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Pleiotropic-Acting Nutrients Require Integrative Investigational Approaches: The Example of Flavonoids

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ABSTRACT: Assessment of the health effects of dietary interventions in humans poses a particular challenge to nutritional and clinical scientists. In contrast to drugs possessing a well-defined molecular mechanism of action, food-derived components act in subtle and pleiotropic ways by nature. Moreover, dietary compounds are mainly not intended to cure a disease but to prevent or beneficially affect situations when the physiology gets slightly out of homeostasis. By the example of a recent clinical pilot study, this paper illustrates an endeavor to find new strategies for the detection of health effects of flavonoids in the human vasculature. Integration of a meticulously selected cluster of relevant biomarkers eventually enabled the beneficial vascular health effects of flavonoids to be revealed. A critical appraisal of this approach for the future is provided.

KEYWORDS: *flavonoids, nutrients, antioxidants, vascular health, inflammation, integration, biomarkers, risk factors, end points, health index, clinical study*

INTRODUCTION

Currently, there is quite some debate as how to evaluate the health-promoting effect and the disease risk-reducing effect of foods and food constituents in humans.[1] Clear evidence-based efficacy should be firmly established before claims are allowed on the benefits of nutraceuticals, nutrients, or food components. We are inclined to define end points in efficacy-testing similarly as in the evaluation of drugs.[2] However, drugs are designed as golden bullets, that is, one compound for one disease via a single molecular target. Drugs are used to treat and cure sick people. The drug effect should preferably be observed directly. A blood pressure lowering effect, the diuretic action, or a cholesterol-lowering effect should instantly become established. Pharmacology, the science of drug action, has strikingly and precisely been described as a form of "selective toxicity".[3] This is in sharp contrast to food-derived compounds, which are used in rather healthy people. In most cases they exert pleiotropic actions; that is, they subtly modulate a multitude of physiological processes. The effects are chronic and are not immediately measurable.[2]

WHAT IS HEALTH?

When we aim for a health-promoting effect of a food or food constituents, it should be clear what we mean by health. The frequently used definition of the WHO is "a state of complete physical, mental, and social well-being" and not merely the absence of disease or infirmity.[4] The first part of this definition of health is part of the founding constitution of the WHO, which dates from 1946. With increasing understanding of disease, not only at societal and individual levels but certainly also at a molecular level, the definition has become flawed.[5] It is now increasingly recognized that health is not a fixed entity but should rather be seen as a dynamic condition, which may differ under various circumstances. Furthermore, health is not a state of perfection but the ability to adapt.[5] Of course, this changing view also has consequences for the prevention or treatment of diseases. Instead of trying to attain perfection, our preventive and therapeutic approaches should rather aim for increasing the aptitude to adapt.

In addition, it becomes increasingly clear that diseases, in particular chronic diseases, are not caused (nor treated or prevented) via a single molecular target. Interestingly, popular blockbuster drugs have often been developed via medicinal chemistry to reach specificity, but after all appeared to deploy pleiotropic actions in the human body. This multitude of actions of those single drugs might even explain their therapeutic effectiveness and hence their success. Good examples in this regard are various cardiovascular drugs such as statins, β-blockers, or calcium antagonists.[6] Statins were originally developed as specific inhibitors of the rate-limiting enzyme of the cholesterol biosynthesis hydroxymethylglutaryl-CoA reductase. However, it became gradually evident that statins exert, among others, antiatherosclerotic, antinociceptive, anti-inflammatory, and antithrombotic effects.[7]

β-Blockers besides specific blockage of β-adrenoceptors have been shown to inhibit lipid peroxidation.[8] This capacity has even been optimized to increase the therapeutic effects of the β-blocker carvedilol.[6] Likewise, the antioxidant activity of calcium antagonists has been suggested to contribute to their clinical efficacy.[6] It appears not only that is disease best counteracted but that health is best established by compounds acting in a pleiotropic manner.

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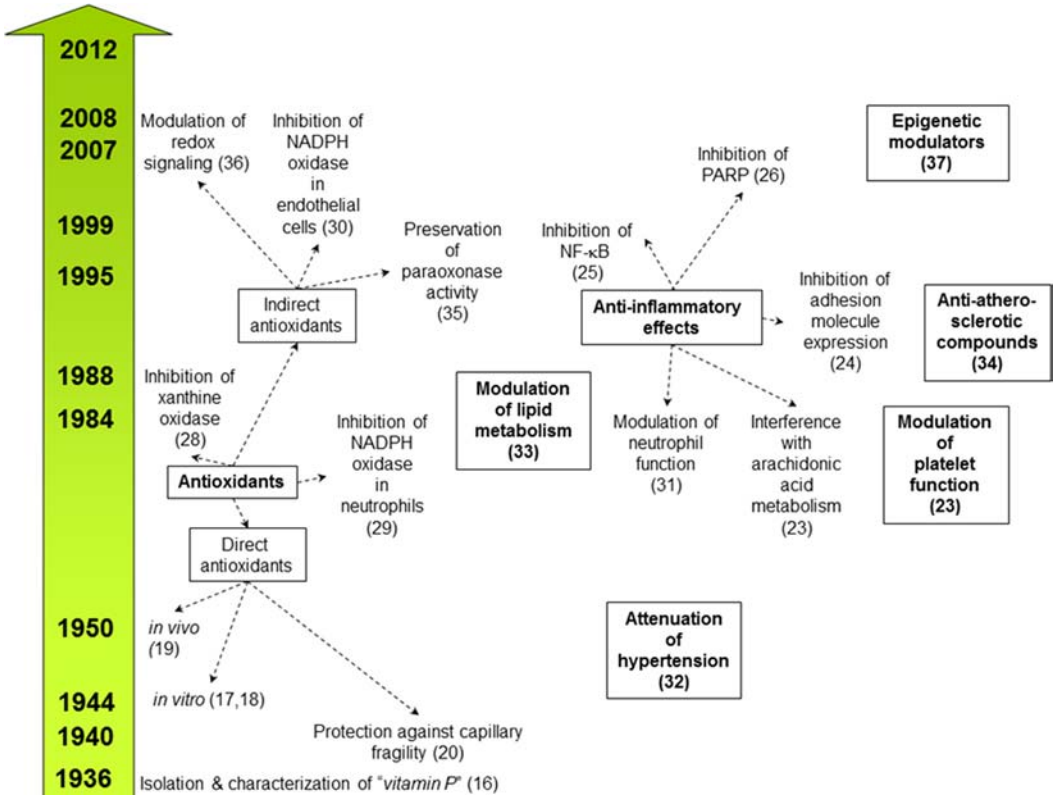

Figure 1. Historical overview of the major discoveries of the molecular mechanisms of action and biological effects of flavonoids since their first description as vitamin P by Szent-Györgyi and colleagues in 1936. The number in parentheses indicates the corresponding literature reference.

HOW TO TEST PHYSIOLOGICAL EFFICACY IN AN APPARENT HEALTHY POPULATION?

The first thing that comes to mind is that the original WHO definition for health mentions a complete well-being. Increasing health even in this seemingly perfect situation is possible when health is defined as the ability to adapt. Even in a total healthy state, a stressor may bring an apparent homeostasis out of balance. The increased ability to withstand such a stressor can then be regarded as a marker for health. Such a stressor might be applied in vivo, for example, within the setting of a glucose tolerance test.

In our clinical pilot study, in which we investigated the vascular health effects of flavanols in smokers (vide infra), we used flow-mediated dilatation (FMD) of the brachial artery as a stress response test.[9] Although the 5 min occlusion of the brachial artery can probably not be regarded as a physiological stressor, the elicited blood flow peak and the consequent arterial dilatation after deflating the cuff is certainly a stress response. Brachial artery FMD is a well-established noninvasive method to assess the function of the vascular endothelium, which appears to be related to cardiovascular risk.[10,11]

A challenge might also be applied ex vivo. In this case after having received the intervention, samples of a volunteer's body compartment such as blood can be subjected to an in vitro challenge. In our clinical trial example, we challenged blood collected from the subjects allocated to the verum or placebo intervention with bacterial endotoxin (lipopolysaccharide (LPS)) to induce an inflammatory response (vide infra). Remarkably, LPS challenges have been even used in vivo with humans.[12]

Another example of an in vitro stress response approach is the measurement of the antioxidant status of plasma. In this case the obtained plasma of the volunteers is subjected to a radical stressor, namely, the green-blue 2,2′-azinobis(3-ethylbenzthiazolidine-6-sulfonic acid) (ABTS) radical. Decolorization of this ABTS radical is a measure of the plasma to withstand radical stress.[13]

Finally, it should also be realized that a smooth transition exists from a pure healthy to an overt-diseased state. Using "slightly unhealthy" volunteers offers the possibility to detect beneficial effects of foods or food-derived components. People with a slightly elevated blood pressure, for example, may respond to blood pressure lowering compounds. Overweight volunteers exhibit a light degree of systemic inflammation. Anti-inflammatory effects of compounds could become apparent in these volunteers.

In this respect healthy smokers are also an interesting alternative group of subjects. Although it may be a matter of debate as to whether "healthy smokers" really exist or rather are an oxymoron, they are well-known to develop, among other symptoms, impaired vascular function, elevated levels of inflammation, and oxidative stress.[14,15] To investigate the pleiotropic health benefits of flavonoids in the human vasculature, smokers thus appear as an ideal study population (vide infra).

MOLECULAR MECHANISMS OF ACTION OF FLAVONOIDS

In the 1920s flavonoids were described as important health-promoting compounds. In that period also vitamins were discovered, and in 1936 flavonoids were indicated as vitamin P.[16] There are not many food-derived compounds that followed the development of biomedical science as closely as flavonoids (Figure 1). The denomination of flavonoids as a

vitamin is very illustrative in this regard. In view of the awareness that vitamin C plays an important role as a vascular protective agent, preventing bleeding from capillaries, it is not surprising that flavonoids were regarded as vitamin C enhancers and were further investigated as protectors against capillary fragility.[17−20] The mode of action remained obscure for a long time. However, after the huge interest in the physiological role of free radicals in the 1980s, the antioxidant function of flavonoids was elaborated (Figure 1). In the beginning free radicals were judged as merely damaging species. They were seen as detrimental to all kind of biomolecules (e.g., fatty acids, proteins, carbohydrates, and ribonucleic acids). Cellular structures and molecules (e.g., membranes, enzymes/receptors, DNA) can indeed be damaged by an overshoot of free radicals, and the antioxidant function of flavonoids was seen as the mode of action of these polyphenols. This concept appeared in particular plausible because it could be easily explained by their natural function. In plants, where they are produced, flavonoids act as antioxidants to protect against radical-inducing UV light.[21]

The biochemistry community already realized from the beginning that free radicals are not only damaging but are also part of the normal physiology. Moreover, on the basis of the reaction rate constants, the physiological probability of pure hydroxyl radical scavenging was difficult to comprehend and could not be shown clinically up to now.[22] Other ways had to be found to explain the beneficial health effects of flavonoids in vivo. This notion slowly penetrated the field of nutritional research, which then started to focus on the anti-inflammatory effects of these polyphenols.[23] This research became feasible because of the accessibility of easy methods to measure, for instance, a wide spectrum of cytokines as well as their gene expression levels.[24] Moreover, the effects of flavonoids on transcription factors steering the inflammatory process, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and its modulator poly(ADP-ribose) polymerase-1 (PARP-1), could be determined and led to a further underpinning of their anti-inflammatory action also in vivo.[25−27] These DNA transcription regulating factors are activated by free radicals, which made it a logical continuation of flavonoid research (Figure 1).

Another investigational route to explain flavonoids' health benefits further focused on the antioxidant activity of flavonoids by studying their influence on radical-producing enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase.[28,29] Interestingly, even metabolites proved to be effective inhibitors, which is meaningful because most polyphenols have a relatively low bioavailability due to rapid biotransformation.[30]

The anti-inflammatory action of flavonoids became further intertwined with their antioxidant effects by the recognition that the function of neutrophils is effectively modulated.[31] This mechanism not only enhances the anti-inflammatory potency but contributes at the same time to a further decrease of free radicals because an important source of radical formation becomes attenuated.

Whereas antihypertensive effects of a specific flavonolic glycoside were already reported in the early 1950s,[32] the reduction of plasma lipids[33] and a decreased risk of coronary heart disease[34] as well as attenuation of LDL cholesterol oxidation by preserving the activity of the enzyme paraoxonase[35] were discovered later as cardiovascular health-promoting effects.

Recent research activities addressed the capability of flavonoids to interfere with intracellular redox signaling cascades and the activity of redox-sensitive transcription factors.[36] To explain the possible long-term health effects of flavonoids, epigenetic modulating activities are now increasingly explored.[37] Also in these cases flavonoid research closely follows the new methodological possibilities that arise and become accessible to a wider scientific community.

The main reason for presenting this historical background on the research efforts around flavonoids is to emphasize that these polyphenols indeed possess a pleiotropic action per se. The intrinsic multiple effects of these food-derived compounds apparently renders them excellently suitable to prevent or delay the onset of complex chronic pathophysiological conditions such as cardiovascular diseases.

It is, however, important to realize that flavonoids are in fact not a homogeneous group of compounds. During the years of flavonoid research multitudinous efforts were dedicated to establish structure−activity relationships (SAR). It could be shown that SAR are applicable for flavonoid subgroups.[38] However, for many biological functions such as anti-inflammatory actions,[39] PARP-1-inhibition,[26] and epigenetic modulation,[40] clear SAR could not be unraveled until now. This emphasizes the need for awareness that the biological activity of a specific flavonoid must not necessarily be representative for the whole class of compounds. A similar perception is required for flavonoid-rich plant extracts, where the entire extract has to be regarded as an individual "active principle".

WHAT IS A SUITABLE CLINICAL END POINT THAT REFLECTS THE PLEIOTROPIC NATURE OF FLAVONOIDS IN HUMANS?

On the basis of the cognition that nutrients and dietary interventions (consisting of numerous individual compounds) affect human health not only by a single mechanism but by a multitude of mechanisms, it appears self-evident that a single clinical end point would never sufficiently reflect this pleiotropic efficacy in humans. Therefore, it is necessary to carefully define a representative panel of end points consisting of risk factors and/or cellular and subcellular markers that are known to play a key role especially in the early development of a disease. Moreover, the selection of those end points is guided by the present knowledge on the molecular mechanisms that are ascribed to the nutrient or dietary compounds in question in previous in vitro and animal experiments.

In a recent pilot study we implemented this strategy practically for the investigation of the effects of an 8 week supplementation of grape seed-derived monomeric and oligomeric flavanols on vascular health of healthy male smokers.[9]

The major study parameters assessed in this trial are displayed in the outer circles of Figure 2. They are either biochemical or functional markers that provide information on the main important pathophysiological processes occurring in the early onset of (cardio)vascular diseases: disturbances in serum lipids as well as in inflammatory, redox, and prothrombotic states, eventually all contributing to an impaired macro- and microvascular function (Figure 2).

Due to the fact that all of these processes are inseparably linked to each other and frequently even amplify each other, the integration of the effects on all these study parameters would enable one to quantify the flavanols' efficacy on vascular health in an integral manner. In search of a pragmatic way to integrate

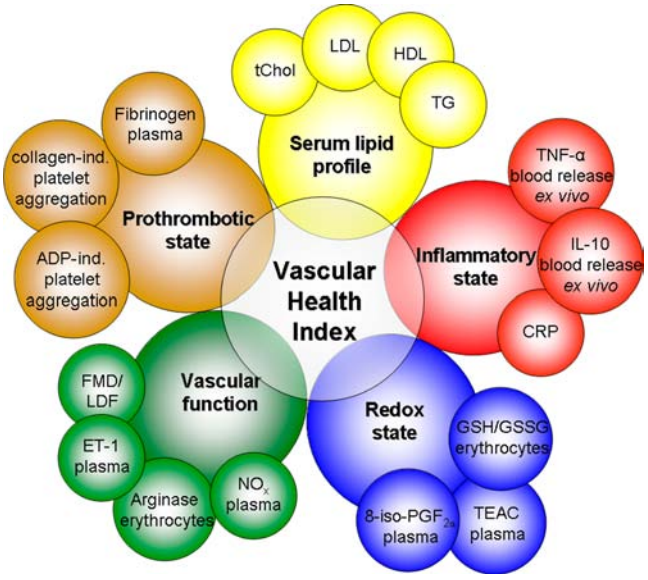

Figure 2. Overview of the study parameters integrated into the vascular health index within a randomized clinical pilot study to assess the vascular health effects of flavanols in male smokers.[9]

the data, we ended up with the concept of defining a global health index as coined by Heany.[2] For each subject we added up the percentage changes from baseline of all parameters for which an increase was expected to reflect beneficial effects on vascular health. Accordingly, parameters for which a reduction was assumed to reflect a beneficial vascular health effect were subtracted. By this simple and straightforward approach we created the so-called vascular health index (VHI). Indeed, the mean VHI increased significantly compared to placebo in the test group that was supplemented with the flavanols for 8 weeks, despite the fact that the alterations in many of the individual parameters did not reach statistical significance.[9]

Thus, the meticulous selection of a cluster of end points forms the sound and plausible platform for the implementation of strategies that facilitate an integration of data for the quantification of the health effects of pleiotropic-acting nutrients.

HOW TO PURSUE THE PROOF OF HEALTH BENEFITS OF FOOD COMPOUNDS?

A drug consists usually of a pure substance being applied in a well-defined pharmaceutical formulation and with a standardized bioavailability. In contrast, dietary interventions can vary considerably with respect to the food matrix, which in turn can influence considerably the bioavailability of individual ingredients. Hence, it appears plausible that differences in food composition can fairly influence the outcomes of clinical trials. In addition, pharmaceuticals are usually developed on the basis of a unique mechanism of action. Consequently, in a situation (an overt-diseased state) that obviously needs correction, the clinical efficacy of a drug can be determined by means of a single end point. Due to the pleiotropicity and subtlety of actions of food-derived compounds, the situation is more complex (Figure 3). Therefore, the question remains how to pursue the proof of their beneficial effects on human health.

Basically, the concept of health benefits of nutrients should be amended by the perception that these compounds may contribute to reset homeostasis on a higher level of "heterostasis" to acquire or maintain optimal health.

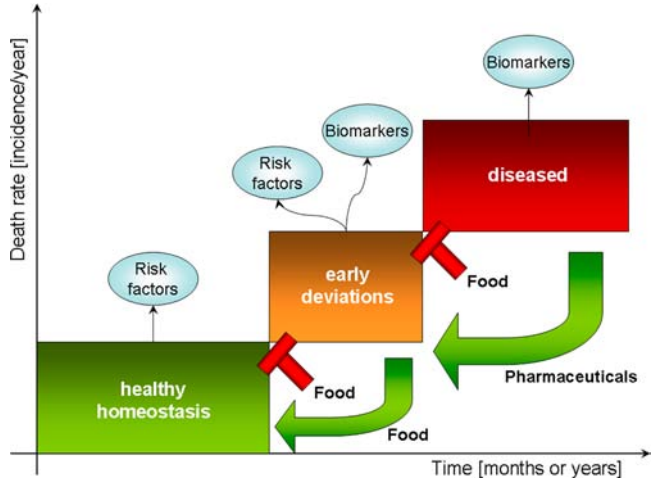

Figure 3. Conceptual view on health and disease, their assessment by means of risk factors and biomarkers, and the modulating roles of nutrients and pharmaceuticals. In a healthy homeostasis, risk factors should be identified that indicate the possible occurrence of early deviations from homeostasis. Biomarkers reflect a state already deviated from homeostasis. Biomarkers can even be used to define the degree of the disease. Food compounds and nutrients might block or delay the transition to a further worsening of the disease state, that is, from homeostasis to early deviations or diseases. Pharmaceuticals are primarily aimed to correct the disease state, whereas nutrients rather function to remediate a slightly imbalanced homeostasis.

With regard to the design of clinical trials to prove the health effects of dietary compounds, we should not hesitate to include volunteers that possess a mild aberrant physiology.[41] Moreover, we should apply stress response tests (briefly, stress tests) in or ex vivo that enable us to study optimization of health as the capacity to adapt to different environmental or intrinsic stimuli. Because the current methodologies for those stress tests are quite limited and still in their infancies, the development of novel approaches will be a work of merit in this field.

The difficult choice of end points needs thorough attention while projecting a clinical trial for a nutritional intervention. Many clinically established biomarkers are indicators of a diseased state and, hence, signs of an already exceeded range of homeostasis. It is therefore imperative, instead of using markers of a diseased state, to find and apply markers, that is, risk factors, which reflect the misbalanced homeostasis and the early developmental state of a disease.

By means of our recent clinical study on the vascular health effects of grape seed-derived flavanols, we could demonstrate that the careful selection of a scientifically meaningful cluster of markers (including well-established cardiovascular risk factors and investigative markers for vascular health) paves the way to define an integrative outcome measure (VHI) for the quantification of flavanols' effects on vascular health. The integration of biomarkers into a health index may be a promising approach, although we are fully aware that it does not enable the prediction of the risk of cardiovascular disease and associated mortality of the investigated volunteers. It is rather a pragmatic, simple, and comprehensible approach to reveal the multitude of mild effects by which flavanols modulate essential (patho)physiological processes in the human vasculature.

Nonetheless, regarding the practical implementation and interpretation of such a health index, there are still numerous questions to be answered:

(1) What is the most accurate set of biomarkers reflecting vascular health? Do we need to include a certain minimum or maximum number of parameters?

(2) Do we need to develop for specific (classes of) dietary compounds a standardized set of biomarkers that should definitely be included in a particular health index?

(3) Do we need to define and apply weighing factors for the integration of the individual biomarkers? How can this be achieved?

(4) How do we deal with differences in time dependence with respect to the change of biomarkers?

(5) Do biomarkers interact, and does this have to be taken into account for integrating them into a health index?

(6) Is a health index dose-dependent?

(7) How can we handle within-person and between-person variability of a health index?

(8) Can a health index be translated into disease risk?

Of course, these questions do not alter when the term "biomarkers" is replaced by "risk factors". Despite these and probably many more issues that need to be addressed by future research, we are convinced that integrative approaches, regardless of how exactly they are practically implemented, will find their way into the clinical assessment of health benefits of foods and nutrients. Our approach demonstrates that data integration can be achieved in a pragmatic manner and does not necessarily require expensive and/or highly sophisticated techniques.

Randomized (placebo-) controlled clinical trials (RCT) are without doubt regarded as the gold standard of clinical efficacy testing. However, due to fundamental differences in the objectives of a trial with a drug (improve or cure a disease) and dietary compounds (maintain or promote health) as well as in the nature of the active ingredients (high-potent monovalent drug vs low-potent, polyvalent food-derived compounds), the definition and handling of clinical end points in RCT with dietary compounds need a critical rethinking.

Although it is without question necessary to investigate the health effects of nutritional compounds with the same scientific diligence as pharmaceuticals, we should be cautious not to restrict ourselves to methodologies that have been developed for different targets. Instead, scientists should be open-minded and creative in the search for new ways to more adequately capture the health-promoting effects of nutrients and food-derived substances in humans. Only those data enable the successful implementation of an effective application of dietary compounds for the general public's maintenance of health.

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ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzthiazolidine-6-sulfonic acid); ADP, adenosine diphosphate; CRP, C-reactive protein; ET-1, endothelin-1; FMD, flow-mediated dilation; GSH, glutathione; GSSG, glutathione disulfide; HDL, high-density lipoprotein; IL, interleukin; 8-iso-$PGF_{2\alpha}$, 8-isoprostaglandine $F_{2\alpha}$; LDF, laser Doppler flowmetry; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO_x, nitrate and nitrite; PARP-1, poly(ADP-ribose) polymerase-1; tChol, total cholesterol; RCT, randomized controlled clinical trials; SAR, structure–activity relationships; TEAC, trolox equivalent antioxidant capacity; TG, triglycerides; TNF-α, tumor necrosis factor alpha; VHI, vascular health index.

REFERENCES

(1) Biesalski, H. K.; Aggett, P. J.; Anton, R.; Bernstein, P. S.; Blumberg, J.; Heaney, R. P.; Henry, J.; Nolan, J. M.; Richardson, D. P.; van Ommen, B.; Witkamp, R. F.; Rijkers, G. T.; Zollner, I. 26th Hohenheim Consensus Conference, September 11, 2010, Scientific substantiation of health claims: evidence-based nutrition. *Nutrition* **2011**, *27*, S1–S20.

(2) Heaney, R. P. Nutrients, endpoints, and the problem of proof. *J. Nutr.* **2008**, *138*, 1591–1595.

(3) Albert, A. *Selective Toxicity. The Physico-Chemical Basis of Therapy*, 7th ed.; Chapman and Hall: London, U.K., 1985.

(4) WHO. *Constitution of the World Health Organization – Basic Documents*; Oct 2006.

(5) What is health? The ability to adapt. *Lancet* **2009**, *373*, 781.

(6) Weseler, A. R.; Bast, A. Oxidative stress and vascular function: implications for pharmacologic treatments. *Curr. Hypertens. Rep.* **2010**, *12*, 154–161.

(7) Adam, O.; Laufs, U. Antioxidative effects of statins. *Arch. Toxicol.* **2008**, *82*, 885–892.

(8) Sitnikova, M.; Ivanov, S. G.; Khmel'nitskaia, K. A. [Pleiotropic effects of β-adrenoblockers in the therapy of cardiovascular diseases: effect on lipid peroxidation, endothelial function, and evolution of atherosclerosis]. *Kardiologiia* **2009**, *49*, 61–66.

(9) Weseler, A. R.; Ruijters, E. J. B.; Drittij-Reijnders, M.-J.; Reesink, K. D.; Haenen, G. R. M. M.; Bast, A. Pleiotropic benefit of monomeric and oligomeric flavanols on vascular health – a randomized controlled clinical pilot study. *PLoS ONE* **2011**, *6*, e28460.

(10) Neunteufl, T.; Katzenschlager, R.; Hassan, A.; Klaar, U.; Schwarzacher, S.; Glogar, D.; Bauer, P.; Weidinger, F. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis* **1997**, *129*, 111–118.

(11) Hashimoto, M.; Kozaki, K.; Eto, M.; Akishita, M.; Ako, J.; Iijima, K.; Kim, S.; Toba, K.; Yoshizumi, M.; Ouchi, Y. Association of coronary risk factors and endothelium-dependent flow-mediated dilatation of the brachial artery. *Hypertens. Res.* **2000**, *23*, 233–238.

(12) Richardson, R. P.; Rhyne, C. D.; Fong, Y.; Hesse, D. G.; Tracey, K. J.; Marano, M. A.; Lowry, S. F.; Antonacci, A. C.; Calvano, S. E. Peripheral blood leukocyte kinetics following in vivo lipopolysaccharide (LPS) administration to normal human subjects. Influence of elicited hormones and cytokines. *Ann. Surg.* **1989**, *210*, 239–245.

(13) Fischer, M. A.; Gransier, T. J.; Beckers, L. M.; Bekers, O.; Bast, A.; Haenen, G. R. Determination of the antioxidant capacity in blood. *Clin. Chem. Lab. Med.* **2005**, *43*, 735–740.

(14) Yanbaeva, D. G.; Dentener, M. A.; Creutzberg, E. C.; Wesseling, G.; Wouters, E. F. M. Systemic effects of smoking. *Chest* **2007**, *131*, 1557–1566.

(15) Celermajer, D. S.; Sorensen, K. E.; Georgakopoulos, D.; Bull, C.; Thomas, O.; Robinson, J.; Deanfield, J. E. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* **1993**, *88*, 2149–2155.

(16) Bentsath, A.; Rusznyak, S.; Szent-Györgyi, A. Vitamin nature of flavones. *Nature* **1936**, *138*, 798.

(17) Somogyi, J. C. An investigation of substances which inhibit vitamin C degradation. *Zschr. Vitaminforsch.* **1945**, *16*, 134.

(18) Richardson, G. A.; El-Rafey, M. S.; Long, M. L. Flavones and flavone derivatives as antioxidants. *J. Dairy Sci.* **1947**, *30*, 397.
(19) Crampton, E. W.; Lloyd, L. E. A quantitative estimation of the effect of rutin on the biological potency of vitamin C. *J. Nutr.* **1950**, *41*, 487−498.
(20) Scarborough, H. Deficiency of vitamin C and vitamin P in man. *Lancet* **1940**, *2*, 644.
(21) Caldwell, M. M.; Robberecht, R.; Flint, S. D. Internal filters: prospects for UV-acclimation in higher plants. *Physiol. Plant.* **1983**, *58*, 445−450.
(22) Hollman, P. C.; Cassidy, A.; Comte, B.; Heinonen, M.; Richelle, M.; Richling, E.; Serafini, M.; Scalbert, A.; Sies, H.; Vidry, S. The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. *J. Nutr.* **2011**, *141*, 989S−1009S.
(23) Landolfi, R.; Mower, R. L.; Steiner, M. Modification of platelet function and arachidonic acid metabolism by bioflavonoids. Structure−activity relations. *Biochem. Pharmacol.* **1984**, *33*, 1525−1530.
(24) Gerritsen, M. E.; Carley, W. W.; Ranges, G. E.; Shen, C. P.; Phan, S. A.; Ligon, G. F.; Perry, C. A. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. *Am. J. Pathol.* **1995**, *147*, 278−292.
(25) Tsai, S. H.; Liang, Y. C.; Lin-Shiau, S. Y.; Lin, J. K. Suppression of TNFα-mediated NFκB activity by myricetin and other flavonoids through downregulating the activity of IKK in ECV304 cells. *J. Cell. Biochem.* **1999**, *74*, 606−615.
(26) Geraets, L.; Moonen, H. J.; Brauers, K.; Wouters, E. F.; Bast, A.; Hageman, G. J. Dietary flavones and flavonoles are inhibitors of poly(ADP-ribose)polymerase-1 in pulmonary epithelial cells. *J. Nutr.* **2007**, *137*, 2190−2195.
(27) Weseler, A. R.; Geraets, L.; Moonen, H. J.; Manders, R. J.; van Loon, L. J.; Pennings, H. J.; Wouters, E. F.; Bast, A.; Hageman, G. J. Poly(ADP-ribose) polymerase-1-inhibiting flavonoids attenuate cytokine release in blood from male patients with chronic obstructive pulmonary disease or type 2 diabetes. *J. Nutr.* **2009**, *139*, 952−957.
(28) Robak, J.; Gryglewski, R. J. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* **1988**, *37*, 837−841.
(29) Tauber, A. I.; Fay, J. R.; Marletta, M. A. Flavonoid inhibition of the human neutrophil NADPH-oxidase. *Biochem. Pharmacol.* **1984**, *33*, 1367−1369.
(30) Steffen, Y.; Gruber, C.; Schewe, T.; Sies, H. Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase. *Arch. Biochem. Biophys.* **2008**, *469*, 209−219.
(31) Busse, W. W.; Kopp, D. E.; Middleton, E., Jr. Flavonoid modulation of human neutrophil function. *J. Allergy Clin. Immunol.* **1984**, *73*, 801−809.
(32) Hellerstein, H. K.; Orbison, J. L.; Rodbard, S.; Wilburne, M.; Katz, L. N. The effect of rutin in experimental malignant hypertension. *Am. Heart J.* **1951**, *42*, 271−283.
(33) Muramatsu, K.; Fukuyo, M.; Hara, Y. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol. (Tokyo)* **1986**, *32*, 613−622.
(34) Hertog, M. G.; Feskens, E. J.; Hollman, P. C.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007−1011.
(35) Aviram, M.; Rosenblat, M.; Billecke, S.; Erogul, J.; Sorenson, R.; Bisgaier, C. L.; Newton, R. S.; La Du, B. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. *Free Radical Biol. Med.* **1999**, *26*, 892−904.
(36) Na, H. K.; Surh, Y. J. Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. *Food Chem. Toxicol.* **2008**, *46*, 1271−1278.
(37) Berletch, J. B.; Liu, C.; Love, W. K.; Andrews, L. G.; Katiyar, S. K.; Tollefsbol, T. O. Epigenetic and genetic mechanisms contribute to telomerase inhibition by EGCG. *J. Cell. Biochem.* **2008**, *103*, 509−519.
(38) van Acker, S. A.; van den Berg, D. J.; Tromp, M. N.; Griffioen, D. H.; van Bennekom, W. P.; van der Vijgh, W. J.; Bast, A. Structural aspects of antioxidant activity of flavonoids. *Free Radical Biol. Med.* **1996**, *20*, 331−342.
(39) Kim, H. P.; Son, K. H.; Chang, H. W.; Kang, S. S. Anti-inflammatory plant flavonoids and cellular action mechanisms. *J. Pharmacol. Sci.* **2004**, *96*, 229−245.
(40) Gilbert, E. R.; Liu, D. Flavonoids influence epigenetic-modifying enzyme activity: structure−function relationships and the therapeutic potential for cancer. *Curr. Med. Chem.* **2010**, *17*, 1756−1768.
(41) Morand, C.; Dubray, C.; Milenkovic, D.; Lioger, D.; Martin, J. F.; Scalbert, A.; Mazur, A. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. *Am. J. Clin. Nutr.* **2011**, *93*, 73−80.