Protection by Flavanol-Rich Foods Against Vascular Dysfunction and Oxidative Damage: 27th Hohenheim Consensus Conference[1]

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ABSTRACT

Criteria for assessing the purported protection by flavanol-rich foods against vascular dysfunction and oxidative damage to biomolecules was the subject of the 27th Hohenheim Consensus Conference held on July 11, 2011. State-of-the-art evidence was put into perspective, focusing on several questions that were followed by a consensus answer. Among the topics addressed were the major sources of flavanols in the human diet, the bioavailability of flavanols, biomarkers for "health benefit," and the biological function of flavanols. Consensus was reached on these topics. No conclusion was reached on the design of randomized, controlled trials for substantiation of health claims for flavanol-rich foods as to the necessity of a study arm with an isolated pharmacologically active compound, e.g., (−)-epicatechin. *Adv. Nutr. 3: 217–221, 2012.*

Introduction

Herein we compile and assess the available evidence on the topic of protection by flavanol-rich foods against vascular dysfunction and oxidative damage, which was the focus of the 27th Hohenheim Consensus Conference, held on July 11, 2011, at the University of Hohenheim, Stuttgart, Germany. There has been a recent surge in the interest in the relationship between polyphenol intake and health issues. This has led to attempts to accumulate scientific evidence of cause-effect relationships for individual chemically identified polyphenol compounds or for classes of compounds in food rich in specific polyphenols. In vitro research on such compounds, either chemically isolated or within the food matrix, has provided considerable insight into potential health benefits of food products in recent years. However, progress in solving the problem of translating that knowledge into beneficial health outcomes related to nutritional supply and demand has been limited. Nonetheless, there is growing attention from diverse fields (e.g., agriculture, phytochemistry, physiology, nutrition sciences and medicine), particularly in regard to flavonoids (1).

The topic has been a matter of debate, centering on the use of appropriate biomarkers on the one hand, and on the question of whether in vitro data (e.g., on antioxidant properties) can be translated into relevant in vivo data. Recent assessments in the literature came to the conclusion that the biological relevance of *direct* antioxidant effects of polyphenols for cardiovascular health has not been established (2,3). Current knowledge and future needs on recommending flavanols and procyanidins for cardiovascular health have been reviewed (4).

The presentation is organized by a consensus reply (in italics) to a given question, followed by further statements that reflect current available literature.

Definitions

Vascular health

Vascular components in disease processes make the cardiovascular circulatory system a prime target for preventive measures, involving conduit vessels as well as the microcirculation. There is a need to identify scientific requirements for monitoring cardiovascular health outcome, using functional and biochemical markers. In a recent publication (5) by the European Food Safety Authority (EFSA)[9] on guidance on the scientific requirements

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[9] Abbreviations used: EFSA, European Food Safety Authority; FMD, flow-mediated dilation; PWV, pulse wave velocity.

for health claims related to antioxidants, oxidative damage, and cardiovascular health, the opinion was expressed that "maintenance of normal blood pressure is a beneficial physiological effect" and that substantiation of health claims in this regard can be obtained from human intervention studies showing a sustained (e.g., 2 mo) lowering of blood pressure. It was stated further (6) that endothelial function per se is not sufficiently defined for scientific evaluation because of a diversity of endothelial functions, including vasomotion, smooth muscle proliferation, thrombosis, inflammation, coagulation, fibrinolysis, and oxidation. However, it was concluded (6) that an improvement of specific endothelial functions (e.g., endothelium-dependent vasodilation) after sustained exposure to the food/constituent (e.g., 1 mo) may be considered a beneficial physiological effect. Similar considerations apply to lowering platelet aggregation.

Oxidative damage

The protection of tissue, cells, and biomolecules such as DNA, proteins, and lipids is considered by the EFSA a beneficial physiological effect (5,6). The scientific substantiation of protection requires an appropriate method of assessment to determine the oxidative modification of the target molecule in vivo by at least 1 appropriate marker.

Flavanols

Polyphenols represent a major class of phytochemicals, and within this class, the flavonoids have attracted much interest in recent years (7). In the chemical category of polyphenols, catechins compose a subgroup of flavanols with catechin, epicatechin, gallocatechin, epigallocatechin, and the respective gallic acid esters at the 3-OH position (catechin gallate, epicatechin gallate, gallocatechin gallate, and epigallocatechin gallate) as major representatives (8). In nature, (+)-catechin and (−)-epicatechin are the major optical isomers. Procyanidins (a subgroup of the proanthocyanidins) are oligomers of parent monomers, the flavanols. Procyanidins are oligomers of catechin and/or epicatechin; among them are the dimeric procyanidins B1, B2, B3, and B4 (4–8 linkage of the C to the A ring) and procyanidins B5, B6, B7, and B8 (4–6 linked) as well as A-dimers, which contain an additional ether bond (2–O7 linked). Higher polymers are also found in natural products.

Redox chemistry of polyphenols is determined by the reactivity of hydroxyl groups attached to the same aromatic ring (9,10). Two aromatic hydroxyl groups are oxidized to the corresponding quinone via an intermediate semiquinone radical, which is the 1-electron intermediate oxidation product. This reaction provides the chemical basis for the antioxidant properties of polyphenols. The antioxidant activity of catechins and procyanidins is determined by the number and localization of the hydroxyl groups (11). Indirect antioxidant effects are mediated by inhibiting/lowering activity of pro-oxidant enzymes, including cyclooxygenases and lipoxygenases, monooxygenases, and xanthine oxidase or NADPH oxidases (12). Conversely, flavonoids can stimulate enzymes involved in antioxidant defense, e.g., by activating gene expression through Nrf2-related mechanisms (13).

What are the major sources of flavanols in the human diet?

Polyphenols are present in a great variety of food sources. Major dietary sources of flavanols are tea, grapes, apples, and cocoa.

Intake of all polyphenols in the general population is currently estimated at ∼1 g/d (2); data on polyphenols in foods are now available in a searchable form online (14), but much information is still lacking, especially on more complex polyphenols such as certain procyanidins, ellagitannins, and black tea thearubigens, and some commonly consumed foods are not included. In addition, polyphenol content and profile of edible plants vary widely because of many factors such as variety or cultivar, growth conditions, crop management, storage, and food processing. Fruits (apples, grapes, pears), tea, wine, and cocoa/chocolate are the main sources of monomeric flavanols (14,15), and large variations in intake were observed (4–121 mg/d) (2). More recently, after the publication of the USDA database for proanthocyanidins, the intake of procyanidins has been estimated in the US and Finland (16,17). It was found to be ∼4–14 times higher than that of the flavanol monomers. Perez-Jimenez et al. (18) analyzed the dietary intake of polyphenols in a French population participating in the SU.VI.MAX (Supplementation en Vitamines et Mineraux Antioxydants) trial. Total intake was 1.2 g/d, containing 99 mg catechins. The major sources of (−)-epicatechin were green tea (28%), apples (24%), cocoa products (17%), and red wine (15%); sources of (+)-catechin were red wine (41%), tea (15%), cocoa products (10%), and peaches (6%). The sole source of (−)-epigallocatechin 3-O-gallate, (+)-gallocatechins, and (−)-epigallocatechins was green tea.

There are many papers reporting the flavanol content of various foods and beverages, and these have been compiled in databases, currently as Phenol-Explorer and the USDA databases [see Ref. (2)]. When interpreting the data, it is important to consider the amount of polyphenols per portion typically consumed. As examples of published data, (−)-epicatechin in dark chocolate is between ∼0.1 and 2 mg/g (19), which translates to between ∼4 and 80 mg per 40-g portion. The average contents of green tea were 0.4 mg of (+)-catechin and 6.7 mg of (−)-epicatechin per gram dry weight (20). On this basis, 2 g of green tea leaves in 200 mL of water with an extraction yield of 95% for (+)-catechin, and 85% for (−)-epicatechin (extraction conditions: 5 min, 80°C), an average cup of green tea would contain ∼0.8 and 11 mg of catechin and epicatechin, respectively.

What is the bioavailability of flavanols?

The bioavailability of flavanols varies over a wide range. In general, absorbed flavanols have a short half-life in plasma. A fraction of ingested flavanols is absorbed intact in the small intestine and undergoes phase II metabolism. The remaining portion is extensively metabolized by the gut flora, and the metabolites are subsequently absorbed.

The absorption and metabolism of epicatechin and catechin are now quite well understood. However, they are still a matter of debate for procyanidins. In general, all polyphenols undergo metabolism either by the intestinal tissues and

liver and, if they are not absorbed in the small intestine, by the gut microbiota. There is no evidence that polyphenols and their metabolites are stored in the body, as they are rapidly excreted in urine and bile with widely varying half-lives (always <48 h). It is important to make a distinction between flavanols (the monomers) and proanthocyanidins (the oligomers) considering bioavailability.

Epicatechin and catechin are absorbed in the small intestine. Many papers have reported their absorption and metabolism in humans, supported by extensive mechanistic studies in rodents and intestinal Caco-2 cells. The absorption of epicatechin from green tea in humans was critically reviewed recently (21), and it is concluded that from a green tea beverage, taking the consensus of publications that measured pharmacokinetics after a single acute dose, the expected maximum concentration in plasma normalized to a 15-mg dose of epicatechin was 127 nM epicatechin, present as 76 nmol/L epicatechin sulfates and glucuronides, and 51 nmol/L as methylated epicatechin sulfates and glucuronides. The proportion of epicatechin that is not absorbed in the small intestine passes to the colon where it is extensively metabolized by gut microbiota into low molecular weight phenolics, which are then absorbed. There is less known about the biological properties of these microbial metabolites (22).

The situation is different for the procyanidins. After consumption of procyanidin-rich dark chocolate, only very low amounts of procyanidin dimer could be detected in human plasma (23). No other procyanidins were detected in plasma. However, although the parent compounds do not seem to be absorbed as such, there is evidence that the breakdown products after catabolism by the gut microbiota are extremely well absorbed. Based on a study on rats using radiolabeled procyanidin B2, >80% of the gut microbiota metabolites of the procyanidin were absorbed and subsequently appeared in the urine (24). Again, the biological properties of the microbiota flavanol metabolites are not well-known, and metabolism by humans may differ significantly from that by rodents. Based on the available information on absorption and metabolism, epicatechin and catechin are absorbed in the small intestine and may potentially act on the vascular endothelium directly, whereas the longer oligomers are not absorbed and so are unlikely to exert effects directly.

Are there suitable biomarkers for a "health benefit"?

There are biomarkers for vascular dysfunction [flow-mediated dilation (FMD), vascular stiffness] and for oxidative damage (DNA oxidation products, lipid peroxidation). Lowering of risk factors (blood pressure, LDL cholesterol) in humans has been described for flavanol-rich food but cannot yet be directly attributed to flavanols per se.

The European Union Regulation 1924/2006 addresses nutrition and health claims made about foods (25). Claims about antioxidants have been classified as health but not nutrition claims and must be scientifically substantiated. Scientific advice is provided by the EFSA. According to the EFSA, maintenance of normal blood pressure is considered a beneficial physiological effect. One parameter for addressing vascular function that can be used as functional biomarker is measurement of FMD of the brachial artery using vascular ultrasound measurements (26,27). It represents the percentage of diameter gain of the artery in response to a temporal occlusion of the vessel by means of inflating a blood pressure cuff. This response represents the endothelium-dependent relaxation of the artery. Evidence from prospective studies suggests that FMD is an independent predictor of cardiovascular events (28).

Vascular aging progressively deteriorates vessel elasticity, which leads to an increase in arterial stiffness. An increase in arterial stiffness leads to functional changes and has detrimental effects on cardiovascular health. A valid measure of arterial stiffness is the aortic pulse wave velocity (PWV). PWV is determined by measurement of the aortic pressure as a function of time, the pulse wave. Left ventricular contraction creates a forward pressure wave, whereas peripheral points reflect this wave. Increased vascular stiffness causes the reflected wave to speed up, causing an increase in aortic systolic pressure. The aortic PWV is considered a suitable measurement of arterial stiffness (29). The applicability of PWV for assessing cardiovascular disease is well established; a meta-analysis of 14 prospective studies in cardiovascular patients as well as the general population showed a pooled relative risk of 2 for cardiovascular events and mortality in subjects with high versus low PWV values (30). Until now, PWV has been little explored in interventions with polyphenols, and only small studies with isoflavones have been reported (31).

The EFSA Dietetic Products, Nutrition, and Allergies Panel also states that protection of DNA, proteins and lipids from oxidative damage is beneficial to human health. For several dietary constituents including vitamin C, selenium, and manganese, sufficient generally accepted scientific evidence of an antioxidant health claim is available. However, at the moment, it is generally considered that there is not yet sufficient evidence available to make a definitive decision about flavanol compounds.

As for oxidative damage, there is a need for reliable biomarkers. In the context of antioxidants, the biomarker concept aims to evaluate the pro-oxidant load, antioxidant defense, and/or the resulting oxidative damage (32,33). The utility and validity of biomarkers for oxidative stress are still a matter debate (34–36). Significant information can only be expected from studies in complex organisms (e.g., animals, humans) when suitable markers are analyzed, whereas data from cell culture and model systems can only be supportive. Addressing different target molecules, various markers have been introduced (37–41). Formation of malondialdehyde, conjugated dienes, lipid hydroperoxides, or F2-isoprostanes is frequently measured to evaluate lipid peroxidation. Oxidative damage of DNA is evaluated by analyses of oxidized DNA bases (e.g., 8-oxo-2′-deoxyguanosine) or, more generally, in the comet assay in which DNA strand breaks can be detected by applying single-cell microgel electrophoresis and analyzing the extent of tailing by damaged DNA. Modifications of the comet assay have been developed that allow the detection of

oxidized DNA bases (42). Protein oxidation has been determined by the analyses of protein carbonyls, which are nonspecifically generated (43). More specifically, oxidized amino acids may be analyzed by means of HPLC-mass spectrometry. Resistance of LDL to oxidative challenge by radicals and modified LDL protein have also been used as indicators of antioxidant defense and prevention of specific protein damage.

A number of assays have been introduced to analyze the total antioxidant capacity of either blood or food samples (44). Among these methods are the ORAC (oxygen radical absorbance capacity), TRAP (total reactive antioxidant potential), TEAC (Trolox-equivalent antioxidant capacity), and FRAP (ferric-reducing ability of plasma) assay. These assays are nonspecific and not suitable as markers for oxidative stress in in vivo studies (45,46). The assays have also been used to estimate the total antioxidant activity of a food or food product, especially in context with flavonoid-rich items. In food samples, glycosides of the flavonoids make a lower contribution to total antioxidant capacity than aglycones, so that deglycosylation in vivo may liberate activity. Conversely, flavonoids are efficiently conjugated in vivo in reactions blocking redox-active hydroxyl groups, which corresponds to inactivation of direct antioxidant activity.

What is the biological function of flavanols with respect to human health?

Biological functions of flavanols can occur at the cellular and systemic levels by modulating cellular signaling and enzyme activities at levels achievable with a normal diet. Randomized, controlled trials show an effect on blood pressure, LDL cholesterol, and FMD.

Although antioxidant properties of flavanols are demonstrable in vitro, there is no evidence that there are direct antioxidant effects of flavanols in the circulation and in tissues in vivo, as recently summarized (2,3). Changes in risk factors (e.g., blood pressure, LDL cholesterol) in humans have been described for flavanol-rich food but cannot yet be directly attributed to flavanols per se. In humans, the evidence of biological effects is almost entirely based on flavanol-rich foods such as chocolates, cocoa, and green and black tea rather than pure compounds. Here, randomized, controlled trials show an effect on blood pressure, LDL cholesterol, and FMD [summarized in Reference (47)]. The molecular mechanism may be via effects on NADPH oxidase [see discussion in Reference (7)].

Is it possible to substantiate the health benefit of flavanols with a randomized, controlled trial?

The answer is affirmative, although no consensus has been reached as to the requirement of a study using an isolated food component (here, a specific flavanol) in addition to a study using a food or food product.

Although there are many human intervention studies on flavanol-rich foods, there are still gaps in our knowledge in this area. Evidence is mostly available for flavanol-rich foods, without absolute proof that the effects are due to the flavanol component. Studies need to be of sufficient length in the future, at least 1 mo and preferably longer. In addition, validated biomarkers should be used when the study is intended to be used for claims. In terms of advancing scientific knowledge, however, we do not discourage the use of "experimental" markers such as metabolomics. The future use of pure compounds or foods as experimental material was discussed, but no consensus reached, although it is clear that better placebo foods are required. We therefore recommend that future studies be randomized, placebo-controlled trials using validated biomarkers of at least 1-mo duration, using foods and a design for which the biological effects of the flavanol component can be readily ascertained. A recent assessment of the scientific substantiation of health claims in the European Union (48) is pertinent to the issue of flavanol-rich food. Likewise, the general question in the debate on scientific substantiation of health claims in terms of evidence-based nutrition was addressed at the 26th Hohenheim Consensus Conference (49).

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