Polyphenols in human nutrition: from the in vitro antioxidant capacity to the beneficial effects on cardiometabolic health and related inter-individual variability - an overview and perspective

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Polyphenols in human nutrition: from the *in vitro* antioxidant capacity to the beneficial effects on cardiometabolic health and related inter-individual variability – an overview and perspective

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Running head: Dietary polyphenols in the past and future

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Abbreviations

LDL – low-density lipoprotein

ROS – reactive oxygen species

PUFA – polyunsaturated fatty acid

4-HNE – trans-4-hydroxy-2-nonenal

RCT – randomized controlled trial

TEAC – trolox equivalent antioxidant capacity

DPPH – 2,2-diphenyl-1-picrylhydrazyl

FRAP – ferric reducing antioxidant power

ADME – absorption, distribution, metabolism and excretion

PKC – protein kinase C

PI3K – phosphoinositide-3-kinase

TAK1 – TGF-beta-activated kinase 1 (mitogen-activated protein kinase kinase kinase 7)

PDK1 – 3-phosphoinositide dependent protein kinase 1

AKT – AKT serine/threonine kinase

NF-κB – nuclear factor kappa B

TNFα – tumor necrosis factor-alpha

MEK2 – dual specificity mitogen-activated protein kinase kinase 2

IKKα – NF-κB inhibitor kinase alpha

ERK – mitogen-activated protein kinase

eNOS – endothelial nitric oxide synthase

AMPK – AMP-activated protein kinase

miRNA – microRNA

p38-MAPK – p38 mitogen-activated protein kinase

HDL – high-density lipoprotein

Abstract

Oxidative damage of cells and tissues is broadly implicated in human pathophysiology, including cardiometabolic diseases. Polyphenols, as important constituents of human diet and potent *in vitro* free radical scavengers, have been extensively studied for their beneficial effects on cardiometabolic health. However, it has been demonstrated that the *in vivo* antioxidant activity of polyphenols is distinct from their *in vitro* free radical scavenging capacity. Indeed, bioavailability of nutritional polyphenols is low, and conditioned by complex mechanisms of absorption, distribution, metabolism and excretion. Nowadays it is commonly accepted that the cellular antioxidant activity of polyphenols is mainly carried out via modification of transcription of genes involved in antioxidant defense. Importantly, polyphenols contribute to the cardiometabolic health also by modulation of plethora of cellular processes that are not directly associated with antioxidant enzymes, through nutri(epi)genomic mechanisms. Numerous human intervention studies have demonstrated beneficial effects of polyphenols on the key cardiometabolic risk factors. However, inconsistency of the results of some studies led to identification of the inter-individual variability in response to consumption of polyphenols. In perspective, a detailed investigation of the determinants of this inter-individual variability will potentially lead us towards personalized dietary recommendations. The phenomenon of inter-individual variability is also of relevance for supplementation with antioxidant (pro)vitamins.

Introduction

Nutrition is essential for maintenance of optimal health. Both under- and overnutrition lead to disease development and increase in the rate of mortality. Both phenomena are still present in the human society, and it is rather difficult to overcome them, despite the unprecedented technological advances in food science the past few decades. In addition, we witness occurrence of obesity accompanied by deficit of essential nutrients and plant food bioactives in low- and middle-income countries, as well as in low-income subgroups in developed countries[1,2]. Other than economic status, it has also been suggested that the educational status has a significant influence on food choices[3].

Plant food bioactives are important constituents of human diet. Although not classified as essential nutrients, they may have beneficial effects on human health. Unlike vitamins, their inadequate intake will not result in specific syndrome of deficit, but rather in increased risk of chronic diseases. The plant food bioactives include terpenoids (isoprenoids), phenolic compounds (including polyphenols), glucosinolates, betalains and others. In this review, we will focus on polyphenols and their effects on human cardiometabolic health. Indeed, there is growing evidence about the preventive effects of polyphenols on cardiometabolic diseases, such as Diabetes mellitus type 2[4] and atherosclerosis[5]. There is also evidence that polyphenols exert beneficial effects on neurodegenerative diseases[6] and some forms of cancer[7], which will not be addressed in this occasion.

Because of their remarkable *in vitro* free radical scavenging properties, early investigators attributed the beneficial effects of polyphenols on human health exclusively to their capacity to scavenge free radicals[8,9]. Regarding their cardioprotective effects, many studies were focused on LDL oxidation, which plays central role in atherogenesis[10]. Indeed, it has been demonstrated that, upon consumption of foods and beverages rich in polyphenols, the resistance of LDL to oxidation increases[11]. Notably, LDL oxidizability is still of interest[12]. Following these observations, polyphenols became an integral part of the theory of oxidative stress.

Therefore, in the first part of this review we will give an overview of the theory of oxidative stress and a summary of the results of antioxidant supplementation studies. In the second part, we will focus on polyphenols and their antioxidant properties, which will be followed by an

overview of their effects on cardiometabolic health, and our current understanding of the inter-individual variability in the response of their consumption.

1. Oxidative stress and antioxidant supplementation

1.1 Definition of oxidative stress and mechanism of lipid peroxidation

The term "free radical" refers to the chemical species containing one or more unpaired electrons, which encompass various oxygen-, carbon-, nitrogen- or sulfur-centered radicals. Among them, the oxygen-centered radicals are the most prevalent in the biological systems[13], including the superoxide radical, and the highly reactive hydroxyl radical. These, along with the non-radical hydrogen peroxide, are the most common reactive oxygen species (ROS). Following Harman's free radical theory of aging (1956)[14], the term "oxidative stress" was first mentioned in 1970, referring to the oxidative challenge of erythrocytes by hydrogen peroxide[15]. In 1985 oxidative stress was defined as an imbalance between oxidants and antioxidants, in favor of the former[16], focusing on the oxidative damage of cells, tissues and organs[17]. Since then, the concept has evolved substantially, leading to redefinition of oxidative stress as a dysfunction of cellular redox signaling and redox control[18,19]. Most recently, a distinction has been made between oxidative eustress, as a physiological condition that is essential for redox signaling, and oxidative distress, as a supraphysiological condition that causes damage to biomolecules[20]. So far, hundreds of thousands of papers have been published demonstrating and confirming the association of oxidative stress with various human diseases, including cardiometabolic ones. Indeed, nowadays it is almost impossible to find a human disease or condition that is not associated with the oxidative stress.

Classical theory describes the oxidative stress as an overall imbalance between the ROS from one side, and the cellular antioxidants from the other, of course in favor of ROS. This imbalance occurs because of an excessive production of ROS, or an inadequate concentration and/or activity of the cellular antioxidants (non-enzymatic or enzymatic), or because of both[21]. The classical understanding of the oxidative stress was a leitmotif of the early attempts to translate the immense amount of scientific evidence into clinical practice. Within these efforts, it seems that the chain reaction of lipid peroxidation attracted most attention. Hydroxyl radical ($HO^{\cdot}$), as the most reactive ROS, reacts in a high rate reaction with polyunsaturated fatty acids (PUFAs), in the first place linoleic or arachidonic acid of membrane phospholipids. Hydroxyl radical

abstracts hydrogen atom from PUFA, which starts the autocatalytic chain reaction of lipid peroxidation. In this initiating reaction, a carbon-centered lipid radical is formed ($L^{\cdot}$), which further reacts with molecular oxygen to generate an oxygen-centered lipid peroxyl radical ($LOO^{\cdot}$). The lipid peroxyl radical can attack another PUFA to generate lipid hydroperoxide (LOOH) and a new carbon-centered lipid radical, which propagates the chain reaction[22]. Lipid hydroperoxides have ability to react with trace metals to generate lipid alkoxyl radicals ($LO^{\cdot}$). Both lipid peroxyl radicals and lipid alkoxyl radicals undergo cyclization and/or degradation, generating a plethora of reactive aldehydes such as malondialdehyde (MDA), trans-4-hydroxy-2-nonenal (4-HNE), trans-4-oxo-2-nonenal (4-ONE), acrolein, glyoxal and many others. What reactive aldehydes will be generated depends on the type of PUFA subjected to lipid peroxidation[23]. Importantly, these reactive aldehydes have ability to oxidize proteins[24] and DNA[25]. Therefore, if present in excess and/or for a longer period, they can induce cellular damage, and consequently induce disease development.

Lipid peroxyl radicals can be scavenged by vitamin E, but also by other nutrients such as polyphenol, or beta-carotene[26], which terminates the chain reaction of lipid peroxidation. Notably, the chemistry behind the scavenging of lipid peroxyl radicals by polyphenols and vitamin E is very similar[27]. Following the reaction of termination by the vitamin E, a relatively stable α-tocopheryl radical is formed (α-tocopherol is the predominant form of vitamin E in human tissues[28]). The α-tocopheryl radical can be oxidized to a stable nonradical quinone, or reduced back (recycled) to hydroqiuinone by vitamin C[29]. However, the biological relevance of the later reaction is not completely clear[30]. Other possibilities for termination of the chain reaction of lipid peroxidation are the following: substrate unavailability, collision of two $LOO^{\cdot}$ molecules or reaction of $LOO^{\cdot}$ with other radicals.

1.2 Antioxidant supplementation studies – hopes and pitfalls

Undoubtedly, if present at high concentrations, ROS, free radicals and reactive aldehydes will cause cellular damage, and eventually disease. Therefore, for early investigators, it seemed logical that supplementation with low molecular weight antioxidants would prevent the cellular damage, resulting in beneficial effects on human health, and decreased mortality. Following this assumption, numerous randomized controlled trials (RCTs) have been conducted with the aim to study the effects of supplementation with vitamin C, vitamin E, beta-carotene, vitamin A and

selenium, alone or in various combinations, on oxidative stress and health maintenance. Some of these studies included patients with various chronic diseases. In others, the relevance of antioxidant supplementation in primary prevention was studied on healthy subjects. A meta-analysis of 67 RCTs was conducted and published in 2008[31], the results of which were discouraging. The same authors repeated the meta-analysis, including the papers that were published subsequently and the updated meta-analysis of 78 RCTs was published in 2012[32]. The results were similar, *i.e.* showing "no evidence to support health effects of supplementation with low molecular weight antioxidants in both primary and secondary prevention". Moreover, it has been demonstrated that supplementation with vitamin E, beta-carotene and vitamin A may slightly increase mortality. After the initial disappointment, a quest for rational explanation has been initiated, and several important issues have been raised, which will be presented in the following paragraphs.

Over-simplification of the theory of oxidative stress has been identified as an important cause of the disappointing results of the antioxidant supplementation studies, which were an attempt to translate the accumulated scientific evidence into practice. This over-simplification has been referred as a "*translational shortcut*"[33]. Indeed, behind the simplified representation of the oxidative stress as an overall imbalance between ROS and antioxidants, there is a complex network of cellular redox regulators. These include antioxidant enzymes and low molecular weight antioxidants that work together, and along with other cellular factors, to establish and maintain the cellular redox balance. Obviously, an over-simplification of this complex network down to unselective supplementation with high doses of free radical scavengers was not effective. Moreover, the finding of increased mortality points out that "natural", which refers to both vitamin E and beta-carotene, is not equal to "safe in high doses". Another potential issue might be the composition and/or quality of supplements, which would be extremely difficult to address from our perspective.

In the antioxidant supplementation studies, an important factor that should have been taken into account is the role of reactive aldehydes as second messengers of free radicals. Indeed, there is evidence that, in its physiological concentrations, 4-HNE acts as a signaling molecule, and as such is involved in many biological processes[34], including adaptive response to other oxidative stressors[35]. Therefore, supplementation with high doses of vitamin E may impair the

physiological role of 4-HNE, potentially leading to adverse effects. It is indicative that an increased mortality was observed in supplementation with vitamin E, beta-carotene, and vitamin A, all of them liposoluble compounds, but not with vitamin C. Indeed, liposolubility aggravates elimination, which may be an important element for development and manifestation of adverse effects.

Vitamin E[36] and beta-carotene[37] have multiple biological functions. Currently, it is not completely understood how these functions were implicated in the results of the RCTs of antioxidant supplementation. However, some aspects have been discussed in a recent review[38]. Namely, it has been demonstrated that beta-carotene inhibits the enzymatic activity of glutathione-S-transferase π, which provides protection against BPDE (benzo[a]pyrene diol epoxide), a toxic metabolite of tobacco smoke. The decreased enzymatic activity of glutathione-S-transferase π has been proposed as underlying mechanism of the increased incidence of lung cancer in smokers supplemented with beta-carotene[39]. On the other hand, it has been demonstrated that vitamin E supplementation has beneficial health effects in specific groups of patients[40], such as patients with non-alcoholic steatohepatitis[41], diabetic patients with haptoglobin 2-2 genotype[42], patients on hemodialysis[43], and patients with oxidative stress induced hypertension[44]. Moreover, some meta-analyses did not confirm the finding regarding the increased mortality in persons supplemented with vitamin E[45,46]. These examples illustrate the need to identify population groups and individuals that will most benefit from supplementation with antioxidant vitamins, as precisely as possible, including the dose and duration of supplementation. To the best of our knowledge, the inter-individual variability of response to low molecular weight antioxidants has not been explored in detail so far. Biomarkers of oxidative stress[47] shall be used for identification of patients that will most benefit of supplementation with antioxidant vitamins. However, the shortcomings of the currently available biomarkers[48] do not allow their effective use in clinic. Therefore, a development of better, biologically much more specific and analytically more precise biomarkers of oxidative stress, is of utmost importance.

2. Polyphenols and their antioxidant properties

2.1 Polyphenols in human diet

Polyphenols are plant food bioactives that possess remarkable *in vitro* antioxidant capacity[49]. They have ability to scavenge lipid peroxyl radicals[50]. As such, they are an integral part of the theory of oxidative stress[51]. Polyphenols are present at relatively high concentrations in human diet. However, depending on individual[52] and national nutrition habits, there is a large difference in daily consumption, ranging from 377±15[53], to 1757±696[54] mg/day. Dietary polyphenols originate mainly from plant based foods including fruits (mainly berries and apples), vegetables (onions, spinach, asparagus, broccoli), spices (black pepper, curcuma, saffron), whole grains, coffee, cocoa, tea, olive and various kinds of nuts[55]. Many of them are also administered as food supplements or herbal remedies. Polyphenols are well studied and classified. They are widespread in the plants, encompassing over 8000 different compounds[56]. However, the number of polyphenols that are relevant to human nutrition is much smaller. Two databases containing comprehensive information on nutritional polyphenols have been developed and are freely accessible: (http://phenol-explorer.eu/)[57] and (https://www.ars.usda.gov/ARSUserFiles/80400525/Data/Flav/Flav_R03-1.pdf)[58].

Regarding the health benefits of consumption of foods and beverages rich in polyphenols, the first epidemiological study demonstrating an inverse association between flavonoid intake and mortality from coronary heart disease was published in 1993[59]. Unfortunately, another translational shortcut was taken here. Since polyphenols demonstrate high *in vitro* antioxidant capacity, it was assumed that the *in vitro* antioxidant capacity of any food or plant extract can be used as a direct determinant (or, in other words, a measure) of their beneficial health effects in humans. Following this concept, a list of *in vitro* antioxidant capacities of foods and beverages (measured with Oxygen Radical Absorbance Capacity assay – ORAC assay) was published[60]. Besides the ORAC assay[61,62], several other methods were also developed, such as TEAC, DPPH and FRAP assay[63]. However, growing scientific evidence started to elucidate some of the numerous and complex mechanisms of absorption, distribution, metabolism and excretion (ADME) of polyphenols, as molecular basis of their low bioavailability. It also became clear that the *in vivo* antioxidant activity of polyphenols involves much more complex mechanisms than the free radical scavenging, and that molecular mechanisms that are not directly associated with the antioxidant enzymes are also involved in their health promoting effects. At the same time, the ORAC list was misused by food and dietary supplement manufacturing companies to promote their products[64]. All this together led to withdrawal of the ORAC list.

2.2 Metabolism of nutritional polyphenols in the human body

Bioavailability of nutritional polyphenols is low, and far from uniform. Their plasma concentrations are at range of nM to low μM[65]. A notable exception is the gastrointestinal tract[66], where the intestinal cells are in contact with high concentrations of polyphenols and other plant food bioactives. In the human body, polyphenols are subjected to complex and extensive metabolic transformations[67], which significantly alter their chemical properties, and sometimes diminish their antioxidant activity[68]. Notably, lack of association between the antioxidant activity and some of the biological effects of polyphenol metabolites has been clearly observed[69]. Therefore, the *in vitro* total antioxidant capacity of foods, beverages or plant extracts, cannot be a direct determinant of their biological effects.

Polyphenols are a heterogeneous family of plant food bioactives, encompassing several classes of bioactive compounds, such as flavonoids, phenolic acids, lignans, stilbenes, and other polyphenols[57]. Because of the heterogeneity in their chemical structures, the metabolism of compounds that belong to different classes and subclasses, or even the metabolism of individual compounds, is different, which has been extensively reviewed elsewhere[67,70]. Here, we will give only a general overview of the metabolism of polyphenols, pinpointing the factors that are relevant for the inter-individual variability in the response to their consumption. In the human diet, most of the polyphenols are found as glycosides. Hydrolysis of glycoside conjugates is a critical step in absorption of polyphenols in the small intestine, following which corresponding aglycones appear in the intestinal cells. In the intestinal cells, these aglycones are further subjected to an initial phase II metabolism, resulting in appearance of sulfate, glucuronide or methyl metabolites. These metabolites enter the circulation and reach the liver, where their phase II metabolism continues; only a small amount is transported back to the lumen of the small intestine, eventually reaching the large intestine. Therefore, after the absorption in the small intestine, polyphenol phase II metabolites are predominantly found in the circulation[71], whereas the appearance of parent compounds, such as (-)-epicatechin-3-O-gallate and (-)-epigallocatechin-3-O-gallate is practically an exception of the general rule. Post-absorption plasma concentrations of phase II metabolites are low, reflecting the low bioavailability of polyphenols, which is further emphasized by their rapid urinary excretion. Plethora of enzymes and transporters are involved in the processes of hydrolysis, absorption, phase II metabolism and

renal excretion of polyphenols[67]. Theoretically, polymorphisms in the genes that code these proteins may be involved in the ADME component of the overall inter-individual variability in response to consumption of nutritional polyphenols. Indeed, it has been demonstrated that COMT (catechol-O-methyltransferase; enzyme that catalyzes methylation of nutritional polyphenols in the human body) genotype (rs4680) may determine the health benefits from green tea intake[72]. However, the existing data is limited, and genetic factors, along with the influence of gut microbiota, should be addressed in more detail future studies[73].

Indeed, only a small portion of the nutritional polyphenols are absorbed in the small intestine, while most of them reach the large intestine where they are subjected to extensive metabolism. Gut microbiota plays an important role in the metabolism of nutritional polyphenols, which results in appearance of plethora of gut microbiota metabolites, such as aglycones, small phenolic acids and valerolactones. Some of these molecules are absorbed in the large intestine, and are further metabolized in the liver. There is strong scientific evidence that gut microbiota metabolites are among the key mediators of the (cardiometabolic) health promoting effects of polyphenols[74,75]. In addition, with identification of different metabotypes[76,77], gut microbiota has been pinpointed as one of the determinants of inter-individual variability in response to consumption of polyphenols.

2.3 Molecular mechanisms involved in the antioxidant effects of polyphenols, and beyond

With evolution of our understanding of oxidative stress (see section 1.1) and elucidation of the complex metabolism of polyphenols in the human body (section 2.2), the concept that health promoting effects of polyphenols are mediated solely by their ability to scavenge free radicals *in vivo* has been abandoned. Indeed, it has been clarified that polyphenol metabolites do not scavenge substantial amount of free radicals *in vivo*, and that α-tocopherol is the key compound that terminates the chain reaction of lipid peroxidation. Hence, summarizing the latest scientific evidence from both fields, the major mechanism by which polyphenols contribute to the cellular antioxidant defense has been proposed recently[78]. Namely, after consumption, low concentrations of mainly metabolized polyphenols reach the cells. Some of them, such as catechin, epicatechin, hydroxytyrosol, delphinidin and carnosic acid are oxidized by free radicals, in free radical scavenging reactions. An important target of the oxidized polyphenols is Keap1 (Kelch Like ECH Associated Protein 1), a cytosolic protein whose function is to regulate

the activity of transcription factor Nrf2 (Nuclear Factor, Erythroid 2 Like 2). Some polyphenols, such as resveratrol and curcumin, bind with Keap1 directly, without prior oxidation. The regulatory function of Keap1 consists of assistance in ubiquitination of Nrf2, which results in its degradation by proteasome. After binding a polyphenolic compound, Keap1 is inactivated, and the half-life of Nrf2 is extended. Therefore, Nrf2 migrates to the nucleus, binds to the ARE – Antioxidant Response Element (also known as EpRE – Electrophile Response Element), and initiate the transcription of genes that are involved in the antioxidant defense and detoxification. Besides Nrf2, other transcription factors, such as c-Maf, c-Jun, c-Fos, Fra1, Bach1, Nrf1 or c-Myc are also involved in the ARE-mediated transcription, in a complex interplay. In addition, there is evidence that protein kinases, such as PKC or PI3K play role in the phosphorylation of Nrf2, which is a critical step for its translocation to the nucleus. Recent findings that polyphenol metabolites have potential to modulate kinases' activities are indicative of multilevel regulation of Nrf2-mediated transcription.

Besides the Keap1/Nrf2/ARE pathway, nutritional polyphenols also modulate a plethora of cellular functions that are not directly connected with the enzymatic antioxidant defense, but are still very closely associated with the subtle regulation of cellular redox balance. Related to the cardiometabolic health, the effects of polyphenols on cellular processes that are involved in chronic inflammation, endothelial dysfunction, impaired insulin signaling, adipose tissue remodeling or mitochondrial dysfunction have been reviewed in detail[79-82]. Similar to what has been demonstrated for Keap1, there is growing body of scientific evidence that molecular mechanisms of health promoting effects of polyphenols involve their binding to specific proteins, and subsequent effects on cell signaling. For instance, *in silico* docking analyses revealed that curcumin also has ability to bind to some kinases, such as TAK1, PDK1, AKT1 and AKT2, which are involved in regulation of the redox-sensitive pro-inflammatory transcription factor NF-κB. Indeed, NF-κB is established as a key mediator of inflammation, which is a common underlying mechanism of cardiometabolic diseases. Accordingly, a pre-exposure of human umbilical vein endothelial cells (HUVECs) to curcumin (1 μM) prior to TNFα stimulation, attenuates the activation of NF-κB. In parallel, curcumin decreases monocyte adhesion and transendothelial migration, and modulates the expression of genes involved in antioxidant defense, metabolism, cell signaling, focal adhesion, intercellular junction and cytoskeleton organization[83], which is altogether pertinent to an improved endothelial function.

Similarly, it has been demonstrated that pre-exposure of HUVECs to physiologically relevant concentrations of plasma anthocyanins' metabolites (including their gut microbiota metabolites), also decreases the TNFα-induced monocyte cell adhesion. Gene expression analysis demonstrated modulation of genes that are involved in cell adhesion, leucocyte transendothelial migration, regulation of actin cytoskeleton or NF-κB signaling. Subsequent bioinformatic analysis and *in silico* docking demonstrated that anthocyanins' metabolites can bind to several cell signaling proteins, including MEK2 and IKKα, and potentially modulate their activity. The results of *in silico* docking were confirmed with Western blot analysis, demonstrating a decreased phosphorylation of ERK and p65 unit of NF-κB, pertinent to the modulation of upstream kinases, and the degreased leucocyte transendothelial migration and inflammation[(84)]. In addition, it has been demonstrated that flavanol colonic metabolites activate eNOS in human endothelial cells via AKT and AMPK signaling, and increase intracellular nitric oxide, which plays a pivotal role in regulation of endothelial function[(85)]. An integrated systems biology approach has been taken recently to elucidate the multi-target and multi-layer cellular effects of circulating epicatechin metabolites on endothelial cells, demonstrating modulation in gene and miRNA expressions, and DNA methylation. *In silico* docking demonstrated favorable binding of epicatechin metabolites to p38-MAPK, which regulates several transcription factors such as NF-κB, CREB1, c-MYC, c-JUN, STAT3 or SP1[(86)].

Molecular mechanisms of the effects of polyphenols on cardiometabolic health have also been studied in other relevant cell models, such as immune cells, adipocytes, hepatocytes, smooth muscle cells and β-cells. Aiming to systematize the current scientific evidence, we recently extracted and analyzed the nutrigenomic data related to the effects of flavanols or their circulating metabolites in these cell models (paper under submission). Thereby, it was noticed that in large number of studies the cells were exposed to plant extracts or high concentrations of pure compounds, which are not physiologically relevant[(73)]. This knowledge gap needs to be addressed in future, in order to better understand the molecular mechanisms of health promoting effects of polyphenols.

Regarding the contribution of nutritional polyphenols to the cellular antioxidant defense, an important consideration is that they are present in the cells at low concentrations. Therefore, they cannot scavenge a significant amount of free radicals. Instead, they modulate transcription of the

genes involved in antioxidant defense and detoxification. On the other hand, if present at high concentrations, polyphenols may induce toxicity. These considerations are integrated in the concept of hormesis[87], which also applies to nutritional polyphenols[88]. Potential toxicity of high doses of polyphenols should be always kept in mind, as over-the-counter supplements (such as resveratrol, curcumin, quercetin or various plant extracts) are widely available. The lesson learned from the antioxidant supplementation studies that "natural" does not always mean "safe in high doses", should be well remembered. As new strategies to increase the bioavailability of polyphenols are developing rapidly[89], safety and toxicity should be carefully investigated. The topic of safety and toxicity[90] becomes even more important when we take into account the inter-individual variability in response, which will be discussed in the following sections.

3. Effects of polyphenols on cardiometabolic health and cardiometabolic risk factors

3.1 Human intervention studies

Following the pioneering study of Hertog et al.[59], which demonstrated an inverse association between flavonoid intake and mortality from coronary heart disease, numerous human studies have been conducted to explore the impact of consumption of polyphenols on cardiometabolic health. In this type of studies, specific biomarkers should be clearly identified and precisely measured. It is above the scope of this review to analyze in detail this large body of scientific evidence. Therefore, in this paper we present only several relevant human studies (Table 1). "Cardiometabolic" is a relatively new term that encompasses a wide spectrum of cardiovascular and metabolic diseases, including coronary heart disease, non-alcoholic fatty liver disease, metabolic syndrome and type 2 diabetes. These diseases are the leading cause of morbidity and mortality worldwide. They all share common risk factors such as overweight/obesity, inflammation, increased blood pressure, and dyslipidemia[91]. Knowing that diet and life-style largely determine the risk for cardiometabolic diseases, one of the main goals in promoting the cardiometabolic health is development of preventive health programs, such as adoption of healthy dietary habits by the majority of population. Moreover, personalized nutrition could be an effective strategy for prevention and treatment of cardiometabolic diseases in future, either at larger population groups or at individual level.

Cardioprotective effects of polyphenols have been extensively studied. In an acute study[92], it has been demonstrated that flavonoid-rich apple improves plasma nitric oxide status and

endothelial function (measured as flow-mediated dilatation – FMD) and lowers systolic blood pressure. These outcomes are among the most important determinants of cardiovascular health. One of the most convincing evidence for cardioprotective effects of dietary flavonoids has also been related to their beneficial effects on endothelial function, as demonstrated in a meta-analysis conducted in 2008[93]. In that sense, the most convincing effect was observed for cocoa flavonoids. Polyphenols have been pointed out as a promising nutritional approach for prevention and treatment of hypertension. Indeed, a large cross-sectional study demonstrated a negative correlation between total polyphenol intake and blood pressure, both systolic and diastolic[94]. Similarly, regular consumption of cocoa product rich in cocoa fiber significantly decreased the systolic and diastolic blood pressure in moderately hypercholesterolemic humans[95]. Another important determinant of cardiovascular (and more broadly, cardiometabolic) health is the level of systemic inflammation of low intensity, which is clinically assessed using plasma C-reactive protein as a biomarker, preferably with a method of high sensitivity (hs-CRP). In that sense, a recent meta-analysis of RCTs demonstrated that resveratrol significantly decreases serum CRP levels[96], thus potentially decreasing the cardiovascular risk.

Cardioprotective effects of nutritional polyphenols are also mediated through their beneficial influence on circulating lipoproteins. As such, nutritional polyphenols exhibit a potential to decrease the atherogenic risk. The effect of epigallocatechin gallate (EGCG) on LDL-cholesterol was summarized in a recent systematic review, demonstrating a significant reduction of plasma LDL-cholesterol in healthy individuals with EGCG intake between 107 and 856 mg/day[97]. It has also been demonstrated that regular consumption of cocoa increases HDL-cholesterol and decreases oxidized LDL in elderly at high risk for cardiovascular disease[98]. Similarly, regular consumption of flavanol-rich cocoa products has been found to increase HDL-cholesterol in both normocholesterolemic and moderately hypercholesterolemic free-living subjects[99,100]. In addition, increase in HDL-cholesterol and decrease in plasma oxidized LDL is linear with the phenolic content of olive oil[101]. Dyslipidemia and oxidative stress, which are well-defined risk factors for atherosclerosis, are also decreased by diet containing products naturally rich in polyphenols[102].

Effects of dietary polyphenols on the metabolic component of the cardiometabolic risk factors have also been explored by many researchers. Indeed, the beneficial effects of dietary

polyphenols on insulin sensitivity, glucose tolerance and insulin secretion may potentially decrease the risk of type 2 diabetes. In that sense, it has been demonstrated that strawberry and cranberry polyphenols improve insulin sensitivity in overweight and obese non-diabetic, insulin-resistant persons[103], and that polyphenols in general decrease blood glucose response during the oral glucose tolerance test in individuals at high cardiometabolic risk[104]. Consumption of flavanol-rich dark chocolate[105], and supplementation with epicatechin[106] improve insulin sensitivity in healthy subjects. Importantly, numerous epidemiological studies and RCTs have demonstrated that consumption of green tea, red wine and cocoa lowers the risk of type 2 diabetes[107]. In addition, higher consumption of anthocyanins and anthocyanin-rich fruit has also been associated with lower risk of type 2 diabetes[108].

Accumulated scientific evidence about the effects of cocoa flavanols and polyphenols in olive on specific aspects of human health was analyzed by EFSA (European Food Safety Authority) Panel on Dietetic Products, Nutrition and Allergies. Regarding the cocoa flavanols[109,110], the Panel endorses the health claim that they "help maintain endothelium-dependent vasodilatation which contributes to healthy blood flow"[110], and regarding the polyphenols in olive, the Panel endorses that "consumption of olive oil polyphenols contributes to the protection of blood lipids from oxidative damage"[111]. Notably, although many human intervention studies demonstrated beneficial effects of polyphenols on cardiometabolic health, and predominantly on cardiometabolic risk factors, there are also studies that reported lack of expected outcomes. For example, red wine polyphenols did not improve obesity-associated insulin resistance[112], and regular consumption of polyphenol-rich apple failed to influence endothelial function in hypercholesterolemic adults[113]. Considering these and other similar findings, it is essential to understand that many aspects should be taken into account in order to predict the effects of dietary polyphenols on cardiometabolic health. The chemical variety of polyphenols, as well as the variety of their nutritional sources and food matrices largely influence the effects of polyphenols in humans. In addition, the inter-individual variability in bioavailability (which is governed by ADME), as well as the inter-individual variability in bioactivity of (the bioavailable) dietary polyphenols, undoubtedly play pivotal role[114]. Besides, the inconsistency of results from human studies might be influenced, at least in part, by the heterogeneity of study populations, study designs and intervention periods.

3.2 Inter-individual variability in response to consumption of polyphenols

Ideally, dietary recommendations for intake of polyphenols (and plant food bioactives in general) should be determined by their: a) bioavailability and b) bioactivity, taking into account the inter-individual variability. In the first place, the inter-individual variability of ADME affects the bioavailability of polyphenols. Further, the bioactivity of the bioavailable polyphenols (and/or their metabolites) is most likely additionally affected by the inter-individual variability of their cellular molecular metabolism in the target tissues, as well as the inter-individual variability of the cellular processes they modulate. All these factors together affect the inter-individual variability in response to consumption of polyphenols. Furthermore, age, gender, health status, genetics and epigenetic factors have been suggested as the main determinants of the inter-individual variability of response[115]. However, the specific contribution of each of these factors is not completely understood. Indeed, we are at the very early stage of the research in this field. The information we currently have about the inter-individual variability was mainly generated during the post-hoc analyses, not from the studies that had initially been designed to study this subject. This issue should be well addressed in future.

To obtain more information about the inter-individual variability in cardiometabolic response of consumption of nutritional polyphenols, four meta-analyses were conducted recently, using the available data from the literature. The meta-analysis of the cardiometabolic health effects of flavonols confirmed that they improve plasma lipid status, decrease systolic and diastolic blood pressure, and plasma glucose. Deeper study of the available data, aiming to address the inter-individual variability, demonstrated that these effects are more pronounced in participants with diagnosed disease or dyslipidemia[116]. The meta-analysis on flavanols also confirmed that they beneficially modulate several cardiometabolic risk factors, such as plasma lipids, body mass index and waist circumference. However, higher efficacy was demonstrated in the participants with body mass index $\geq$ 25 kg/m^2 and in non-medicated individuals, revealing the inter-individual variability[117]. Similarly, the beneficial effects of ellagitannins and anthocyanins on selected cardiometabolic risk factors were found to be more prominent in overweight and obese subjects[118], and the effects of hydroxycinnamic acids may be greater in individuals at higher cardiometabolic risk[119].

These meta-analyses are of high importance because they introduce and emphasize the significance of the inter-individual variability in response to consumption of dietary polyphenols to the scientific community. However, their main limitations were the following: a) unavailability of individual data, pre- and post-intervention, and b) low number of studies. Importantly, even though facing these limitations, the authors were able to demonstrate significant inter-individual variability of several crucial cardiometabolic risk factors, which opens perspectives for further research in the field. However, in order to be able to conduct more robust meta-analyses in future, availability of individual data should be encouraged, of course in compliance with ethical standards. Understanding the factors that determine the inter-individual variability in response to consumption of polyphenols will lead us towards tailoring of personalized dietary recommendations, at both population group and individual level.

Conclusion and perspective

In this review, we aim to address the key aspects of the impact of polyphenols on human cardiometabolic health, historically and in perspective, as summarized in Figure 1. Historically, there was a tendency to attribute the positive effects of polyphenols on human cardiometabolic health exclusively to their free radical scavenging capacity. Nowadays we understand that they express their antioxidant activity mainly through modulation of transcription of genes involved in antioxidant defense and detoxification. Apart from their antioxidant activity, polyphenols also contribute to the cardiometabolic health by modulating plethora of cellular processes that are not directly associated with the antioxidant enzymes, which are subject to extensive research. In the cells, dietary polyphenols and/or their metabolites are present at low concentrations. Administered at very high concentrations, polyphenols may cause toxic effects in humans. Informed about the beneficial effects of polyphenols on human cardiometabolic health, the temptation among the general population for taking supplements of pure compounds or plant extracts, which are readily available as over-the-counter pharmaceutical forms, is realistic. However, the vigilance for their potential toxicity, if taken at high doses, should remain high.

Growing scientific evidence demonstrates the beneficial effects of dietary polyphenols on human cardiometabolic health. Numerous randomized controlled trials report improvement of the key biomarkers of cardiometabolic health such as blood pressure, flow-mediated dilatation, blood lipids, blood glucose, and insulin sensitivity. The inconsistency of the results of some studies

triggered interest towards post-hoc analysis of individual data, which led to identification of the inter-individual variability in the response. Resolving the puzzle of the inter-individual variability will lead us towards personalized dietary recommendations, with ultimate goal to decrease the risk for cardiometabolic diseases and preserve the health for better aging. For this purpose, future studies shall be specifically designed to address the various aspects of the inter-individual variability in response to consumption of polyphenols, and plant food bioactives in general.

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Conflicts of interest

None.

Authorship

TR, VM and DM conceived the structure, wrote the manuscript and approved the final version.

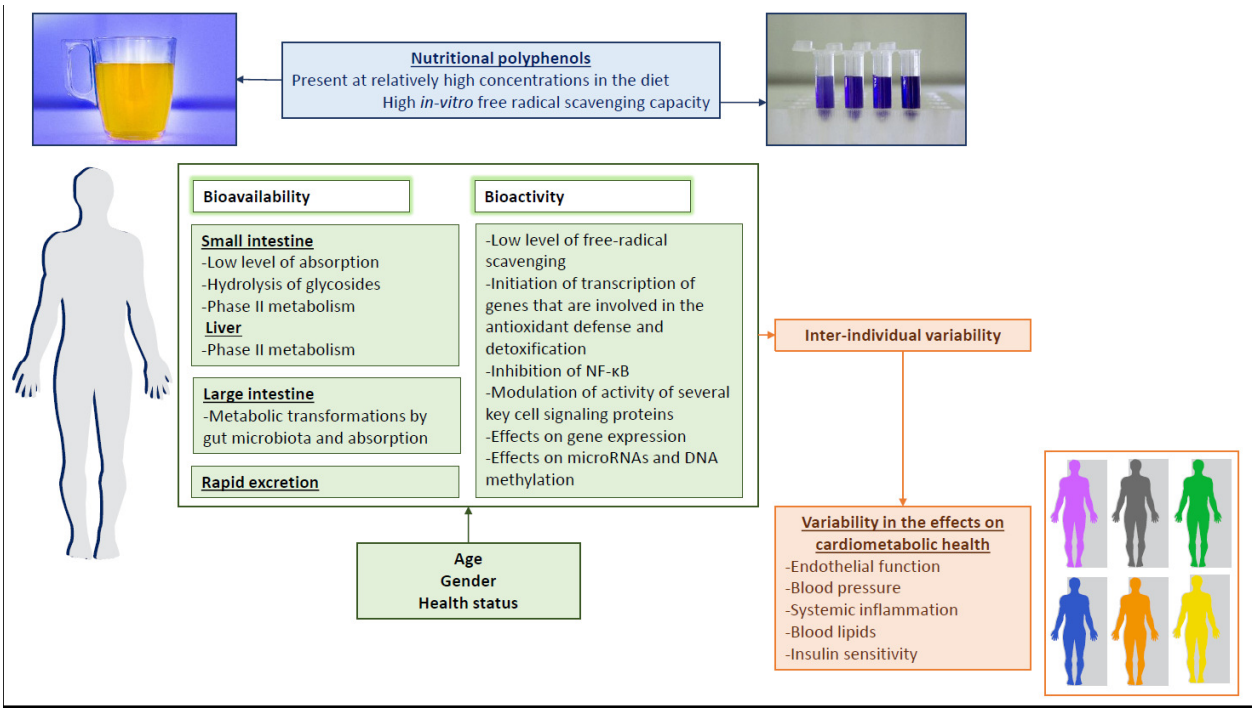

Figure 1. Polyphenols in human diet – *in vitro* and *in vivo*.

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1 **Table 1. Overview of the protective effects of polyphenols on cardiometabolic health - evidence from human studies.**

Study design	Polyphenol, and dose	Duration	Participants (N, and health status)	Cardiometabolic outcomes	Reference
Observational study of the subcohort of the Polish arm of the HAPIEE cohort	Dietary data were collected by using a food frequency questionnaire consisted of 148 food and drink items. The polyphenol content in foods were obtained from the Phenol-Explorer database.	4 years	5806 adults aged 45-69 years recruited in the urban area of Krakow, Poland. Healthy volunteers free of diabetes.	When comparing extreme quartiles, intake of total polyphenol was inversely associated with the risk of type 2 diabetes.	(4)
Observational study of the subcohort of the PREDIMED trial	Two Mediterranean diets: -extra-virgin olive oil (1L/week for all the family) or -extra-virgin olive oil plus mixed nuts (30 g/day, as 15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts).	1 year of intervention	1139 older volunteers at high risk of cardiovascular disease within the PREDIMED trial.	Participants in the highest tertile of changes in total urinary polyphenol excretion showed: -decrease in plasma levels of inflammatory biomarkers (vascular cell adhesion molecule 1, intercellular adhesion molecule 1, interleukin 6, tumor necrosis factor alpha, monocyte chemotactic protein 1), -decrease in systolic and diastolic blood pressure and -increase in plasma high-density lipoprotein cholesterol.	(5)
Controlled randomized clinical study	300 mg of green tea polyphenol extract (Polyphenon E) twice a day.	1 week	Twenty-two male volunteers (12 smokers and 10 non-smokers) aged between 22 and 32 years.	Increased resistance of LDL to oxidation.	(11)
Randomized, cross-over controlled trial	25 mL/d raw high-polyphenol-content olive oil (HPCOO; 366 mg/kg) or low-polyphenol-content olive oil (LPCOO; 2.7 mg/kg).	3 weeks	25 healthy European men, aged 20-59 years.	-Plasma apoB-100 concentrations and the number of total and small LDL particles decreased after the HPCOO intervention, which increased with LPCOO intervention. -LDL oxidation lag time increased with HPCOO intervention. -Lipoprotein lipase gene expression tended to increase after the HPCOO intervention and did not change after the LPCOO intervention.	(12)
Longitudinal investigation study	Mean estimated flavonoid intake was 26 mg/day.	5 years	805 men aged 65-84 years.	Flavonoid intake was significantly inversely associated with mortality from coronary heart disease and showed an inverse relation	(59)

				with incidence of myocardial infarction.	
Double-blind, crossover, dose-response, randomized, placebo-controlled trial	Pomegranate extract. Dose 1: 160 mg phenolics/day or Dose 2: 640 mg phenolics/day.	3 weeks	Forty-nine participants (body mass index > 27 kg/m^2).	After Dose 2, total cholesterol, LDL-cholesterol, small LDL-cholesterol, non-HDL-cholesterol, apolipoprotein-B and oxidized LDL-cholesterol decreased, but only in urolithin metabotype B subjects.	(76)
Double-blind, placebo-controlled crossover study	Soy isoflavones (80 mg aglycone equivalents).	Acute study	Male equol producers and non-equol producers; 14 per group, at moderate cardiovascular risk.	Arterial stiffens (carotid-femoral pulse-wave velocity) significantly improved in equol producers at 24 h, which was significantly associated with plasma equol concentrations.	(77)
Randomized controlled crossover (Latin-square) designed study	120 g of apple flesh and 80 g of apple skins that provided 184 mg of quercetin and 180 mg of (-)-epicatechin.	Acute study	Healthy men and women, N=30.	Increased flow-mediated dilatation. Decreased pulse pressure and systolic blood pressure.	(92)
Cross-sectional substudy of the PREDIMED trial	Intake of fruit, vegetables, coffee, wine.	5-year follow up study	589 volunteers (men and women) free of cardiovascular disease at baseline and fulfilled at least one of the following two criteria: (1) type 2 diabetes mellitus and/ or (2) three or more coronary heart disease risk factors.	An inverse association was observed between urinary total polyphenol excretion (TPE) and the prevalence of hypertension. Participants in the highest quartile of urinary TPE had a reduced prevalence of hypertension compared to those in the lowest quartile. Systolic and diastolic blood pressure were inversely associated with urinary TPE after adjustment for potential confounders.	(94)
Non-controlled, non-randomized, open intervention trial	Two servings per day cocoa product which provided 12 g of dietary fiber and 283 mg of soluble polyphenols.	2 months	21 volunteers (women and men), moderately hypercholesterolemic, non-vegetarian, non-smoker, between 18 and 45 years old, with a body mass index under 30 kg/m^2, not suffering from any other chronic pathology.	Glucose, systolic and diastolic blood pressure, and malondialdehyde decreased, while HDL-cholesterol slightly increased.	(95)
Randomized, crossover and controlled clinical trial	500 mL of skimmed milk/day with or without 40 g of cocoa powder that provided 495.2 mg of total polyphenols and 425.7 mg of	4 weeks	Forty-two high-risk volunteers (19 men and 23 women), mean age 69.7 years.	Increased HDL-cholesterol. Decreased oxidized LDL.	(98)

	proanthocyanidins.				
Controlled, cross-over studies in free-living condition	Soluble cocoa product rich in dietary fibre (DFCP) providing daily 10.17 g, 43.8 mg and 168.6 mg of total-dietary-fibre, flavanols and methylxanthines, respectively and a product rich in polyphenols (PPCP) providing daily 3.74 g, 45.3 mg and 109.8 mg of total-dietary-fibre, flavanols and methylxanthines, respectively.	4 weeks	N=44, men and women, between 18 and 55 years old, normocholesterolemic and moderately hypercholesterolemic subjects, non-vegetarian, non-smoker, not suffering from any chronic pathology or gastrointestinal disorder, body mass index was under 30 kg/m^2.	Both products increased HDL-cholesterol concentrations, whereas only DFCP decreased glucose and IL-1β levels in all subjects.	(99)
Non-randomized, controlled, crossover study of free-living individuals	Soluble cocoa product providing 45.3 mg of flavanols per day, in milk.	4 weeks	Normocholesterolemic (N=24; 25.9 ± 5.6 years) and moderately hypercholesterolemic (N=20; 30.0 ± 10.3 years) volunteers.	Increase in HDL-cholesterol. Decrease in IL-10.	(100)
Randomized, crossover, controlled trial	25 mL of 3 olive oils. Olive oils had low (2.7 mg/kg of olive oil), medium (164 mg/kg), or high (366 mg/kg) phenolic content but were otherwise similar.	3 weeks	200 healthy male volunteers.	A linear increase in HDL-cholesterol levels was observed for low-, medium-, and high-polyphenol olive oil. Total cholesterol/HDL-cholesterol ratio decreased linearly with the phenolic content of the olive oil. Oxidative stress markers decreased linearly with increasing phenolic content.	(101)
Randomized controlled study	Diet rich in polyphenols.	8 weeks	Eighty-six individuals of both sexes, aged 35-70 years, with a large body mass index (27-35 kg/m^2) and large waist circumference (men, >102 cm; women, >88 cm) and any other component of the metabolic syndrome.	Decrease of fasting plasma triglyceride concentrations. Decrease of postprandial plasma triglyceride total area under curve. Decrease of urinary 8-isoprostane.	(102)
Parallel, double-blind, controlled and randomized clinical trial	Strawberry and cranberry polyphenols, 333 mg/day.	6 weeks	Free-living insulin-resistant overweight or obese human subjects (N=41).	Increased insulin sensitivity.	(103)

Randomized controlled study	Diet rich in polyphenols.	8 weeks	Eighty-six individuals of both sexes, aged 35-70 years, with overweight or obesity (body mass index 27-35 kg/m^2) and large waist circumference (men, >102 cm; women, >88 cm) and at least one more component of the metabolic syndrome.	During OGTT, polyphenols significantly reduced plasma glucose total area under curve and increased early insulin secretion. Improved post-challenge oral glucose insulin sensitivity.	(104)
Randomized, crossover, controlled trial	100 g dark chocolate bars containing ≈500 mg polyphenols.	15 days	15 healthy persons (7 men and 8 women) aged 33.9 ± 7.6 years.	Improved insulin sensitivity. Decreased systolic blood pressure.	(105)
Randomized, double-blind, placebo-controlled, crossover trial	(-)-epicatechin, 100 mg/day.	4 weeks	Thirty-seven apparently healthy men and women aged 40-80 years with a systolic blood pressure between 125 and 160 mm Hg at screening.	Reduced insulin resistance.	(106)
Prospective cohort studies	Flavonoid intakes were assessed using food frequency questionnaires. For quantification of the flavonoid content in various food sources, a comprehensive database of levels of individual flavonoids in foods was established predominantly on the basis of the USDA flavonoid content of the foods database.	1984-2008 1991-2007 1986-2006	70359 women in the Nurses' Health Study (NHS; 1984-2008), 89201 women in the NHS II (1991-2007), and 41334 men in the Health Professionals Follow-Up Study (1986-2006) who were free of diabetes, cardiovascular disease, and cancer at baseline.	Higher intakes of anthocyanins were significantly associated with a lower risk of type 2 diabetes, after multivariate adjustment for age, body mass index, and lifestyle and dietary factors.	(108)