/d, as compared with subjects that did not drink tea [86]. Even when the differences in systolic BP and diastolic BP detected were small, the authors claim that these could be significant on a population/wide basis.

4.1.2. Isolated Flavonoids: (+)-Catechin, (-)-Epigallocatechin Gallate, and Quercetin

A reduced number of studies have been carried out using isolated compounds to reduce BP. (+)-Catechin and (-)-epigallocatechin gallate (EGCG) are two flavanols that have been assayed in humans (Table **1**). Diet supplementation with catechin (75 mg/d for 24 w) was effective reducing systolic BP in both, obese and near-obese Japanese children [69]. Treatment of overweight or obese males with 800 mg/d of EGCG for 8 w resulted in a small but significant reduction in diastolic BP [76]. It is interesting to point that for adults in US, the estimated daily intake of total flavonoids is about 190 mg/d, from which flavanols account for an 83% [87].

The ingestion of flavonols was estimated in 12.9 mg/d [87]. When quercetin, which is a representative of this subfamily, was administered to healthy subjects, it was found to decrease BP [74, 75] (Table **1**). In the latter study, the administration of quercetin (150 mg/d for 6 w) decreased systolic BP only in apo ε3 group but not in carriers of the ε4 allele. By contrast in a study in which 1.0 g quercetin/d was administered for 28 d, no changes in BP were observed [72].

4.1.3. Soy and Soy-Based Food

A survey among 45694 participants in the Shanghai Women's Health Study, concluded that intake of soy foods was inversely associated with both SBP and DBP, particularly among elderly women [88]. When a soy-based food was studied in a Japanese population, it was found an inverse correlation (borderline in its significance) between soy consumption and BP in men, and no correlation in women [89]. The American Heart Association (AHA) has analyzed an important number of studies concluding that the efficacy and safety of soy isoflavones supplements in food or pills cannot be confirmed. By contrast, soy products as tofu, butter, nuts, and burgers, should be beneficial for cardiovascular and overall health, but not because of isoflavones but because of the high content of polyunsaturated fats, fiber, vitamins, and minerals, and low content of saturated fat [90].

Isoflavones have marked chemical similarities with estrogens and have been reported to have both pro- and anti-estrogenic effects. Considering that estrogen replacement therapy has been shown to attenuate BP elevation in postmenopausal women, several studies on BP modulation by soy-derived products in the diet were carried out on peri-menopausal and postmenopausal women, with no conclusive results (Table **1**). Concerning the action of pure isoflavones, when two diets supplemented with soy protein with high and low isoflavone content were studied, the supplementation was associated with the reduction of BP only in males. However, this effect was not influenced by the amount of isoflavone administered [80].

Table 1. Effects of Treatments with Polyphenol-rich Foods and Beverages, and Purified Compounds on Blood Pressure, Nitric Oxide and Oxidative Stress in Humans

Polyphenol Source	Daily Dose	Subjects	Lentgh	Effects on BP	NO Related Parameters	OS Related Parameters	Ref.
Grapes and wine							
Concorde grape juice	5.5 ml/kg	HT	8 w	↓BP	--	--	[46]
Red wine extract tablets	510-765 mg polyphenols	NT	4 w	No effect	--	--	[47]
Cocoa and chocolate							
100 g chocolate	500 mg polyphenols	HT (153/84)	2 w	↓SBP↓DBP	--	--	[48]
46 g chocolate	213 mg procyaninids + 46 mg epicatechin	NT (121/68)	2 w	No effect	↑FMD	No effect on LDL oxidation & p8IP	[49]
105 g chocolate	168 mg flavanols	NT (123/72)	2 w	↓DBP ↓medianBP	--	↓ plasma MDA ↑VitE/LDL ↑VitE/colesterol	[50]
100 g chocolate	500 mg polyphenols	NT (SBP 114)	2 w	↓SBP	--	--	[51]
100g chocolate	88 mg flavanols	HT (142/92)	2 w	↓SBP	↑FMD	--	[52]
6.3 g chocolate	30 mg polyphenols	PreHT & HT (148/86)	18 w	↓SBP↓DBP	↑S-nitrosothiols	No effect on p8IP	[53]
150 ml of cocoa drink	900 mg flavonols	HT (141/91)	2 w	No effect	Improved Bad	-	[54]
37 g chocolate + 237 ml of cocoa beverage	≈ 800 mg procyanidins	NT (old)	6 w	No effect	--	--	[55]
22 g chocolate x 2	360 mg flavonols	NT (high cholesterol)	8 w	↓SBP↓DBP	--	--	[56]
100 g chocolate	140 mg flavonols	HT (140/91) impaired glucose tolerance	2 w	↓SBP↓DBP	↑FMD	--	[57]
74 g chocolate or 240 ml of cocoa beverage	≈800 mg flavanols	NT (overweight) (125/69)	acute	↓SBP↓DBP	↑FMD	--	[58]
Cocoa drink	321 mg flavanols	Medicated diabetic	4 w	No effect	↑FMD	--	[59]
Cocoa beverage	805 mg flavanols	Overweight adults	6 w	No effect	↑FMD	--	[60]
Chocolate	750 mg polyphenols	PreHT	8 w	No effect	--	--	[61]
Cocoa drink	701 mg flavanols	Men and PMW overweight or obese	2 h	↓BP	↑FMD	--	[62]
Cocoa beverage	1052 mg flavanols	HT men and PMW	3-6 w	↓BP	--	--	[63]
Cocoa beverage	33; 372; 712 mg flavanols	HT men and PMW	3-6 w	No effect	--	--	[63]
Black and green tea							
6 mugs black tea	--	NT	4 w	No effect	--	--	[64]
5 cups black or green tea	--	HT	30 min 60 min/1w	↑SBP No effect	--	--	[65]
Black tea	450 mg flavanols 700 mg flavanols	HT (137/78) (CAD) HT (137/78) (CAD)	2 h 4 w	↑SBP No effect	Improved BAD	--	[66] [66]

(Table 1) contd….

Polyphenol Source	Daily Dose	Subjects	Lentgh	Effects on BP	NO Related Parameters	OS Related Parameters	Ref.
Green tea extract	583 mg catechins	NT & HT (obese)	2 w	↓SBP	--	--	[67]
3 glasses of black tea	318 mg catechins	Men	24 w	No effect	--	--	[68]
Green tea extract	576 mg catechins	Obese children	24 w	↓SBP	--	--	[69]
Green tea extract	714 mg polyphenols	NT	3 w	No effect	No effect	--	[70]
Black tea	100-800 mg flavonoids	NT	5 w	↓SBP↓DBP	↑FMD	--	[71]
Purified compounds							
Quercetin capsules x 4	1000 mg	NT (122/79)	4 w	No effect	--	--	[72]
Quercetin	150 mg	Overweight or obese	6 w	↓SBP	--	--	[73]
Quercetin tablets	730 mg	Pre HT (137/86) HT (148/96)	4 w 4 w	No effect ↓BP	--	No effect	[74]
Quercetin	150 mg	Overweight	6 w	↓SBP ↓DBP (in ε3 for Apo E)	--	--	[75]
EGCG tablets x 2	800 mg	Overweight or obese	8 w	↓SBP ↓DBP	--	--	[76]
Soy and soy based products							
20 g soy protein	34 mg phytoestrogens	Perimenopausal W	1 dose/6 w 2 doses/6 w	No effect ↓DBP	--	--	[77]
40 g soy protein	118 mg isoflavones	NT men & PMW	12 w	↓SBP↓DBP	↓FMD in men	--	[78]
500 ml soy milk x 2	--	HT men	12 w	↓SBP↓DBP	--	--	[79]
50 mg soy protein	73 or 10 mg isoflavones	Men and PMW	4 w	↓BP in men	--	--	[80]
Soy protein	43.5 mg isoflavones	Perimenopausal W	48 w	No effect	--	--	[81]
Soy protein	99 mg isoflavones	PMW	48 w	↓BP in equol producers	↑endothelial function in equol producers	--	[82]
40 g soy protein	118 mg isoflavones	HT men & PMW	12 w	No effect	--	--	[83]
25 g soy nuts	101 mg isoflavones	PMW	8 w	↓SBP↓DBP	--	--	[84]

The numbers between parentheses in the third column indicate the initial BP values of the group of subjects under study.
NO: Nitric Oxide; OS: Oxidative Stress; FMD: Flow-mediated dilation; BAD: Brachial artery diameter; PMW: postmenopausal women; NT: normotensive; HT: hypertensive; p8IP: plasma 8-isoprostanes; CAD: coronary artery disease.

4.1.4. Conclusions from Intervention Trials

Table **1** summarizes an important number of the intervention studies where the effect of polyphenol-rich foods and beverages (and several purified compounds) were studied. An adequate interpretation of those results has to take in consideration the experimental design and many other factors, as the fact that in many of these studies BP was not the primary outcome. Normotensive and mild-hypertensive groups are the most studied groups, since interventions on hypertensive groups require the restrain from antihypertensive treatment during the study. Comparative analysis among doses employed is difficult because of the variety in polyphenol sources and the lack of uniformity in the composition expression; however, it is possible to approximate that in most of the studies the tested doses were around 100-500 mg of flavonoid/d (1.4-7.2 mg/kg/d). Regarding treatment periods, single dose experiments were designed to measure BP between 30 min and 120 min post flavonoid consumption, and longer studies can be considered as short-term (1-12 w) and long-term experiments (18-48 w).

Hooper *et al.* (2008) reviewed the effectiveness of different flavonoid subclasses and flavonoid-rich food sources on cardiovascular disease, and one of the risk factors measured was BP [91]. Through the analysis of 133 trials they con-

cluded that chocolate decreased DBP and SBP, soy protein isolate (and no other soy derived products) decrease DBP, while acute treatment with black tea increased SBP and DBP. In the same direction, another meta-analysis about the effects of cocoa and tea on BP, showed that foods rich in cocoa may reduce BP while tea intake appears to have no effect [92]. It should be important to point out that in this work only short-term treatments (a median duration of 4 w) were compared.

Several of the studies included in Table **1** have correlated the BP-lowering effects of polyphenols and polyphenol-rich foods with NO related parameters, and a few with ROS. The most common NO-dependent outcome was flow mediated dilation (FMD), which was improved by polyphenol intake in most of the cases. One trial found increased circulating levels of S-nitrosothiols associated to the decrease in BP in hypertensive and prehypertensive subjects treated during 18 w with low doses of flavanol rich chocolate [53]. Additionally, the effects of flavonoid rich foods or beverages (or purified compounds) were analyzed in subjects for factors associated to NO and/or ROS, but BP was not measured. The oral ingestion of a high-flavanol cocoa drink (917 mg of flavonols) resulted in a transient increase in NO-derived species concentration in plasma and urine of healthy subjects [93], associated with the improvement of FMD, and the levels of plasma flavanols. More recently, the single oral ingestion of 200 mg of quercetin or EC was also associated with increased plasma and urine NO metabolites [94].

In brief, the data of these studies appear to provide some association between polyphenol consumption and decrease in BP in hypertensive subjects; however those studies are not enough to advance in the understanding of mechanistic aspects underlying the antihypertensive/hypotensive effect of polyphenols. In this regard, a high number of animal studies has been developed to explore the role of NO, ROS and other BP modulators.

4.2. Polyphenol-Rich Beverages and Foods: Studies in Animals

4.2.1. Studies with Polyphenols from Red Wine, Tea, and Cocoa, and Purified Compounds

Different animal models of hypertension have been used to test the antihypertensive effect of polyphenol rich foods/beverages or purified compounds. Considering both, the hypertension model and the determined parameters it is possible to build few hypothesis on the action of polyphenols.

L-NAME-induced hypertension is a model where a non selective inhibitor of NO synthase (L-NAME) induces an increase in arterial BP. Red wine polyphenols (40 mg/kg/d) administered simultaneously with L-NAME prevented the increase in BP associated with an increase in NOS activity and eNOS expression, and a reduction in oxidative stress in aorta and heart left ventricle [95].

DOCA-salt hypertensive rat is a model characterized by a suppressed plasma renin level due to sodium retention. Red wine polyphenols (40 mg/kg/d) administered by gavage during 5 w prevented the increase in SBP induced in those rats [96]. The treatment produced detectable levels of catechin in plasma and was associated with decreases in oxidative stress parameters (plasma MDA and urinary isoprostane F2α). Aorta NOX expression and activity were increased in DOCA-salt rats, and the polyphenol treatment prevented the increase in NOX activity and in the expression of the aortic NOX subunit, p22(phox). No parameter related to NO metabolism was measured in this work.

SHR (spontaneously hypertensive rats) is a genetic and multifactorial model of hypertension similar to essential human hypertension that has been vastly used to test polyphenol antihypertensive effects. Many reports only showed the antihypertensive effects of polyphenol rich foods or purified compounds, as the flavanone hesperidin [97, 98], chrysin [99], and polyphenol rich cocoa powder [100]. In another group of studies in SHR, the flavonoids baicalein and quercetin [101], and grape polyphenols (carried out on estrogen-depleted female SHR rats) [102] provided antihypertensive effects associated with decreases in oxidative stress parameters. More mechanistic aspects, including endothelial dysfunction studies, have also been studied in SHR rats. Glucosyl hesperidin (50 mg/kg diet for 8 w) produced antihypertensive effects associated with decreased urinary levels of oxidative stress and mRNA expression of NOX subunits, with no effect on eNOS expression in aorta [103]. Red wine polyphenols (40 mg/kg for 5 w) produced similar decreases in BP and oxidative stress in ovariectomized and sham rats [104]. Beverages prepared with water added with 3.5 g/L thearubigins, 0.6 g/L theaflavins, 0.5 g/L flavanols, and 0.4 g/L catechins (as representative of black tea), and water added with 3.5 g/L catechins, 0.5 g/L flavonols and 1 g/L polymetric flavonoids (as representative of green tea) attenuated the increase in BP developed by stroke-prone SHR rats [105]. Unexpectedly, these effects were associated with decreased plasma concentration and urinary excretion of NO metabolites. One of the major flavonoid compounds in tea, ECGC, administered by gavage (200 mg/kg/d for 3 w) to SHR rats prevented systolic BP increases. NO involvement was suggested by the abolishment of the antihypertensive effects when the rats were concurrently given L-NAME [106].

Hypertension model produced by Ang II infusion was used to test both, red wine polyphenols [107] and green tea extracts [108]. Red wine polyphenols administered in drinking water during one week were able to avoid the BP increase mediated by Ang II, as well as the vascular O_2^- production and the expression of NOX subunits, nox1 and p22(phox). Green tea extract administered during 2 w, also produced a correction in BP associated to a decreased expression of NOX subunit gp91(phox), Rac-1 translocation, and NOX activity.

Hypertension associated to obesity and/or metabolic syndrome was also a model to study polyphenol antihypertensive actions. Hypertensive fructose-fed rats (60 % w/w fructose in the diet) were provided with three different grape extracts: a red grape skin polyphenolic extract enriched in anthocyanins, a grape seed extract enriched in procyanidins and rich in galloylated procyanidins, and a commercial preparation rich in catechin oligomers [109]. Extracts enriched in anthocyanins and catechin oligomers corrected high BP and cardiac hypertrophy. By contrast, the extract enriched in procyanidins and gallolylated procyanidins did not affect BP. Interestingly, the three extracts decreased O_2^- production by

the heart left ventricle and the thoracic aorta, and the expression of gp91(phox) in the left ventricle. Resveratrol (10 mg/kg/d for 8 w) dramatically decreased BP in obese Zucker rats in association with an increased aorta eNOS exp-ression [110]. Identical resveratrol dose administered for 6 w reverted increases in BP and plasma MDA, and decrease of eNOS activity in mesenteric bed and heart in fructose-fed rats (10 % w/v fructose in drinking water) [111].

Studies in normotensive animals demonstrate a hypotensive effect for red wine extracts. Short-term administration (7 d) of red wine extract rich in polyphenols produced a progressive BP decrease in normotensive rats [112, 113]. This hypotensive effect was prevented by L-NAME, suggesting the involvement of NO [112], and was associated with decreased levels of MDA and 4-HNE with no effect on eNOS expression [113]. A diet enriched with dealcoholized red wine fed to rats during 10 d, increased NOS activity and decreases NADPH-induced O_2^- production in aortic segments [114]. In parallel experiments, diets enriched with quercetin or catechin produced similar increases in NOS activity with non significant effect on NADPH-induced O_2^- production, even when the quercetin- and catechin-rich diets administered to rats contained 1000 times more of these constituents than the dealcoholized red wine enriched diet.

Quercetin is the only flavonoid that was administered pure to test its antihypertensive effect in various rodent models of hypertension. The results on BP have been reviewed and allowed the following conclusions [115]: i) long term administration of quercetin (usually by a daily single oral dose of 10 mg/kg) exerts antihypertensive effects, independently of hypertension origin; ii) these effects appear after 1-2 w of treatment; and iii) quercetin does not exert hypotensive effect. Mechanisms proposed included a reduction in oxidative stress and particularly a reduction in O_2^--driven NO inactivation. This interpretation is based on the association of BP reduction with the down-regulation of p47(phox) NOX subunit, increases in eNOS activity and decreases in NOX activity [116, 117], decreased oxidative stress markers [118-121] and increased NO metabolites [122].

4.2.2. Studies with Soy Products and Isoflavones

As it was previously mentioned, results from a meta-analysis [90] suggest that the antihypertensive effect of soy foods is mostly associated with soy protein and not with isoflavones. However, an important number of studies have been carried out in experimental models of hypertension in rats by using isoflavones providing some interesting results. Additionally, mechanistic aspects have been studied using soy based diets, or protein or isoflavone components separately. Soy based diets attenuated the development of hypertension in male and female SHR rats [123], in ovariectomized female SHR rats [124, 125], and in male SHR rats [126]. There is no agreement about the role of NO in this protective effect: Martin *et al.* [124] concluded that the antihypertensive effects did not involve NO. Park *et al.* [126] suggest that the effect is mediated by a decrease in oxidative stress and the augmentation of NO production. A study in male Wistar rats maintained during gestation and adult life (12-16 mo) with a soy enriched diet showed increases in eNOS and antioxidant gene expression in the vasculature and other tissues. These increases result in reduced oxidative stress, increased NO bioavailability, and decreased BP [127]. Isoflavones have been proposed as the responsible for those effects via modulation of the key transcription factors NFκB and Nrf2 [128].

5. POLYPHENOL METABOLISM

It should be noted that these effects of polyphenols are strongly associated to their metabolism. Dietary polyphenols can be absorbed in the upper digestive tract as they are present in plants or foods, or after being partially altered by the present bacteria in the lower intestine. After absorption, glucuronidation, sulfation and methylation are the most common processes. Several end products (occasionally together with the free parent compound) have been detected in plasma and could potentially be responsible to produce a biological effect.

Specific metabolism is crucial for obtaining a biological action of foods and many times of single compounds. For example, the effects of caffeinated coffee consumption on BP and the risk of developing hypertension were explored in several studies with conflicting results. A possible explanation for those discrepancies is the one based on the polymorphisms of cytochrome P450 1A2, which is the main enzyme responsible for the metabolism of caffeine. It has been shown [129] that the risk of hypertension associated with coffee intake varies according to cytochrome P450 1A2 genotype: carriers of *1F allele ("slow" caffeine metabolizers) were at increased risk, whereas individuals with *1A/*1A allele ("rapid" caffeine metabolizers) did not show that association. Then, the presence of caffeine (and theobromine) in chocolate or coffee could be masking the benefits associated to the antihypertensive effects of polyphenols. Another example is the metabolization of the isoflavone daidzein to equol [130]. This metabolite is produced by the intestinal flora in 100 % of rodents, but only in ≈30% of humans [82]. This allows segregating subjects that are equol producers and non equol producers. In an intervention study in which soy was supplemented in the diet (99 mg isoflavones/d in 202 postmenopausal women, 60/75-y old for 12 mo) it was observed a decrease in BP and an improvement in endothelial function in the equol producers subjects, and an increase in BP and deterioration of endothelial function in the non equol producers [82]. Because of the number of subjects, these differences were not statistically significant; however, the results allow speculating that the possible benefits of soy products on BP are limited to subjects able to metabolize isoflavones. A final example highlights the importance of the metabolization processes at cellular level. Studies carried out on cell cultures have suggested that EC needs to be methylated to inhibit NOX activity and this inhibition results in NO preservation [131]. Results suggest that (-)-EC metabolism mediated by catechol-O-methyl transferase (COMT) produces 3'and 4'-O-methyl-EC, two compounds that share a structural feature with apocynin, which is an inhibitor of NOX. The effect of EC protecting NO levels in HUVEC cells was attenuated in the presence of 3, 5-dinitrocatechol, an inhibitor of COMT [132, 133].

CONCLUDING REMARKS

Sufficient NO bioavailability is associated with normal vasodilation and consequently, normal BP. Failure to gener-

ate NO or enhanced NO consumption can then, lead to hypertension. ROS, and essentially O_2^-, are ubiquituous and effective "consumers" of NO. Polyphenols in general, shared chemical structures that allow them to act as classic antioxidants, i.e. free radical scavengers and/or redox-active metal chelator. Even though those antioxidant reactions are thermodynamically favored, the actual concentrations of polyphenols in most tissues make quantitatively irrelevant these direct antioxidant mechanisms [134, 135]. Then, other biochemical mechanisms, probably related to specific polyphenol-protein and polyphenol-lipid interactions can partially explain the observed in vivo effects of polyphenols decreasing ROS steady-state levels. These mechanisms should be more consistent with the in vivo polyphenol level observed in most human and animal tissues. Considering those concepts we can hypothesize on the actions of polyphenols that concord in increasing NO availability, and affect normal BP maintenance are Fig. (**2**) i) increase of eNOS expression and/or activity; ii) decrease the expression and/or activity of NOX which is a major source of O_2^- in the vasculature; and iii) modulate enzymes and receptors associated to ROS production, e.g. angiotensin converting enzymes, AT1 receptor, angiotensin II-mediated pathways, etc.

A full range of health benefits can be associated to plant polyphenols present in fruits and vegetables that are part of human diets. Grape, tea, cocoa, and soy products and many of their components have been extensively associated to the regulation of BP. At this point we have no definitive but encouraging data to start considering diets that can provide a benefit to hypertensive subjects, and those benefits will be more significant in people that do not have controlled his/her elevated BP. However, many questions remain open, and we have in front a long way to finally define a precise antihypertensive diet or to incorporate a single compound that can provide definitive antihypertensive benefits.

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