JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

REVIEW

pubs.acs.org/JAFC

Antioxidants in Foods: State of the Science Important to the Food Industry

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ABSTRACT: Antioxidant foods and ingredients are an important component of the food industry. In the past, antioxidants were used primarily to control oxidation and retard spoilage, but today many are used because of putative health benefits. However, the traditional message that oxidative stress, which involves the production of reactive oxygen species (ROS), is the basis for chronic diseases and aging is being reexamined. Accumulating evidence suggests that ROS exert essential metabolic functions and that removal of too many ROS can upset cell signaling pathways and actually increase the risk of chronic disease. It is imperative that the food industry be aware of progress in this field to present the science relative to foods in a forthright and clear manner. This may mean reexamining the health implications of adding large amounts of antioxidants to foods.

KEYWORDS: antioxidant, cancer, diabetes, exercise, food, gene activation, iron, nrf2, oxidative stress, reactive oxygen species, selenium, thiols

INTRODUCTION

Antioxidants have a long history of use in the nutrition/health community and food industry. The traditional understanding has been that antioxidant chemicals promote health by removing reactive species that may otherwise exert harmful metabolic effects. By this view, most free radicals and reactive oxygen species (ROS; chemically reactive molecules that contain oxygen) were considered to be harmful,[1] implying that maximizing antioxidant concentrations could minimize the risk for chronic disease. However, as we uncover the complexity of cellular defenses and cell signaling pathways, the role of oxidative stress, and the defense system that eliminates it, it has become more complicated.

The term "antioxidant" also has become ambiguous as it has different connotations to different audiences. For example, to biochemists and nutritionists, the term often suggests a compound capable of quenching metabolically generated ROS. However, to some food scientists the term implies a substance used for functional characteristics (e.g., retard oxidation), whereas others may understand the term as describing foods or substances with high values for in vitro measures of radical quenching ability, such as the oxidative radical absorbance capacity (ORAC) test.[2] The importance of the term is evidenced by a search of the PubMed database using the terms "antioxidant" and "food", which yielded over 36000 hits.

The following review concerns antioxidants that are claimed to have health-promoting abilities and does not address antioxidants used in food manufacturing for technical functionality. Recent evidence has changed many views regarding how such antioxidants function and their optimal dosages. It now appears that ROS can have important functions in normal metabolism and that many benefits of antioxidants are not from direct radical scavenging (and thus are unrelated to tests such as ORAC). This presents a problem and an opportunity to the food industry; that is, how should the most healthful levels and types of antioxidants for food applications be determined? This review contrasts the traditional view of "antioxidants" with recent findings that are challenging our overall understanding.

TRADITIONAL ANTIOXIDANT THEORY

Harman first postulated in 1956[1] that free radicals and ROS underlie progressive damage that characterizes the process of aging. Although the causality of oxidative damage and aging was never firmly established, the theory spawned at least 7000 scientific publications. Later modified to emphasize mitochondria as the site of production of most ROS,[3] the antioxidant theory is based on the tenet that continuous generation of free radicals from inefficiently coupled oxidative phosphorylation continuously bombards the mitochondria with oxidative attacks and that the balance between these attacks and the cellular antioxidant defense network determines the overall progression of damage to DNA, lipids, and proteins. This theory has been implicated as causal in many chronic diseases such as cancer and cardiovascular disease.

Received: April 6, 2011
Revised: May 31, 2011
Accepted: May 31, 2011
Published: May 31, 2011

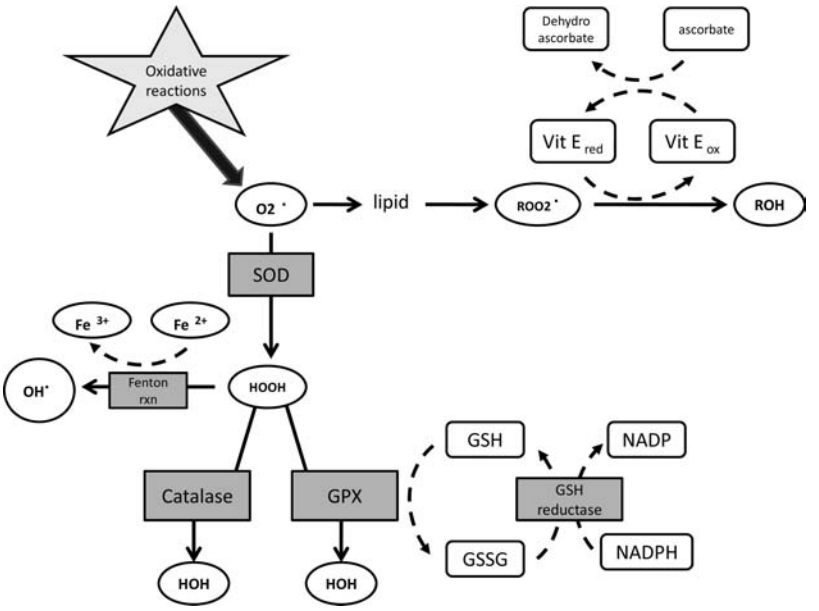

Figure 1. Simplified view of traditional antioxidant theory. $O_2^{\bullet}$, the superoxide anion, may be produced by multiple reactions within the cell. This reactive oxygen species (ROS) may be converted to other destructive species such as hydrogen peroxide (HOOH) or lipid peroxides ($LOO^{\bullet}$) that may, in turn, cause cellular harm. Direct reacting antioxidants such as vitamin E and antioxidant enzymes such as superoxide dismutase (SOD), catalase, or glutathione peroxidase (GPX) reduce such oxidants; other cofactors such as glutathione (GSH), ascorbate, and NADPH and enzymes such as glutathione reductase (GSH reductase) are important for regenerating reducing molecules/enzymes.

The traditional understanding of the "antioxidant network" is well reviewed[4] and is discussed in brief. A simplified scheme of "cellular antioxidant defense" is depicted in Figure 1. Oxidative reactions produce ROS such as the superoxide anion, and these ROS can interact with biological molecules, especially lipids, forming other radicals. The chain of radical formation can be autocatalytic once started, and some antioxidants are referred to as "chain-breaking" because of their ability to stop the process. "Direct" antioxidants are substances such as vitamin E that have the ability to absorb and delocalize an electron, thus changing a radical to a less energetic intermediate; tests such as ORAC measure the ability of a substance to do this.[2] Other antioxidants catalyze the reduction of ROS mediated through enzymes such as catalase and glutathione peroxidase (GPX) or through cofactors or reductants, such as GSH. The antioxidant network is thought to work at multiple biological levels with multiple overlapping layers of protection.

FOOD ANTIOXIDANTS

Many dietary compounds are capable of negating the danger of ROS: vitamin C, tocopherols (vitamin E), carotenoids, polyphenolics, etc. It has been suggested that including these compounds in foods will enhance their capacities to support protection against ROS damage and reduce the risk of chronic disease.[5] These putative benefits delivered via foods helped spawn a large antioxidant functional food market, spurred by a health-conscious and aging U.S. population. Examples of products that are marketed on the basis of high ORAC values and antioxidant benefits include whole foods and beverages (e.g., acai berry, gogi berry, green tea) as well as isolated substances sold primarily as dietary supplements (e.g., vitamin C, lycopene, selenium) or added to foods (e.g., vitamin E). In 2007, antioxidant ingredients and supplements represented a $3.7 billion market with growth in the U.S. increase of approximately 3%/year.[6]

Epidemiological evidence seems supportive of antioxidant-rich foods mitigating the risk for chronic disease, but interventional trials have been inconsistent. As an example, epidemiological studies have shown apparent protective effects of fruits and vegetables.[7] Such evidence was the impetus for several intervention trials with high doses of β-carotene, a powerful antioxidant abundant in fruits and vegetables; however, these trials found no convincing evidence of benefit and, in fact, demonstrated increased risk for lung cancer.[8] Similar results have been reported for other antioxidants including vitamins E[9] and C.[10] In fact, meta analyses of multiple antioxidant supplementation trials concluded that supplementation with β-carotene, vitamin A, and vitamin E increased mortality.[11,12] Such reports present a conundrum: benefits appear to be realized by consuming "antioxidant-rich" foods, whereas interventions with specific antioxidants have not proven to be beneficial. There are many possibilities for such discrepancies; however, the intention of this review is not to re-examine old findings, but instead to present an overview of newer findings. Much of the new information suggests a fundamental limitation of the traditional antioxidant theory.

Emerging science is challenging the traditional understanding of oxidative stress and aging. In addition to direct quenching of oxygen radicals, it now appears that antioxidant function also may involve effecting the expression of multiple genes encoding enzymes with antioxidant function, sequestering pro-oxidants, altering ratios of reducing substrates, and effecting cell signaling. It also is becoming clear that many health benefits from antioxidants in the diet may occur in response to relatively low exposures. Moreover, it now appears that a certain level of ROS is needed to stimulate many of these processes, and removal of too many ROS may have deleterious implications to the organism. These changes in thinking have direct implications for the addition of antioxidants to food. The following presents an overview of these new findings and hypotheses related to antioxidants

that may be delivered by foods/diet. This review concerns antioxidants that are claimed to have health-promoting abilities and does not address antioxidants used in food manufacturing for technical functionality.

NATURAL COMPOUNDS MAY ACT AS ANTIOXIDANTS THROUGH GENE EXPRESSION PATHWAYS

Some substances that exhibit antioxidant activity function as inducers and/or cell signals, leading to changes in gene expression, which result in the activation of enzymes that eliminate ROS and/or toxins, including those involved in initial events in cancer. Early studies showed that flavones and related compounds,[13] phenolic antioxidants,[14] and butylated hydroxyanisole (BHA)[15] decreased experimentally induced carcinogenesis and/or elevated the activity of protective enzymes. Similar experimental effects were noted for compounds from cruciferous vegetables.[16] Specifically, dietary antioxidants[17] and compounds from crucifers[18] elevated phase II detoxifying enzymes such as glutathione *S*-transferase (GST) activities and protected against mutagens. These findings suggested that phenolic antioxidants possess potent anticarcinogenic actions mediated through altered gene expression.

Many dietary substances induce phase II detoxifying enzymes; these include compounds such as curcumin, coumarins, and 1,2-dithiol-3-thiones,[19] and the potency of induction parallels their reactivity as Michael acceptors.[19] The mechanistic basis for these effects has been shown to be coordinate regulation through a consensus *cis* acting-element (5′-GTGAC*nnn*GC-3′, where *n* can be any nucleotide) at the 5′-flanking promoter region of many genes called the antioxidant responsive element (ARE)[20] or the electrophile response element (EpRE).[21] Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or Nrf2) was demonstrated to be the protein that transcriptionally induced the ARE and thus regulated detoxifying enzymes.[22] Under unstimulated conditions, Nrf2 is bound to cytoskeleton-related Kelch-like ECH-associated protein 1 (Keap1) or inhibitor of Nrf2 (iNrf2),[23,24] and Keap1 targets Nrf2 for ubiquitination and degradation.[25] The interaction of these multiple components of the ARE system are shown in Figure 2.

ARE activation begins with cellular signaling cascades generated by compounds with "chemical redox stress" that also have sulfhydryl modifying ability.[26] Compounds such as green tea polyphenols,[27] BHA,[28] *tert*-butylhydroquinine,[28] and phenethyl isothiocyanate[29] alter cysteine residues on Keap1[30] and activate kinase pathways.[26] These events result in the release and translocation of Nrf2 into the nucleus where, in conjunction with small Maf proteins, it binds to the ARE and up-regulates the transcription of target genes.[31] Redox status also modifies the nuclear import/export of Nrf2.[32] Hence, when cells are exposed to antioxidant compounds generating oxidative stress, redox-sensitive Nrf2 ubiquitination is impeded, translocation of Nrf2 is increased, and Nrf2 import/export rates to/from the nucleus are altered, leading to increased free Nrf2, resulting in enhanced transcription of Nrf2-ARE-mediated antioxidant enzymes. The physiological consequences of the system are demonstrated by reports that Nrf2 knockout mice are more prone to oxidative stress-induced lung damage,[120] chemical-induced carcinogenesis,[33] and chemical-induced inflammation.[34]

ANTIOXIDANT FUNCTION BY SEQUESTRATION OF POTENTIAL OXIDANTS

Accumulating data are suggesting that yet another facet of antioxidant action is by substances that regulate ROS-generating

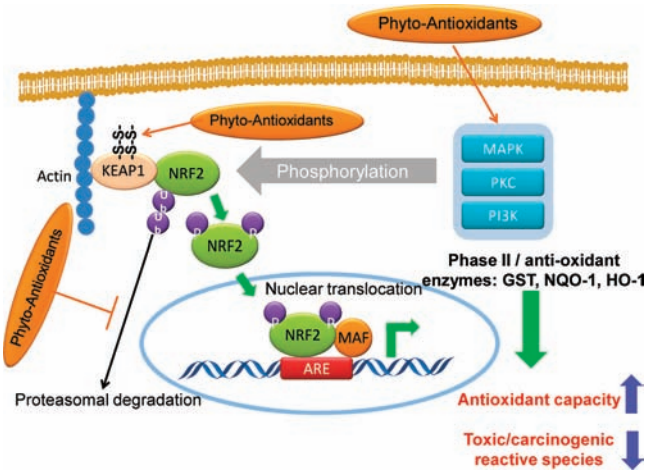

Figure 2. Antioxidant activity mediated through gene expression. The antioxidant response element (ARE) in the promoter region of select genes allows for coordinated up-regulation of antioxidant and detoxifying proteins in response to dietary constituents (phyto-antioxidants). This up-regulation is mediated through nuclear factor (erythroid-derived 2)-like 2 (Nrf2) that may be activated directly or induced by series of protein kinases. Phosphorylation of Nrf2 at serine and threonine residues by kinases such as phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC) results in release of Nrf2 from Keap1 and subsequent nuclear translocation of Nrf2. In the cell nucleus translocated Nrf2 interacts with small MAF protein, forming a heterodimer that binds to the ARE sequence in the promoter region and up-regulates transcription of many genes encoding detoxifying enzymes.

compounds and, thus, preempt oxidative insult. A well-characterized system is the regulation of cellular iron.

Iron, an essential nutrient, exists in multiple redox states, making it valuable for cellular metabolism. However, these same properties also make iron a catalyst in reactions that generate dangerous ROS. Consequently, cells have evolved systems to carefully manage iron metabolism, and the Nrf2 system is intimately involved in this regulation. The ARE-containing genes that control iron homeostasis include ferritin (iron storage),[35,36] heme oxygenase-1 (heme catabolism),[37] and ferroportin (cellular iron export).[38] Functioning together, these genes reduce potential oxidative stress by limiting the amount of free Fe within the cell (Figure 2).

Ferritin. Ferritin reduces the potential of iron-catalyzed oxidation by compartmentalizing iron within the cell; up to 4000 iron atoms are sequestered within a single ferritin protein.[39] Mammalian ferritin has 24 subunits from two gene products, ferritin H and L,[40] regulated by iron via transcriptional and translational mechanisms. Translational regulation is mediated by a portion of the 5′ untranslated region called the iron response element that works in concert with the iron response element binding proteins 1 and 2.[41] Alternatively, transcriptional regulation is mediated through the Nrf2/ARE axis.[35,36] Ferritin H transcription is activated by multiple substances including *t*-BHQ,[42] oxidants such as H_2O_2,[43] and phase II-inducing compounds such as *t*-BHQ, oltipraz, and 1,2-dithiole-3-thione.[44]

Ferritin is transcriptionally and translationally regulated by iron availability.[45] Transcriptional regulation was suggested by heme and protoporphyrin IX (a heme precursor) induction of ferritin H mRNA, whereas transcription inhibitors ablated the effect.[46] Direct evidence for transcriptional regulation by the ARE was demonstrated by Hintze and Theil,[36] who found that

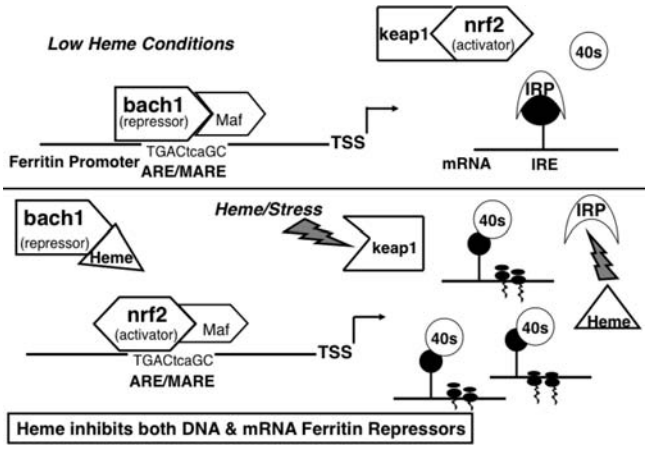

Figure 3. Heme-mediated transcriptional and translational regulation of ferritin genes. Heme increases ferritin transcription by binding to and ultimately leading to the degradation of the ARE transcription repressor Bach1. Heme also increases ferritin translation by inhibiting interactions between the iron responsive element (IRE) and the IRE binding proteins 1 and 2, allowing ferritin mRNA to be translated. By simultaneously increasing both transcription and translation, heme synergistically increases ferritin expression and rapidly increases cellular iron storage capacity.

reporter constructs containing the ferritin L promoter region were responsive to the ARE inducers sulforaphane and heme. Translational regulation was demonstrated in a similar manner as reporter constructs containing the iron responsive element, but a mutated ARE, were equally responsive to inorganic iron and heme. Constructs with both the ARE and the iron responsive element had a 3-fold higher induction, demonstrating cellular dependence on both methods of regulation (Figure 3).

Heme also regulates transcription through interactions with the repressor protein Bach1 that is constitutively bound to the Maf recognition element (MARE),[47] a promoter region with sequence homology to the ARE[48] and referred to as the MARE/ARE.[35,49] Cysteine−proline regions in the C-terminal region of Bach-1 bind heme,[49] lessening the interaction between Bach1 and the MARE/ARE,[35,49] resulting in Bach1 degradation[50] and subsequent transcription of MARE/ARE-containing genes.

Heme Oxygenase-1 (HO1). Control of HO1, the rate-limiting enzyme in the catabolism of heme to ferrous iron and biliverdin,[51] serves an antioxidant function because heme is a powerful pro-oxidant.[52] The HO1 promoter contains several Bach1/Nrf2 binding sites that are induced by heme;[53] Bach1 knockdown, as well as dietary ARE inducers such as sulforaphane and allyl isothiocyanate, increases HO1 expression.[35,54] HO1 is one of the genes most responsive to transcriptional regulation by heme and Bach1.[35]

Ferroportin. Control of intracellular iron is also regulated by the export of iron from the cell, and the transmembrane protein ferroportin is the only dedicated export pathway.[55] A MARE/ARE sequence responsive to Bach1/Nrf2 binding has been reported in the ferroportin promoter,[38] and ferroportin expression is induced by heme and sulforaphane. Induction results in increased iron export and decreased intracellular iron concentrations, thus lessening the potential of iron-catalyzed ROS.

In summary, the ARE-Nrf2 axis has proven to be a primary means of regulating the production of proteins that regulate the intracellular iron. Although none of these proteins have direct antioxidant function, the system reduces the concentration of free iron within a cell, and this has an overall effect similar to many other antioxidants, that is, a lessening of oxidative stress.

ANTIOXIDANT FUNCTION THROUGH REDOX REGULATION OF THIOLS

Thiols have great redox capability, and the balance of oxidized: reduced thiols may play a key role in regulating many cellular pathways associated with oxidation. Cellular redox balance serves to both quench excess ROS and signal apoptosis when the degree of damage exceeds the capacity for repair.

In the presence of excess ROS, sulfur amino acids (methionine and cysteine) in proteins are major targets for oxidation.[56] Methionine sulfoxide reductase, with reducing power from thioredoxin, reverses methionine oxidation. When cysteines oxidize, they typically form sulfenic acid. Unquenched, due to insufficient GSH, oxidation of cysteines may continue to *irreversible* sulfinic and sulfonic acid formation.[56] More commonly, cysteine oxidation to sulfenic acid rapidly binds GSH or forms disulfide bridges across proteins.[56]

An example of disulfide bridge formation changing protein activity is the oxidation of the apoptosis (programmed cell death) trigger Bax; sulfur bridge oligomerization of oxidized Bax activates it and induces apoptosis.[57] Thus, a cell undergoing uncontrolled oxidation may be targeted for elimination. Glutathiolation activates many cell signaling proteins including several in the MAPK pathways; as previously discussed, redox regulation of cell signaling may modify risk for chronic disease.[56] Healthy resting cells have ∼1% of proteins glutathiolated; after an oxidative insult, the sudden increase in GSSG results in increased glutathiolation. Restoration of GSH levels allows GSH to react with these glutathiolated proteins and regenerate a reduced sulfhydryl. This process also generates GSSG, which can be reduced to GSH by glutathione reductase. Thus, glutathiolation may act as a "thiol switch" that, much like phosphorylation, turns enzyme activity on or off reversibly.[58]

Many transcription factors, including p53, NFkB, c-Jun/AP-I, and Nrf2, also are regulated by glutathiolation.[59] Whereas changing the cytosolic redox potential to a more oxidized state improves passage of these transcription factors into the nucleus, they must be reduced to bind DNA and alter gene expression. Although GSH is present in the nucleus, nuclear redox is tightly regulated by proteins and peptides, including the couplet thioredoxin/thioredoxin reductase.[60] The Nrf2 system is particularly involved with this pathway; oxidation of sulfhydryls or covalent binding of electrophiles in the Nrf2 tethers protein KEAP-1 and frees Nrf2, allowing it to migrate to the nucleus and activate the ARE.[61] Nrf2 then induces multiple systems that lower the reduction potential and restore the redox balance in the cell. Nrf2 translocation is also under redox regulation; it passes from cytosol to the nucleus in the oxidized form, but must be reduced for DNA binding to occur. Therefore, if oxidation is severe and the nucleus is in a more oxidized state, Nrf2 will not bind the ARE and will not activate enzymes that restore redox balance; instead, oxidized thioredoxin releases ASK-1, promoting apoptosis and ridding the body of the oxidatively damaged cell.[62]

Diet influences thiol redox regulation at multiple points. If dietary cysteine, required for GSH synthesis, is limiting, tissue GSH levels fall and the cell cannot prevent oxidative damage.[63] Dietary selenium is also essential, although if there is sufficient cysteine and the direct-acting vitamin E, selenium requirements

are minimized.[60] In addition, other food components, typically electrophiles, may trigger the Nrf2 system and enhance the many endogenous pathways for maintaining redox control. Best-known is the aliphatic isothiocyanate from broccoli, sulforaphane, which binds to the most abundant cellular nucleophile, GSH.[64] The loss of GSH relative to oxidized glutathione (GSSG) raises the redox potential to a more oxidized state, triggering multiple thiol switches. Whether some proteins are more susceptible to cysteine oxidation, disulfide bond formation, or glutathiolation is an active area of research.

A key barrier to research in these areas is the lack of simple, rapid methods that measure cellular redox state. The emerging fields of proteomics and metabolomics may provide information on glutathiolated protein profiles and identification.[65] Tests such as ORAC that are used by industry to promote products rich in polyphenols measure only direct-acting dietary components able to quench ROS in vitro.[2] However, due to the low levels of these "direct-acting" dietary antioxidants found in plasma after ingestion (often $^{1}/_{1000}$ of the concentration of antioxidant vitamins), ROS quenching by dietary antioxidants may play a very minor role in antioxidant balance. This is supported by the finding of a lack of correlation between ORAC antioxidant capacity of a series of broccolis, measured in vitro, and the ability of extracts from these broccolis to control intracellular oxidative stress.[66] The ability of food components to trigger endogenous redox control may be of greater impact, and in fact, some polyphenols from fruits and vegetables trigger the Nrf2 system and thus alter thiol redox. For example, feeding quercetin to mice raised the GSH:GSSG ratio in liver and decreased the resting level of protein disulfides in liver and plasma.[67] Similarly lycopene, which upon oxidation becomes an electrophilic unsaturated ketone, interacts with Keap1 and triggers the Nrf2 system.[68]

■ ROS MAY FUNCTION AS ANTIOXIDANTS THROUGH CELL SIGNALING: THEORY OF HORMESIS

Clarification and expansion of the definition of "antioxidant" are important, but changing the definition does not address the question of "how much of an antioxidant is optimal?" Many foods and dietary supplements are marketed on the assumption that any addition of antioxidants is beneficial; however, accumulating research suggests that it is redox balance that is most important, and changing the balance toward either oxidation or reduction may be deleterious. The theory of hormesis addresses this point; a certain level of ROS may be essential, because at low levels ROS may function to trigger antioxidant responses.[69]

It is known that vigorous physical exercise increases cellular ROS production, for which reason many exercising individuals take antioxidant supplements. The field was surprised in 2009 when Ristow and colleagues[70] found that antioxidant treatment antioxidants (1000 mg of vitamin C + 400 IU of vitamin E/day) blocked the positive effects of exercise on the cell signaling of insulin-dependent glucose uptake by muscle. These results and others suggest that exercise-generated ROS may regulate redox potential via the mechanism of hormesis,[69] and thus it may be postulated that exercise and exercise-generated redox stress generate antioxidant function. An extension of this postulate is that removal of too many ROS from a system may not be beneficial but, in fact, may be a health detriment.

Intracellular ROS may stimulate gene expression of antioxidant and immunoreactive proteins such as cytokines, chemokines, and transcription factors.[71] Several redox-sensitive cell signal pathways are involved including nuclear factor (NF)κB, MAPK, phosphoinositide 3-kinase/Akt, p53 and the heat shock response.[72] Multiple metabolic events promote the production of a number of chemical species such as H_2O_2, NO, Ca^{2+}, and cytokines that activate these pathways; H_2O_2 is the most common.[71]

ROS may influence transcription factor binding through several ways: (a) activation of kinases and signaling cascades through sequential phosphorylation; (b) modulation of phosphatase activity; and (c) control of synthesis and degradation of transcription factors. Also, ROS may modulate the activity of Krebs cycle enzymes that generate NADH and $FADH_2$, thus influencing production of superoxide anion and H_2O_2 in the electron transport chain. This pathway can provide feedback on the production of ROS and indirectly influence redox signaling.

Goodyear et al.[73] reported activation of kinase signal pathways in rat skeletal muscle after treadmill running. Subsequent studies have shown that MAPK signal transduction pathways can be activated by contraction in skeletal muscle. Biological implications of MAPK activation are widespread and include regulation of glucose transport, muscle and heart hypertrophy, angiogenesis, and vascular adaptation.[74] NFκB binding, also elevated in rat skeletal muscle after exercise,[75] was accompanied by increased MnSOD mRNA and protein. Ji et al.[76] found that increased NFκB binding after acute exercise was accompanied by other cell signals in the muscle nucleus; NFκB activation was attenuated by antioxidant treatment and mimicked by lipopolysaccharide, a known H_2O_2 generator. High doses of *tert*-butyl hydroperoxide had little effect on NFκB, suggesting that signals were mediated by H_2O_2 instead of general oxidative stress. In contrast, muscle unloading dramatically decreased NFκB activity, suggesting that muscle contraction or nerve stimulation is required for signaling activity.[77]

Antioxidant enzymes can be up-regulated by chronic exercise training;[78] one such enzyme is superoxide dismutase (SOD)[75] (primarily manganese SOD as CuZnSOD did not respond). Induction by exercise may be mediated through tumor necrosis factor-α and interleukin-1, which in turn activate protein kinase C and NFκB-induced kinase, leading to signal cascades. The MnSOD promoter contains NFκB and AP-1 binding sites. Treadmill running induced a 2-fold increase in MnSOD mRNA in rats,[79] but ROS suppression abolished or severely attenuated enzyme and mRNA expression.

GSH is critical in muscle antioxidant defense during exercise, and the rate-limiting enzyme for GSH synthesis, glutamylcysteine synthetase, is induced in rats by endurance training.[80] The enzyme is composed of a catalytic heavy-chain and regulatory light-chain subunits.[81] Heavy-chain subunit expression is regulated by redox-sensitive mechanisms, a variety of oxidants, phenolic antioxidants, TNFα, interleukin-1β and possibly NFκB. Both heavy and light chain gene promoters contain the ARE binding site.

ROS and cell signaling pathways have been shown to play a vital role in mitochondrial biogenesis of rodent muscle, and endurance exercise and stimulated muscle contraction activate mitochondrial protein synthesis and proliferation.[82] These events may be important for mediating mitochondrial adaptations to exercise, including enhanced/elevated oxygen consumption, expression of energy-generating enzymes, fatty acid oxidation, and changes in mitochondrial morphology. ROS induced by sprinting activate pathways that stimulate mitochondrial biosynthesis, whereas reducing ROS generation by allopurinol

attenuated some pathways.[83] However, whether antioxidant supplementation can abolish mitochondrial biogenesis is equivocal.[84] It should be emphasized that redox signaling is activated by specific signaling agents (such as H_2O_2) and not general oxidative stress; thus, the effects of antioxidant supplementation are dependent on how it interferes with specific oxidative species.

In summary, physical exercise affects intracellular antioxidant levels and capacity primarily by activation of redox-sensitive cell signaling pathways; as such, exercise functions as an internal regulator of redox homeostasis. Caution should be used in supplementing exogenous reductant and antioxidants due to the potential inhibition of and interference with the hormetic effect exerted by ROS.

SELENIUM AND DIABETES: DOES THE REMOVAL OF TOO MANY ROS RESULT IN METABOLIC DYSFUNCTION?

Subsequent to 1957 and its determination to be an essential nutrient, Se has been widely studied for its function as an antioxidant.[85] Many of the biochemical functions of Se are a result of its function in 25 selenoproteins, and many of these are involved in redox control. For example, the selenoprotein GPX reduces peroxides,[85] and thioredoxin reductase reduces thioredoxin.[86] Public interest in supplementation of Se was spurred by a report of a remarkable decrease in cancer mortality in persons consuming 200 μg of Se daily (or approximately 4 times the daily requirement; the NPC trial).[87] Se supplementation had also been reported to reduce diabetic risk and act as an insulin-mimic.[88] Animal model and epidemiologic investigations reported correlations between Se deficiency and abnormal glucose or lipid metabolism,[89] as well as low plasma Se levels or selenoperoxidase activity in diabetic subjects.[90] Thus, supplementing Se was perceived as an effective strategy to prevent and treat diabetes.

However, mice overexpressing the Se-dependent cellular glutathione peroxidase-1 (GPX1; involved in reduction of peroxides), the most abundant selenoprotein, developed type 2 diabetes-like phenotypes.[91] A strong positive correlation between erythrocyte GPX1 activity and insulin resistance in nondiabetic pregnant women was reported in 2003.[92] Moreover, posthoc analysis of the NPC trial revealed a >2-fold increase in type 2 diabetes incidence in the Se versus the placebo group,[93] resulting in a hazard ratio of 2.7 in subjects in the highest tertile of plasma Se concentrations. A similar, but nonsignificant, increase was seen in subjects supplemented with 200 μg/day of Se in the 35533 participant Selenium and Vitamin E Cancer Prevention Trial (SELECT).[94] Recently, regression analysis of the ORDET cohort study[95] comprising 7182 women from northern Italy for a median follow-up of 16 years indicated that the odds ratio for diabetes comparing the highest to the lowest quintile of Se intake was 2.39 (P for linear trend = 0.005); and the odds ratio for diabetes associated with an increase in Se intake of only 10 μg/day was 1.29. Cross-sectional analyses within the U.S. Third National Health and Nutritional Examination also revealed a strong positive correlation between serum Se concentrations and the prevalence of type 2 diabetes.[96] Notably, high body Se status also was associated with adverse plasma lipid profiles in adults in the United States, the United Kingdom, and Taiwan.[97−99]

In contrast, mixed or positive effects of Se on decreasing diabetic risk have been shown in several studies in the United States and Europe.[100,101] Additionally, supplementing 200 or 800 μg Se/day for 5 years to men with prostate cancer did not significantly change serum glucose concentrations compared to placebo controls.[102] Apparently, more basic and clinical research is needed to elucidate the role of Se in glucose metabolism and the optimal level of Se intake for preventing diabetes.[90]

These findings raise the question of whether very low ROS production may pose a metabolic risk. As previously discussed, ROS are regulators of cellular physiology and altering intracellular ROS status may affect many metabolic pathways. Dysregulation of ROS may dysregulate insulin function/glucose metabolism in at least three ways.[90] First, insulin signaling is regulated by a balance between the activity of protein kinases (phosphorylation) and phosphatases (dephosphorylation). Oxidative inhibition of protein phosphatases by H_2O_2 prolongs phosphorylation of insulin signaling proteins such as insulin receptor and protein kinase B after insulin stimulation. In the case of GPX1 overexpression, diminished intracellular ROS results in attenuated insulin-stimulated phosphorylation of these two signal proteins, presumably by removing the inhibition of the basal levels of ROS on the protein tyrosine phosphatases.[91] In contrast, knockout of GPX1 enhanced mouse resistance to high-fat diet-induced insulin resistance,[104] and knockout of CuZnSOD improved insulin sensitivity.[105] These examples illustrate the importance of basal or slightly elevated ROS levels in insulin signaling under nondiabetic conditions; different outcomes may appear after prolonged exposure to ROS at excessively high levels or during late stage of diabetes.

A second reason why ROS dysregulation may affect glucose metabolism is that mitochondrial ROS are signals for glucose-stimulated insulin secretion (GSIS),[106] and within a physiological range, GSIS is proportional to mitochondrial ROS.[107] Mitochondrial uncoupling protein 2 inhibits GSIS and mitochondrial potential,[108] and its production and function are regulated by antioxidant enzymes and ROS.[103] Overproducing GPX1 down-regulates the protein, accelerating GSIS and leading to hyperinsulinemia.[103] In contrast, knockout of SOD1 alone or together with GPX1 up-regulates the protein attenuating GSIS.[105]

Finally, ROS dysregulation may alter transcriptional factors such as pancreatic duodenal homebox-1 and forhead box A2 that play crucial roles in β cell differentiation, survival, and function.[109] These factors are highly regulated by ROS and antioxidant enzymes at the epigenetic to post-translational levels. Diminished islet intracellular ROS by GPX1 overexpression caused hyperacetylation of histones in pancreatic duodenal homebox-1, resulting in elevated mRNA and protein and decreased degradation[103] and, subsequently, hypertrophy of islet β cell mass and hyperinsulinemia. Knockout of SOD1 alone or together with GPX1 produced reversed changes in the islet/insulin phenotypes.[105]

CLASSICAL TOXICITY DUE TO ANTIOXIDANT SUPPLEMENTATION

Cell culture and in vivo animal studies indicate that some antioxidant substances can cause classical toxicity, particularly at high levels of intake. For example, the cell death proteins, caspase-3 and JNK, were activated by green tea epigallocatechin-3-gallate (EGCG) in a dose- and time-dependent manner, especially at higher doses.[110] EGCG was also found to damage mitochondria, and JNK mediated EGCG-induced apoptotic cell death in HT-29 cells.[111] It is possible that low concentrations of EGCG activate MAPK, leading to ARE-mediated gene expression, whereas higher concentrations and sustained activation of

MAPKs lead to apoptosis.[112] Isothiocyanates induce apoptosis through caspase-3 in HeLa cells,[112] and this may be a distinct mechanism for their chemopreventive functions.[112] Butylated hydroxyanisole (BHA, a common food preservative) exerted dose-dependent toxic effects in HepG2 and HeLa cells[113] and induced apoptosis in freshly isolated rat hepatocytes.[114] Collectively, many antioxidant compounds are able to confer beneficial as well as potential harmful effects depending on physiological conditions and the dosages used.[115]

IMPLICATIONS TO THE FOOD INDUSTRY

"Antioxidant", especially as the term pertains to substances with the capacity to influence health and well-being, in contrast to antioxidants that are used for technical functionality in the food industry, often is used as a marketing tool by the food industry. The use of the term in advertisements or on package labeling has been used to market whole foods or beverages, additives to foods (especially in "functional foods"), or dietary supplements. The advertised benefits of antioxidants include slowing the aging process and decreasing the risk of chronic disease. Scientific understanding of the functions and health roles of antioxidants is changing, and nutritional messages used to market antioxidant-containing foods often are not supported by contemporary evidence.[116] This raises several issues for the food industry:

(i) The term "antioxidant" is indistinct and sends multiple messages. For example, in food processing "antioxidants" may be ingredients added to retard spoilage, whereas the term may be synonymous with "high ORAC value" to some, and it may imply endogenous compounds such as glutathione, vitamin E, and selenium to others. New findings now show that the term can be applied to many compounds not previously considered to be antioxidants (including some that are chemically pro-oxidants) because they have surprisingly strong antioxidant activity in vivo. To prevent confusion among consumers, a more specific nomenclature may be necessary.

(ii) Available scientific evidence does not necessarily support the assumption that all ROS present health risks and should be reduced to as low levels as possible. This has implications for the fortification of foods with antioxidants, as well as for the consumption of many dietary supplements. A corollary is that supplemental doses of some antioxidants may block beneficial actions of other physiological processes, and at high doses some antioxidants may be toxic.

Studies have shown that a sizable portion of the public exhibits "consumer backlash" against nutritional messages.[117] Many are confused with the changing messages within scientific, health, and policy circles, and many do not think that government should be involved in something as personal as food choice. This places much of the responsibility for clear messages regarding the benefits of specific foods and nutrients on the food industry. Clarifying the message regarding antioxidants without engendering consumer backlash will be important.

The present understanding of antioxidant function by the public is a consequence, in part, of relying on incomplete and preliminary data to develop overarching hypotheses and messages. Nutrition professionals urge an "evidence-based" approach that places much reliance on human studies for developing/assessing nutritional messages for public consumption.[118] The food industry may find that a similar approach,[119] although presenting many initial marketing challenges, results in a more attentive and enthused consumer.

Staying abreast of the science of antioxidants provides both a challenge and an opportunity to the food industry. The challenge is to determine whether antioxidants actually deliver the health benefits that are claimed by marketing campaigns, whereas the opportunity will be the chance to gain consumer confidence by presenting a forthright and clear message of the benefits (or detriments) of such foods.

DISCLOSURE

The views presented in this paper are those of the authors and are not those of the U.S. Department of Agriculture.

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