Chapter 2
Antioxidants: A Premature Scientific Hypothesis that Reshuffled the Traditional Food Pyramid

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The curious story of dietary antioxidants, i.e., reducing agents found in considerable amounts in edible plants and often not necessarily understood fully in the context of redox chemistry, can be traced back to the famous statistical analysis of carcinogenic risk factors by Doll and Peto (1981). Their work revealed among others the positive correlation between cancer incidence and meat consumption. In particular, the statistical strength of meat's impact on human health created a kind of a shock that such an obvious component of the human diet may be as carcinogenic as cigarette smoke; unfortunately subsequent epidemiological studies were repeatedly confirming this worrisome association (WCRF/AICR 1997). The emerging results of consecutive statistical analyses derived from ever larger human studies, however, uncovered another, more optimistic association, i.e., a decreased risk of cancer (and also of other chronic diseases) in populations whose diets were rich in plant-based foods, especially in all types of colorful fruits and vegetables, and thus rich in (redox active) secondary metabolites (for sources see Explanatory Box 1).

Explanatory Box 1: Sources of Secondary Metabolites

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of the organism they originate from. They are found extensively in nature, especially, but not exclusively in plants, where they serve numerous and often diverse purposes,

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ranging from host defense and clearing competitors from respective pastures to attracting mates, pollinators, and distributors of their seeds. Numerous examples of redox active secondary metabolites will be discussed as part of this and subsequent chapters of the book. Here, we will briefly mention a few of the most obvious and perhaps exciting ones. Well-known examples of common redox active secondary metabolites include certain vitamins, such as ascorbic acid (vitamin C), which is contained in high amount in citrus fruits (such as oranges, lemons, mandarins), as well as α-tocopherol (vitamin E) and its isomers, which are found in many vegetable oils and in grape seeds. In many fruits, nuts, and vegetables, these vitamins are joined by the large and diverse family of polyphenols, which are found, for instance, in grapes, tea and coffee, cacao, apples, tomatoes, soy beans, peanuts, almonds, and in pomegranates. Colorful berries, such as blueberries, black currants, chokeberries, raspberries, goji berries, and jostaberries, are particularly rich in polyphenolic flavonoids—and indeed, their color is often due to the presence of such compounds.

Such colorful vegetables, fruits, and berries also contain a range of additional redox active groups of compounds, including anthocyanidins (for instance found in cranberries) and proanthocyanidins. As many of these compounds are poorly water soluble and located in the skin or seeds, caution should be taken as juices derived from such fruits, etc., may not be particularly rich in these substances. This also holds true for resveratrol and other stilbenes, which are found in the skin of red grapes and in red (but not in white) wine. Other redox active secondary metabolites are more omnipresent and easier to access for consumption, including many flavonoids. Catechins, for instance, are present as catechin gallate in green or black tea, and as (+)-catechin and (−)-epicatechin in cacao, while quercetin is a predominant component in apples and in many berries.

Many of these more readily available metabolites have been considered as beneficial ingredients in ‘functional foods’. In common perception, quercetin is the ‘healthy ingredient’ in apples, apple juice, and apple wine, (epi) catechins are the beneficial ingredients of cacao and (hot) chocolate, and resveratrol turns wine and xanthohumol (a prenylated chalconoid contained in hops) turns beer healthy. Such ‘health claims’ ascribed to a particular ingredient of a natural product are often problematic, of course, especially once processed foods rich in sugar or (often alcoholic) beverages are concerned. Nonetheless, many redox active secondary metabolites can indeed be found in food stores. Prominent examples include the various sulfur-containing secondary metabolites, i.e., organic sulfur compounds, such as allicin and polysulfanes in garlic and onions, cabbage and dithiins, and asparagusic acid in asparagus, allyl isothiocyanate (mustard oil) in mustard seeds, sulforaphane in broccoli and lenthionine, a rather unusual organic compound, in Shiitake mushrooms (chemically, lenthionine is a relative of varacin, a cyclic polysulfane found in marine ascidiacea from the family *Polycitor*).

Often, spices and flavorings represent a valuable source of redox active secondary metabolites with suspected health benefits, which include curcumin from the turmeric root, capsaicin from chili peppers, and hydroxytyrosol (and derived compounds) found in olive oil. From a nutritional and culinary perspective, the use of such spices—instead of salt—is therefore advisable for a number of good reasons. Such considerations also include the extended use of vegetables as part of our daily diet. Indeed, rather common vegetables sometimes are surprisingly rich in redox active secondary metabolites. Worth mentioning are, for instance, beetroots, a very tasty and healthy vegetable used extensively in Eastern European cuisine, which should not be confused with 'beefroots' or 'meatroots'. These roots contain a fair amount of the 'antioxidant' betalain betanin, which gives beetroots their characteristic color (and not an anthocyanidine). Common tomatoes are also rich in 'antioxidant' substances, including lycopenes and tomatines. Indeed, α-tomatine is a fine example of a hybrid molecule, where elements of a steroid merge with aspects of polyphenolic redox activity. Similar redox active steroidal structures are found in ginseng (ginsenosides) and other saponin-containing plants (such as the soapwort plant) and in lower organisms.

As the hunt for new and perhaps more efficient 'antioxidants' is currently on, ever more exotic sources and molecules are being investigated, including propolis and many colorful flowers (such as the purple coneflower). Indeed, the flowers of plants, together with the petals and leaves, should not be underestimated as they often contain more biologically active ingredients than the corresponding fruits or seeds (phytol will be discussed later on). Some of these flowers—or products thereof—are even edible, for instance dandelion flowers and elderflower (extracts). Redox active metabolites found in flowers often differ from the ones found in fruits and berries, and some of these substances, such as aurones, therefore will be discussed in more detail as part of subsequent chapters.

In parallel, research on the molecular foundations of chronic diseases, such as cardiovascular or neurological disorders, diabetes, and cancer, lead to the observation that all of these illnesses may have one particular risk factor in common, namely a disturbance in redox homeostasis, commonly referred to as 'oxidative stress' (OS, see Chap. 1). OS describes a condition in which the production of damaging reactive oxygen species (ROS, present in the body as an inevitable consequence of respiration, exacerbated by the inflammatory response and by exogenous factors such as environmental pollutants) exceeds the capacity of the body's antioxidant defenses to neutralize them (see Chap. 5).

The two sets of scientific observations became combined into one hypothesis which elegantly explained how different diseases may be prevented simultaneously by plant-borne secondary metabolites with divergent chemical structures. The presumption was that if OS brought about by an organism's weakened antioxidative

barrier accelerates processes leading to the diseased state, the factors neutralizing oxidants should prevent or at least slow down disease progression. Numerous plant secondary metabolites are characterized by a low reduction potential, so their health promoting capability emerging from epidemiological investigations was immediately associated with antioxidant activity, a property reflected by the variety of chemical structures. There were also two additional circumstances which warranted the antioxidant hypothesis instantaneous public attention and scientific consensus. First, the promotion of low caloric plant foods was in line with the strategy of reducing obesity that began to dramatically increase at the end of the twentieth century. Second, the measurement of—the total of—redox active substances (or their 'redox capacity'), be it in mixtures like those in foods or after purification, is very straightforward. Soon, a number of easy and convenient methods to assess antioxidant activity was developed which could be adopted by any research or industrial laboratory.

Once this antioxidant hypothesis was formulated and its implications deduced, scientists worldwide began to verify it in the course of specifically designed research projects involving a plethora of *in vitro* and *in vivo* models, as well as controlled human intervention studies and clinical approaches. This part of the antioxidant story does not differ from scientific deductive business as usual—take a hypothesis, examine experimentally its assumptions, and either confirm initial assumptions or reject or at least modify them. This time, however, the story leaked out of research laboratories virtually with a massive tsunami effect. The developed societies, in particular, despite their access to good nutrition and most of all advanced and extensive medical care, and despite experiencing a reasonably comfortable life with corresponding eagerness to enjoy attractive leisure activities, turned out not only not to be devoid of life-threatening illnesses, on the contrary, the richer the country, the more chronic diseases seemed to take their toll, with serious forms of cancer included. In response, the wealthy societies demanded from the scientific community that a miraculous though scientifically supported cure would be elaborated to ensure a long and healthy lifetime. Antioxidants were just what could satisfy these expectations. First media, then consumers, and soon after industry embraced the combat against OS as the straight route to trouble-free longevity. Antioxidants and free radicals began to be pronounced even by those who had no knowledge of the true meaning of these terms—let alone redox chemistry. The chemical definition of a reducing agent had undergone multitude of reinterpretations depending on to whom it was addressed (see also Chap. 4). In food chemistry, *antioxidants are compounds that are used to increase the 'oxidative stability' of foods*. In the case of medical community, according to the Webster New World Medical Dictionary: *antioxidant is a substance that reduces damage due to oxygen, such as that caused by free radicals; well-known antioxidants include enzymes and other substances, such as vitamin C, vitamin E, and beta-carotene, which are capable of counteracting the damaging effects of oxidation*. The Internet portal 'About.com Chemistry' in the Chemistry Glossary Index also gives the definition stressing antiradical action of an 'antioxidant' as: *an enzyme or other organic molecule that can counteract the damaging effects of oxygen in tissues; although the term technically applies to molecules reacting with oxygen, it is often applied to molecules that protect from any free radical* (*molecules with unpaired electron*).

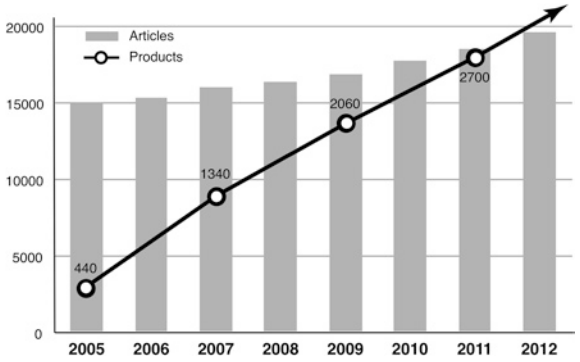

Fig. 1 The number of scientific articles listed in PubMed containing the term 'antioxidant' among keywords and products marketed with the information on antioxidant content according to Global New Product Database

These blurred definitions no longer were related strictly to the reduction potential of compounds. Actually, the term antioxidant has no longer retained a proper chemical connotation at all. Its popular meaning has become equivalent to 'bioactive phytochemical' or 'bioactive food component' regardless of redox activity.

Perhaps the most unexpected development was, that the very word 'antioxidant' occurred to be an extremely well selling marketing slogan. Indeed, the number and variety of products launched bearing a distinct antioxidant claim has been on the constant increase, as has the number of research papers found in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) containing the term 'antioxidant' among the keywords (Fig. 1).

Furthermore, the American Chemical Association noticed a shift in researchers' interest from detrimental to various beneficial food components with papers specifically related to antioxidants appearing in the ACS supported food journals in growing quantity: 45 (5 %) in 1997, 362 (20 %) in 2008 and 391 (18 %) in 2010 (Seiber and Kleinschmidt 2012). The ability to scavenge free radicals (used interchangeably with ROS) by a given food component or phytochemical became synonymous with the term 'functional food', which implies that a food product offers to consumers some benefits beyond simple nutrition. In 2005, in the food and beverage industry, another slogan was coined—'superfruit' or less frequently 'superveg'—to annotate the edible plants with particularly high antioxidant activity (determined usually by the ORAC or FRAP method).[1]

At a rather breathtaking pace, the antioxidant hypothesis was translated into a flourishing business whose annual market value according to Euromonitor (December 2013) in Europe alone is estimated at about 34 billion Euro.

[1] ORAC stands for 'Oxygen Radical Absorbance Capacity' and describes a method of measuring antioxidant capacities in biological samples *in vitro*. ORAC values of fruits and vegetables were published by the United States Department of Agriculture (USDA), but were withdrawn in 2012 as biologically invalid (USDA, Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, 2010), stating that no physiological proof *in vivo* existed in support of the free-radical theory. The Ferric Reducing Ability of Plasma (FRAP, also Ferric ion Reducing Antioxidant Power)-assay is an standard antioxidant capacity assay that uses Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, water soluble analog of vitamin E) as a standard benchmark.

Research in the field of food and nutrition, as well as some branches of experimental medicine focussing on bioactive phytochemicals, have been developing at the same speed (see Explanatory Box 2). Numerous mechanisms have been discovered by which phytochemicals could potentially influence the human organism, from altering genome function to preventing or fighting microbial threat. The antioxidant hypothesis, however, has resisted scientific verification in human studies. While it is clear from *in vitro* and *in vivo* experiments that antioxidative nutraceuticals (another term born as a result of the antioxidant hypothesis) can reduce oxidative damage, trials completed in the mid-1990s showed no protection against cancer or cardiovascular disease by high-dose supplements of beta-carotene; to make matters worse, such high doses of this particular 'antioxidant' actually seemed to increase the risk of lung cancer in smokers. Two subsequent meta-analyses of human cohort and case–control studies with vitamin E (Miller et al. 2005) and with a range of micronutrient supplements (Bjelakovic et al. 2007) also concluded that low doses of antioxidants have no significant effect, and high doses might actually increase mortality and incidence of disease. Further large, long-term studies conducted to test whether antioxidant supplements (vitamin C, A, E, beta-carotene) taken alone or in combination, and for periods of at least a few years, may help prevent chronic diseases in people, neither gave any promising results.

Explanatory Box 2: The Origin of Modern Pharmaceuticals

This chapter opens the discussion on potential health benefits associated with redox active secondary metabolites. Indeed, such compounds often exhibit 'antioxidant' properties *in vitro* and hence are suspected of chemopreventive or otherwise protective properties. Despite the rather complex issues surrounding the 'antioxidant hypothesis' (several of which will be discussed critically as part of this chapter), the more general idea that natural products could act as protective or even therapeutic agents is not unreasonable. Indeed, a brief look at the origins of currently licensed and used pharmaceutical agents demonstrates that about a quarter to a third of our drugs are of natural origin. Such agents are either genuine natural compounds, such as the alkaloid chinin, or semi-synthetic compounds based on natural products and modified to increase activity or bioavailability or to reduce side effects, such as acetyl-salicylic acid (Aspirin). These traditional, small-molecule natural products, which also include many anticancer drugs and antibiotics, have recently been joined by other 'biologicals', i.e., larger molecules with a pronounced and often selective action, including peptides (here a traditional example is insulin) and (native or modified) antibodies. These natural products in a narrower sense of the word are joined by various mimics, i.e., synthetic compounds intentionally resembling (aspects of) a natural substance.

The success of natural products in drug development can be explained by a number of factors. Natural products have often developed during evolution to endow their producers with a particular advantage. These compounds possess

high and often selective activity (for instance against predators, bacteria, fungi), which can be exploited for human uses as well. At the same time, Folk Medicine has in a way already tested such compounds for centuries, and these unintentional 'clinical trials' in humans provide extremely valuable information about activity, application, dosage, and possible side effects.

Nonetheless, a continued importance of natural products as leads in drug development is by no means self-evident. At the end of the last century, powerful new methods were introduced to drug development which in this field could well have pushed aside natural products research altogether. Here, computational methods, in silico drug design, combinatorial chemistry with consecutive compound libraries, and fast screening methods relying heavily on robots have provided powerful and fast new tools to generate and screen immense numbers of new, synthetic compounds. In reality, neither the more logical, rational design approaches nor the more random gunshot methods relying on massive numbers of compounds and screens, however, have resulted in the kind of successes that have been anticipated at the beginning of the 1990s. Indeed, among the over 200 recently produced new therapeutic agents, about one-third can still be classified as 'natural', and two-thirds as synthetic. In fact, natural product (re-)search for biologically active ingredients is currently experiencing a significant renaissance at several ends. Such natural products not only provide leads for new therapeutics, but also for eco-friendly pesticides gentle to the environment and food-chain and as part of functional foods and food supplements in the context of a healthy and healthily aging society. Here, and also increasingly in the field of cosmetics, 'naturals' are clearly superior to 'synthetics' for a number of reasons, which also address issues other than pure efficiency, such as safety, protection of the environment, social acceptance, sustainability, and often (local) economic wealth generation cycles. Some of these issues will be discussed as part of the chapters dealing with product development and commercialization. It should be noted that redox active secondary metabolites are of particular interest here, as they are able to modulate numerous biological processes, some of which are central to the prevention of or fight against diseases and also play a major role in the aging organism. Last but not least, the sheer richness of such metabolites, especially in our food, evidently provides considerable scope for their extensive practical uses and widespread applications.

The question emerges what has gone wrong. Why have strategies used successfully during the development of medical treatments failed in the case of antioxidant food components? Are the reducing phytochemicals indeed without any impact on human health or have the essential points been overlooked while designing the human studies? Some researchers are inclined to admit that beneficial biological properties observed for certain antioxidant food components are associated not that much with their redox activity, but are determined by other,

more specific pharmacophoric features of their chemical structure. Others, me in that number, attempt to recognize shortages in the current human studies and to propose ways of overcoming them in future investigations. Two recent papers from the Bast group have explained why at least in the case of flavonoids a pleiotropic research approach is required (Weseler and Bast 2012). These authors have formulated ten major misconceptions concerning antioxidants (Bast and Haenen 2013). Here, I will try to add some thoughts that have not been raised in the mentioned papers, concentrating rather on some basic needs of living organisms than on antioxidants themselves.

Let us recall the famous essay by Dobzhansky (1973)—"Nothing in Biology makes sense except in the light of evolution". I would paraphrase this title for the sake of the current chapter—"Nothing makes sense in Biology but in the context of chemistry". Should antioxidants be placed on such chemistry backgrounds and why may this be important for interpreting former and planning subsequent experiments? The frequently raised issue concerning antioxidants is their source; a number of sources have been listed with particular emphasis on colorful plants. Nonetheless, the simplest answer is that reducing compounds are actually omnipresent in aerobic organisms. They are found in prokaryotic microorganisms, fungi, plants, and animals including humans, because substances controlling the reduced state in a cell are prerequisites of survival. As soon as oxygen appeared in the Earth's atmosphere, the single-celled organisms had to protect themselves against its toxicity. And this has not changed until now. Availability of free oxygen, however, created also an opportunity for fundamental improvement in efficiency of life's energy-generating systems.

The route from protection against oxygen to its successful utilization may be traced along the evolutionary timeline of proteins containing the metallo-porphyrin component. For example, hemoglobins are found in organisms representing all kingdoms and are involved in different aspects of oxygen interactions with the organism: scavenging to prevent toxicity, transport, storage, redox reactions, and energy production (Hardison 1999). These proteins are not alone in their functions. They are accompanied by low molecular weight reducing compounds, whose reduction potential may decide with which type of ROS they ultimately interact with most efficiently. What could not be taken into account when proposing the antioxidant hypothesis was that oxygen-derived species are not only unwanted by-products of oxidative metabolism. Ample evidence obtained in recent years documents that ROS can have not only deleterious effects, but also constitute necessary elements in chains of signal transduction pathways (Bartosz 2009). Their main and widely recognized effects concern the inhibition of protein tyrosine phosphatases, thus enhancing the activity of protein tyrosine kinases involved, i.e., in mediation of the effects of growth factors and cytokines (Forman et al. 2010). There is evidence that ROS are produced by specialized enzymes (NADPH oxidases, Nox) not only in the context of defense (in phagocytes), but also in many other cell types, and here for signaling purposes (Ago et al. 2011). In their recent review, Hernandez-Garcia et al. (2010) describe the most important functions of ROS for life, which include regulation of cell division, cell

differentiation, transport, and apoptosis. It thus becomes evident that the ingested antioxidants play not necessarily only beneficial roles associated with protection against oxidative damage, but their overabundance may actually become disruptive once the redox homeostasis of the cell is deregulated.

Furthermore, the most recent discoveries have pointed to the epigenetic importance of 5′-methylcytosine (5-mC). Cytosine methylation has long been known to modulate gene expression and consequently cellular identity. This DNA modification, often embracing promotor regions, is a stable chemical alteration that represses transcription in eukaryotic organisms (Klose and Bird 2006). The reverse process, the removal of such methyl groups, allows cells to regain the ability to express silenced genes, or to recover their totipotent potential in the case of germ cells. While some demethylation may occur via a passive process deleting 5-mC from DNA upon repeated replications, active demethylation remained elusive up to 2011. Here, current evidence supports the existence of iterative oxidation of 5-methylcytosine to 5-carboxycytosine (Nabel et al. 2012). The first step of this process generating 5-OH-methylcytosine still seems to be involved in suppression of gene expression (Tahiliani et al. 2009). The subsequent products of oxidation—5-formylcytosine and 5-carboxycytosine—were demonstrated to be substrates for base excision repair (BER) and have been immediately accepted as intermediates of *active demethylation* (He et al. 2011; Ito et al. 2011). A similar role has been also proposed for 8-oxoguanine, which up till now was regarded solely as a marker of genotoxic exposure to ROS. Currently, however, it is considered as an element of redox sensitive epigenetic regulation (Guz et al. 2012). The dual role of genotoxins—including agents known to induce DNA oxidation—as possible methylome disruptors and remediators has been suggested as well (Lewandowska and Bartoszek 2011). An altered pattern of DNA methylation is observed in many diseases, especially in cancer and neurological disorders, which are prevented by dietary antioxidants. This association may point to the role of redox homeostasis in the maintenance of the correct shapes of human epigenomes over lifetime involving pro- and antioxidant mechanisms not predicted before (Brewer 2010).

It follows that oxygen based redox processes are not only important for the harvest of energy, but are behind the most vital mechanisms ensuring proper function of cells, tissues and the organism as a whole. It can be speculated that discrete values or levels of the cellular redox potential controlled by different ROS trigger certain sets of mechanisms, e.g., via redox sensitive transcription factors influencing gene expression. Fine-tuning of these levels may be offered by sulfur containing compounds creating a larger thio-redox system that would be able to respond to even tiny changes in the organism's homeostasis (such a system will be introduced in Chap. 7 in form of the 'cellular thiolstat'). So should not the reduction potential of dietary antioxidants be considered while discussing their impact on human wellbeing? If the major endogenous reducing agent GSH is taken as a reference point, could any exogenous compound with the higher reduction potential (and usually lower bioavailability) really make a noticeable difference in the neutralization/sequestration of excessive ROS as long as the physiological content of GSH is not significantly impaired? So far, such rather fundamental considerations are not

taken into account at all. The antioxidant activity of dietary redox active components is simply based on their ability to reduce either free radicals or oxidants that are not even found in nature. In contrast, the relation between TEAC[2] or ORAC values or physiologically relevant thio-redox potentials, in the sense sketched out above, has neither been established nor even considered in earnest.

As indicated above, in living organisms the inevitable formation of ROS during oxidative respiration is used to control other biological processes, which can be dependent for instance on energy supply, like cell growth and division. In this particular case, the increased ROS concentration might signal the energetic readiness of the cell for proliferation. Such a multidirectional exploitation is also executed in the case of cellular antioxidants. GSH, apart from being a thiol-based redox buffering molecule, is also a major player in the detoxification of xenobiotics, as well as of potentially undesirable products of the metabolism. Here, it reacts with electrophilic substances which otherwise could bind to nucleophilic centers of proteins and nucleic acids hence endangering their proper structure. Moreover, hydrophobic compounds conjugated to GSH are substrates for efflux pumps present in membranes regulating the cholesterol level and preventing intracellular accumulation of toxic substances. Similarly, flavonoids abundantly produced in plants not only protect these plants against UV exposure but also help to capture additional energy from sun radiation, attract pollinators, and discourage pathogens. In view of the apparently failing antioxidant hypothesis, it has been postulated that health promoting properties of plant secondary metabolites in the human organism are connected with other aspects than protection against ROS. The food synergy concept, for instance, additionally stresses the evolutionary importance of non-random mixtures of numerous molecules that occur naturally in foods and affect human health in a concerted fashion (Jacobs et al. 2012). But can the antioxidant hypothesis be regarded as properly tested at the current stage and therefore should it be abandoned altogether due to the lack of immediate confirmation?

The above-mentioned human studies, which provided a negative message on health benefits of supplementation with antioxidants, did not consider the relation between the reduction potential of compounds tested and for instance that of GSH. But this is not the only problem with the design of these investigations. All antioxidants used were vitamins (A, C, E, β-carotene), nutrients which are necessary for the organism's survival and have to be absorbed from dietary sources in which their concentration naturally is not very high. It can be presumed that in the case of such indispensable compounds, specialist mechanisms have evolved to ensure their efficient uptake from the alimentary tract. In the case of supplementation, such mechanisms will enable relatively efficacious absorption of the vitamins ingested, hence resulting in their enhanced impact on the thio-redox homeostasis. Yet, it is not unequivocally proven that a low reduction potential is indeed relevant for their function or whether it may actually lead to undesirable

[2] TEAC stands for 'Trolox Equivalent Antioxidant Capacity', most commonly measured using the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) decolorization assay.

side effects, as discussed recently for vitamin C (Kuo 2013). The negative results observed in the case of supplementation with vitamins may be thus explained in at least two ways. First, one related to their antioxidant nature, when a substantial bioavailability may lead to an imbalance in cellular thio-redox status impairing various redox dependent processes. Second, the excessive supply of vitamins may deregulate some of the organism's functions related directly to their physiological role. In contrast, non-nutrient antioxidants, such as phenolic plant components, are extremely poorly absorbed and it can be hypothesized that their bioavailability might be more flexibly adjusted to the actual requirement of maintaining a proper thio-redox status. The latter is in a way confirmed by observations during research aimed at the alleviation of side effects of anticancer therapy. The experiments carried out with the aid of *in vivo* models demonstrated efficacy of synthetic antioxidants, as well as of various non-nutrient antioxidant phytochemicals, in situations of OS. For instance, a diminished cardiotoxicity of doxorubicin has been observed. This drug causes the formation of ROS and hence significant side effects which may be counteracted by the use of certain antioxidants. Clearly, such a cardioprotective effect was seen for reducing compounds with divergent chemical structures (flavonoids, lycopene, betalains, *N*-acetylcysteine, etc., Piasek et al. 2009). In clinical practice, antioxidants (but not vitamins) are routinely administered to patients along with the respective anticancer drug to curb ROS and thereby prevent cardiac damage.

Another hint concerning the importance of thiol-specific redox modulatory effects for health benefits of phytochemicals comes from epidemiological studies on the relationship between cancer incidence and dietary pattern. The most consistent inverse correlation between risk of cancer and the amount of food consumed was observed for *brassica* vegetables. It is generally accepted that anticarcinogenic action of these plants is brought about by organic sulfur compounds—*glucosinolates*—that are degraded by the enzyme myrosinase to a number of products among which isothiocyanates exhibit the strongest chemopreventive potential (Dinkova-Kostova and Kostov 2012). The mechanism of action of these thiol-specific modifiers is strictly related to thio-redox homeostasis. Isothiocyanates influence the thiolstat by their ability to react with SH groups of cysteine residues in glutathione, as well as 'antioxidant' proteins including thioredoxin. Most importantly, these phytochemicals, by reacting with specific SH groups, are able to restructure protein complexes releasing nuclear factors such as Nrf2 and NF-κB. As a result, the former is translocated to the nucleus and triggers the expression of cytoprotective genes mediated by the antioxidant response element (ARE). The inhibited translocation of NF-κB prevents the expression of pro-inflammatory genes. Both mechanisms are of key relevance in the prophylaxis of chronic diseases which explains the above-mentioned beneficial role of diet rich in *brassica* vegetables revealed by epidemiological observations. To conclude, the best established chemopreventive dietary ingredients are those that display the ability to influence the cellular (or more generally the organism's) thio-redox status. Can this observation be translated into a more general statement, i.e., are only the substances that are capable of impacting on the thio-redox balance also able to bring about a significant health benefit? The answer to this question remains to be

determined. It is noteworthy, however, that the chemopreventive efficacy, that is the limitation of cancer incidence in humans, was seen for whole vegetables and not for isolated phytochemicals.

The two promising lines of evidence described above suggest that we may indeed be missing some important pieces of the redox conundrum. The discrepancy between experimental research and epidemiological studies vividly demonstrates that there are major gaps in our current understanding of the role reducing agents play in human wellness. Consequently, the proper exploitation of this issue needs some verified tools to predict the biological activity of antioxidants in the human organism, both when still in the digestive tract and after absorption. It will also be necessary to predict and further investigate the ability of such antioxidants to protect foodstuffs against oxidative spoilage which so far has represented a major interest in food science.

Maybe it would be justified to forget for a while about the antioxidant hypothesis in its current shape and form. Then, taking advantage of the vast amount of data collected over the past two decades, it may be advisable to carry out a shift from the deductive to a more inductive approach and scrutinise without wishful thinking what story all these results are really telling. Here, one should take into account the expanding knowledge on possible evolutionary roles of ROS and mechanisms controlling the thio-redox homeostasis—this time also not forgetting about chemistry.

Finally, let us admit that the perhaps premature antioxidant hypothesis has an unprecedented positive impact on human nutrition, lifestyle and, most of all, the awareness of responsibility for one's own health. Millions of people have revolutionized their personal food pyramids and adopted diets richer in fruits and vegetables. Even more astonishing is the fact that the food producers, instead of sticking to the cheapest solutions, began the search for ingredients and technologies leading to the production of health-related quality, or as this industry prefers to call it, functional foods. Furthermore, a growing number of nonprofit organizations have been founded in cooperation with research institutions and governmental agencies to advocate a healthier lifestyle. Education on proper nutrition, rich in plant antioxidants, is now beginning as early as in the kindergarten. Would it be possible to create such a compelling impact without the catchy term ANTIOXIDANT? That is indeed very doubtful. Therefore, it is the great responsibility of the academic community not to waste these achievements for the sake of a not fully recognized and, in any case debatable, scientific accuracy.

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Author Biography

Agnieszka Bartoszek graduated from the Chemical Faculty, Gdańsk University of Technology (GUT) in 1981, receiving the degree of MSc in Organic Chemistry and the title of Engineer. In the same year, she enrolled for doctoral studies at the Department of Pharmaceutical Technology and Biochemistry, GUT, where she joined the research team headed by Prof. Jerzy Konopa and was involved in research on the mechanism of action of DNA-reactive antitumor compounds. In 1990, she received her PhD degree based on a thesis concerning DNA adduct formation by a group of antitumor derivatives of 1-nitro-9-aminoacridine, which was analyzed by the then just developed ^{32}P-post-labelling assay. During 1988–1989, she worked for the Imperial Cancer Research Fund in Edinburgh, at the Laboratory of Molecular Pharmacology and Drug Metabolism, then Medical Oncology Unit. Her major research concerned the biological consequences of activation of antitumor drugs doxorubicin and mitomycin C by NADPH cytochrome P450. In 1990, she obtained an academic teacher position at the Chemical Faculty, GUT, where she works until now and is currently involved in several research projects and extensive teaching.

Agnieszka's main research interests initially concentrated on the mechanism of action of antitumor drugs and compounds, especially their capability of binding to DNA. Another line of scientific investigation, adopted after moving to the Department of Food Chemistry, Technology and Biotechnology, GUT, concerns chemopreventive properties of selected food components and dietary intervention during cancer chemotherapy. The projects on the chemopreventive potential of traditional foods resulted in a close cooperation with the food industry. They are aimed at the design and elaboration of technologies for health-oriented functional food products enriched in plant-based anticarcinogenic phytochemicals.

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