# Health Benefits of Dietary Antioxidants: Controversy and Validation

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## Abstract
The Institute of Medicine defined dietary antioxidants according to their ability to decrease the adverse effects of reactive oxidant species on normal physiological function in humans. While health benefits of dietary antioxidants are generally accepted, they may not provide prevention/protection against chronic diseases through antioxidant actions. Even though the most important end outcomes are reduction in the risk of studied diseases, a complete assessment of oxidative stress status reflecting the action and efficacy of dietary antioxidants shall be performed to establish mechanism of actions. In this context, clinical significance of dietary antioxidants in metabolic syndrome is discussed.

**Glossary of Abbreviations**
**CVD:** Cardiovascular Diseases
**DPPH:** Diphenyl-Picrylhyzdrazyl
**FRAP:** Ferric Reducing Antioxidant Power
**iPF$_{2\alpha}$:** F2α-isoprostanes
**MetS:** Metabolic Syndrome
**NADPH:** Nicotinamide Adenine Dinucleotide Phosphate
**ORAC:** Oxygen Radical Absorbance Capacity
**oxLDL:** Oxidized Low-Density Lipoprotein
**PC:** Protein Carbonyls
**RNS**: Reactive Nitrogen Species
**ROS:** Reactive Oxygen Species
**RSS:** Reactive Sulfur Species
**TEAC:** Trolox Equivalent Antioxidant Capacity
**TRAP:** Total Radical-Trapping Parameter
**WHO:** World Health Organization

## Introduction
Reactive oxidant species, including reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS), are electrophiles that take one or two electrons from a nucleophile without forming an adduct.[1] They are produced as part of normal physiology, but uncontrolled production in the human body has been appreciated for being responsible for the etiology of some diseases, e.g., cancer, obesity, diabetes mellitus, metabolic syndrome, cardiovascular diseases (CVD), neurological disorders, hypertension, and cataracts.[2] The development and progression of diseases occur particularly when the production of reactive oxidant species overwhelm the capacity of the antioxidant defense network, called oxidative stress.[3] One simple way to strengthen the defense is through increased consumption of dietary antioxidants. Dietary antioxidants, ranging from essential antioxidant nutrients, i.e., vitamins C and E and selected minerals (cofactors of the antioxidant enzymes), and nonessential phytochemicals, commonly are present in many plant foods and medicinal plants, and they are appreciated to play an important role in the prevention and treatment of reactive oxidant species-related chronic diseases.[4-6] Thus, consuming more dietary antioxidants is believed to be preventive or even therapeutic against diseases with the reactive oxidant species etiology. Nevertheless, observational and clinical data on the effect of dietary antioxidants is mixed at best. Some studies even showed that increased mortality was associated with supplemental antioxidants.[7-9]

Antioxidants have been implicated in reducing the risk of CVD and diabetes via their ability to scavenge reactive oxidant species even though it is equally important to recognize that they can improve health via mechanisms other than this function.[5,10] This antioxidant-reactive oxidant species relationship has given rise to hypotheses that oxidative stress may be linked to early events in the development of metabolic syndrome (MetS) and its sequelae, i.e., type 2 diabetes and CVD, via dynamic interactions between oxidative stress and the constellation of risk factors of MetS, which include dyslipidemia, hyperglycemia, hypertension, and obesity.[11] Examining antioxidant defense and biomarkers of oxidative stress in the development of MetS and the progression to the sequelae offers a robust approach to testing these hypotheses, as well as to validating health benefits of dietary antioxidants.

In this brief review, we consider evidence from human studies validating whether there is a role of antioxidants and reactive oxidant species in the etiology and/or progression of MetS and consequent risk for diabetes and CVD.

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## Reactive Oxidant Species and Antioxidants

Reactive oxidant species are atoms, molecules or ions with unpaired electrons that are very unstable and highly reactive molecules. While they prefer to accept an electron from other susceptible molecules, they can donate an electron to other molecules.[12] They are mainly derived from three elements, oxygen, nitrogen and sulfur, to form reactive oxygen species, reactive nitrogen species and reactive sulfur species, respectively. The common ROS existing in the human body are superoxide anion ($O_2^-$), hydroperoxyl radical ($HO_2$), hydroxyl radical (OH), nitric oxide (NO), hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and peroxynitrite (ONOO-).[6] Reactive oxidant species are constantly produced in the body via both enzymatic and nonenzymatic mechanisms.[1,3] The enzymatic productions include respiratory burst of immune cells responding to infections, induction of xanthine oxidase during hypoxia and prostaglandin synthesis; the nonenzymatic production includes superoxides produced in the mitochondria when electrons leak out from the electron transport chain during ATP production and hydroxyl radicals from $H_2O_2$ via the Fenton reaction mediated by ferrous ions. Their formations are further increased when human bodies are exposed to X-rays, ozone, smoking, and air pollution.[1,3] It has been estimated that about 1 to 3% of oxygen we inhale becomes reactive oxidant species, and the number is even higher during exercise.

To protect against constant attacks from reactive oxidant species, the human body is equipped with a relatively effective antioxidant defense system. Nevertheless, some reactive oxidant species can still escape from the defense system and attack adjacent susceptible molecules. The antioxidant defense system can be classified into endogenous and exogenous categories. The endogenous system can be further separated into three subcategories: antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase, thioredoxin reductase, quinone reductase); proteins (ferritin, transferrin, ceruloplasmin); and small-molecule antioxidants (glutathione, thioredoxin, lipoic acid, ubiquinol, uric acid). This endogenous system can be enhanced when the body is stimulated by an array of stimuli, such as exercise and dietary polyphenols. The exogenous system is solely derived from foods, particularly fruits, vegetables, whole grains, and nuts, and is comprised of vitamins C and E, carotenoids, phenolic acids, flavonoids, and so on. To enhance the antioxidant defense, increasing consumption of dietary antioxidants appears generally effective. There are several definitions for dietary antioxidants. The Institute of Medicine defined dietary antioxidant as "a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species or both on normal physiological function in humans," which was made based on three criteria: (1) The substance is found in human diets, (2) the content of the substance has been measured in foods commonly consumed, and (3) in humans, the substance decreases the adverse effects of reactive oxygen and nitrogen species *in vivo*.[13] Slightly different from this definition, Khlebnikov, et al.[14] defined antioxidant as "any substance that directly scavenges reactive oxidant species or indirectly acts to upregulate antioxidant defenses or inhibit reactive oxidant species production." In our perspective, the latter definition can properly cover substances displaying a weak radical scavenging capability but capable of boosting overall antioxidant capacity by inducing endogenous antioxidant defense system.

Plant foods are associated inversely with the risk of many chronic diseases, most likely attributed to their high nutrient density and phytochemicals. Among the essential micronutrients, vitamins C and E are well recognized for their antioxidant activity in people valuing health promotion and prevention through diets. Vitamin C is effective for scavenging $O_2^-$, $^1O_2$, OH, and RNS.[15] Vitamin E acts as a potent lipophilic antioxidant to terminate the propagation of lipid peroxidation by donating its phenolic hydrogen to the peroxyl radicals forming tocopheroxyl radicals that despite also being radicals are very unreactive to continue the oxidative chain reaction. In addition to these vitamin antioxidants, vitamin A effectively scavenges peroxyl radicals similar to vitamin E but more potently.[16] Although it is not commonly appreciated as an antioxidant, vitamin K was reported to inhibit lipid peroxidation.[17] Some essential dietary minerals do not act as direct radical scavengers but are an indispensable part of antioxidant enzymes (metalloenzymes), e.g., selenium for glutathione peroxidase and thioredoxin reductase, iron for catalase, zinc and copper for cytosol superoxide dismutase, and manganese for mitochondrial superoxide dismutase.

In addition to being rich in antioxidant vitamins and minerals, plant foods contain an array of phytochemicals that benefit human health, including phenolic acids, flavonoids, carotenoids, phytosterols, and fibers. These phytochemicals are widely investigated for an array of putative bioactions, e.g., antioxidation, anti-inflammation, antiproliferation, and vasodilation. Of these bioactions, antioxidant activity is commonly studied for its contribution to health promotion and prevention. Carotenoids are a group of lipophilic pigments that are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. The most common carotenoids include lycopene, α- and β-carotene, zeaxanthin, and lutein. The primary antioxidant action of carotenoids is ascribed to the singlet oxygen quenching activity.[18] After the quenching reaction occurs, excited carotenoids dissipate the newly acquired energy through a series of rotational and vibrational interactions with the solvent to return to the unexcited state. The antioxidant activity of carotenoids has been seen *in vitro* or preclinical models, but *in vivo* antioxidant action



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has not been clearly illustrated in humans.[18] Polyphenolics, including phenolic acids, flavonoids, tannins, and lignans, possess potent antioxidant properties, especially *in vitro* settings, as well as in animal models administered with doses larger than those humans normally consumed in diets or dietary supplements. Polyphenolics are capable of scavenging a wide range of reactive oxidant species, including $O_2^-$, OH, HOCl, peroxynitrites, and peroxyl radicals.[19,20] Furthermore, they chelate transition metal ions (iron and copper) to avert transient metal induced radical productions.[21]

Total antioxidant defense, including the endogenous and exogenous systems, works in a cohesive network to scavenge reactive oxidant species, but some of them inevitably escape the defense and cause oxidative damages. In the aspect of antioxidant enzymes, superoxide dismutase reduces $O_2$. to $H_2O_2$, which is then reduced to water mediated by catalase or glutathione peroxidase. In the aspect of small molecular antioxidants, vitamin E reduces a free radical and then becomes a weak free radical itself. Subsequently, vitamin E radicals are recycled or reduced back into the original form by vitamin C, lipoic acid or coenzyme Q10.[1,3] Vitamin C radicals can then be reduced by glutathione, in which the oxidized form is reduced via the glutathione reductase in the expense of NADPH, which is generated from the pentose phosphate pathway using glucose as the substrate. Polyphenolics behave as a potent radical scavenging antioxidant in *in vitro* experimental settings. In fact, their radical scavenging action is greatly limited *in vivo* mainly because of their low concentrations in the circulation and target tissues (<1 μmol/L, which is much lower than that of vitamins C and E) and extensive transformation to a form losing its radical scavenging moieties.[22] Opposite to the perception of being a strong radical scavenging antioxidant, polyphenolics contribute to the antioxidant defense by upregulating the endogenous antioxidant system, e.g., increasing glutathione production and activity of glutathione peroxidase.[23] Although the exact mechanism(s) to explain the upregulatory effects of polyphenolics has not been elucidated, it has been postulated that imposing a mild oxidative challenge due to their fast oxidation to generate $H_2O_2$ and other oxidation products elicits the induction.[3] Nevertheless, the *in vivo* antioxidant action of polyphenolics appears under rigid scrutinization; this potential health benefit shall occur in the gastrointestinal tract when they are present in the original form at larger concentrations.[24]

## Tools to Assess Oxidative Stress

Oxidative stress is a consequence of a battle between the antioxidant defense and reactive oxidant species. Some of the reactive oxidant species can escape the defense and attack and induce oxidation of susceptible macromolecules (lipids, DNA and proteins). The quantity of the resulting oxidation products depends on the balance between the antioxidant defense capacity and the amount and reactivity of reactive oxidant species. In theory, oxidative stress status in the body can be best revealed when the element of the antioxidant defense network, reactive oxidant species and the oxidized products are all assessed. However, it is almost impossible to measure highly reactive oxidant species in the human body as they survive for less than a second after formation. Quantifying small-molecule antioxidants and measuring activity of antioxidant enzymes can be reasonably performed though their measurements appear cumbersome because each must be assessed individually. Therefore, the products derived from reactive oxidant species attacks are one of the best biomarkers for the evaluation of oxidative stress status in the human body.

Valid biomarkers can adequately reflect the status of relevant physiological functions, relate to established pathological signs or demonstrate whether an agent has a beneficial, untoward or null effect on health promotion and disease prevention or a therapeutic value in disease treatment. As reactive oxidant species attack nucleophiles to acquire electrons, the consequence of the attacks is the production of an array of oxidized products. Assays for determining oxidation products of lipids, proteins and DNA have been reviewed elsewhere.[25,26] Briefly, malondialdehyde (MDA) and F2α-isoprostanes (iPF2) are two common biomarkers of lipid peroxidation, with the latter being more sensitive and specific. MDA is more commonly determined in studies than $iPF_2$ because of the relatively low cost and high throughput for its determination in different matrices. However, its validity and specificity have been frequently questioned due to a crossreaction of 2-thiobarbituric acid (forming a pink adduct with MDA) with other substrates (other alkanals, protein, sucrose, amino acids, sialic acid, urea, acetaldehyde-sucrose, and reducing sugars). Proteins can be damaged directly by free radical attacks or indirectly via reactions with the secondary byproducts of lipid peroxidation, leading to the formation of oxidatively modified amino acids and protein carbonyls (PC), respectively.[27] PC is the most commonly performed biomarker for protein oxidation in human studies, which can be measured using colorimetric assay, enzyme-linked immunosorbent assay (ELISA) or HPLC method.[28,29] Compared to PC, individual oxidized amino acid products, e.g., chlorotyrosine (Cl-tyr), 3-nitrotyrosine (N-tyr) and dityrosine (di-tyr), are more indicative of specific radical reactions with proteins.[30] In particular, tyrosine-oxidized products are formed exclusively *in vivo* and not subject to artifact.[29] Methods using HPLC equipped with electrochemical detection (ECD) or GC-MS have been developed for the quantification of oxidized amino acids. Oxidized DNA bases reflecting DNA damage and indicating cancer risk can be assessed using different technologies, e.g., HPLC-ECD, GC-MS, LC-MS, and ELISA. Further, radical-induced DNA strand breaks in individual cells can be semiquantified using single cell gel electrophoresis, also

called a "comet" assay.[31] Of oxidized DNA bases, 8-hydroxyguanonise (8-oxo-dG) is the most commonly measured.

Analysis of individual small molecular antioxidants is time consuming and normally requires using assays with a chromatographic separation prior to the quantification. Thus, it is very cumbersome and expensive to quantify all possible antioxidants in foods and human fluids. Further, some antioxidant phytochemicals in foods remain to be characterized. With this notion in mind, over the years many easy-to-perform and cost-effective methodologies have been developed to provide a collective measure of antioxidant potency of all antioxidants in foods or plasma/serum, such as the oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), diphenyl-picrylhydrazyl (DPPH) quenching, trolox equivalent antioxidant capacity (TEAC), and total radical-trapping parameter (TRAP).[32] These assays have been used to reveal the association of consumption of antioxidant-rich foods with risk of diseases and to assess the effect of nutrients, foods or diets on antioxidant status in human interventions. Among these assays, ORAC receives the most recognition because the assay has been frequently used by food companies to generate consumer messages for product promotion. Application of ORAC, FRAP and other TAC assays in food science research remains appealing because TAC assays can inform TAC value of foods. However, there is great concern that dietary TAC may not have a direct link with TAC in plasma/serum because dietary antioxidants are subject to extensive modifications by mastication, pH in the gastrointestinal tract and metabolism mediated by the gut bacteria and endogenous detoxification mechanisms.

The link between the accumulation of biomarkers for oxidative damage and production of a particular free radical is indirect because a change in clearance can dramatically alter the level of the marker with no change in production of a given free radical. Consequently, results of oxidative damage products need to be assessed cautiously with a clear understanding of what the methods used can reveal. It also is important to understand that the capacity of the antioxidants *in vivo* is determined not only by the reactivity toward radicals but also by several other factors such as concentration, distribution, localization, fate of antioxidant-derived radical, interaction with other antioxidants, and metabolism. No single biomarker could reliably evaluate overall oxidative stress in both local and systematic levels. The biomarkers should be selected considering the function and indications to be evaluated. Furthermore, combining evaluation of both "footprint" biomarkers of reactive oxidant species and antioxidant defenses, including both enzymatic and nonenzymatic protection, is the best way to describe the role of free radicals and antioxidants in biology and medicine.

## The Role of Antioxidants in Metabolic Syndrome

Metabolic syndrome (MetS) is a disorder with a constellation of metabolic abnormalities. The World Health Organization (WHO)[33] and the National Cholesterol Education Program Adult Treatment Panel (ATP III)[11] defined the criteria of MetS with a specific focus on dyslipidemia, hyperglycemia, hypertension, and abdominal obesity. It shall be noted that the diagnosis criteria may differ between health organizations. The key components of MetS are risk factors for type 2 diabetes mellitus and CVD.[11] Further, MetS is associated with other medical conditions, such as fatty liver, polycystic ovary syndrome, cholesterol gallstones, and obstructive sleep apnea.[34] The prevalence of MetS is approximately 25 to 30% (~47 million adults) in the United States[35] and 13 to 16% in China.[36] Along with the dramatic increase in overweight/obese populations in developing and developed countries, the prevalence is anticipated to be increasing exponentially. Thus, it becomes a critical issue to find dietary approaches to prevent both its development and progression to more serious health complications.

Circulating oxidized low-density lipoprotein (oxLDL), a biomarker for atherosclerosis due to its involvement in the formation of foam cells in the vessel wall, is associated with increased incidence of MetS, as well as its components of abdominal obesity, hyperglycemia and hypertriglyceridemia.[37-39] Van Guilder, et al.[40] noted that there was a stepwise increase in the circulating oxLDL from healthy individuals to obese and then to obese plus MetS. Importantly, the magnitude of lipid peroxidation appears greater in people with two or more components of MetS than in those with one or less. Konukoglu, et al.[41] found in a study with 25 nonobese normotensives (BP, 95.5/75.4; BMI, 21.5; age, 54.5), 25 nonobese hypertensives (BP, 165.5/105.3; BMI, 23.5; age, 56.5), 35 obese normotensives (BP, 109.5/75.5; BMI, 32.5; age, 60.5), and 45 obese hypertensives (BP, 168.8/115.6; BMI, 33.8; age, 58.5) that obesity and hypertension were associated with greater oxidative stress status, assessed using MDA, than either one alone. Similarly, Stojiljkovic, et al.[42] found increased urinary $iPF_2$ excretion in 10 obese hypertensives with insulin resistance (BMI, 31; BP, 131/88; age, 40) compared to 12 healthy normotensives without insulin resistance (BMI, 22; BP, 106/72; age, 38). All these data indicate that biomarkers of radical oxidized products are elevated in people with MetS or with its key components. However, it shall be noted that these increases may simply indicate there is an increased production of reactive oxidant species in people with MetS, but it cannot illustrate whether the increases are the consequence of weak antioxidant defense capacity resulting from lower intake of dietary antioxidant or a MetS-induced increase in the production of reactive oxidant species. Furthermore, the association between enhanced oxidative stress and MetS is



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not unidirectional. Sjogren, et al.[43] concluded from a small cross-sectional study of 289 healthy older men (62 to 64 y) that circulating oxLDL and urinary $iPF_2$ were not elevated in people with MetS as compared to those who had none, one or two key signs of MetS. In the same cohort, they also found that $iPF_2$ was inversely related to dietary vitamin C and β-carotene intake, implicating a more significant role of dietary antioxidants in enhanced oxidative stress status than the pathogenic conditions of MetS.

Small-molecule antioxidants, including vitamins C and E, carotenoids, polyphenols, α-lipoic acid, glutathione, uric acid, and ubiquinols, contribute to the body's antioxidant defense network complementing the enzymatic antioxidants. If the etiology or progression of MetS was associated with oxidative stress, then an increased utilization and requirement of lower concentrations in a vicious oxidative stress status would be anticipated for these compounds. Indeed, serum antioxidant status has been noted to be inversely associated with MetS in several studies. Using data from the National Health and Examination Survey 2001-2006, Beydoun, et al.[44] found the prevalence of MetS was 32.0% among men and 29.5% among women aged 20 to 85. In this cohort, they found that MetS was associated with lower serum carotenoid status, even after controlling for total cholesterol and triglycerides, than those without the condition. Likewise, serum vitamin C exhibited an inverse linear association with MetS and related conditions like insulin resistance and hyperuricemia. In contrast, after controlling for serum lipids, vitamin E was observed not to have a significant relationship with MetS. In the other study using the data of the Third National Health and Nutrition Examination Survey (1988-1994) including 8,808 U.S. adults aged ≥20 y, Ford, et al.[45] found that concentration of retinyl esters, carotenoids and vitamins C and E was significantly lower in people with MetS than without the condition. In a Chinese cohort with 17% MetS prevalence (n = 2069), Wei, et al.[46] found that the low intake of vitamin C, adjusted with energy intake, was associated with the incidence of MetS but was neither selenium nor vitamin E intake. Likewise, Sohrab, et al.[47] noted that there was a negative association between dietary flavonoid intake and MetS incidence. As cross-sectional studies do not allow inference about progressive occurrence of these associations, it is unclear whether the lower status of circulating antioxidants are the result of increased production of reactive oxidant species caused by MetS or the direct outcome of lower fruit and vegetable intake. Especially since evidence showing that people with MetS consumed less dietary antioxidants adds to the complexity of the interaction.[46]

Total antioxidant capacity is referred to be an integrated measure of all small-molecule antioxidants in both realms of food and nutrition sciences. Bahadoran[48] found that people in the highest quintile of dietary TAC at baseline, calculated using the USDA ORAC database, tended to have a lower incidence of MetS at the three-year follow-up. Because of being rich in micronutrients and phytochemicals, the incorporation of a variety of high TAC plant foods to daily diets of people with MetS shall be encouraged. However, it shall be noted that consumption of high TAC foods is not necessary to relate to high plasma/serum TAC value because of poor bioavailability and extensive metabolism of some dietary antioxidants. In terms of associations between plasma/serum TAC and MetS, clinical evidence is mixed. Korkmaz, et al.[49] found that serum FRAP value was larger in 55 patients with MetS than in 20 healthy controls, primarily attributed to larger circulating content of uric acid, which can contribute to ~60% FRAP value. In contrast, using the other TAC assay, Venturini, et al.[50] observed that plasma TRAP value of people with MetS was lower than healthy controls even though uric acid level was larger. Thus, though TAC assays prove to be cost effective and high throughput to assess the overall status of small-molecule antioxidants, their clinical significance and application in MetS require more validation in future studies. Selection of TAC assay(s) must take biochemical and physiological phenotype of subjects into account.

MetS appears to associate with enhanced oxidative stress manifested with increased radical-induced oxidized products and/or decreased antioxidant defense capacity. This association proffers the potential of dietary antioxidants for MetS prevention and treatment. However, the efficacy of the antioxidants relies on the premise that reactive oxidant species play an etiological role in the development of MetS and progression to more serious complications, such as type 2 diabetes and CVD. If reactive oxidant species had a direct contribution, increased antioxidant defense capacity mediated by any means should have decreased MetS incidence in prospective studies or the number of key components of MetS in intervention studies. However, as little direct evidence is available to substantiate the contribution of dietary antioxidant to reducing the risk of MetS, further research in this area is warranted.

The significance of dietary pattern in health promotion and prevention is emphasized over individual foods or nutrients in the 2015 Dietary Guidelines for Americans, in which a Mediterranean diet rich in fruits, vegetables, nuts, and whole grains is mentioned. Velázquez-López, et al.[51] examined in a nutritional therapy trial the impact of the Mediterranean diet on the key components of MetS in 29 obese children (age: 11 y; BMI: 27 kg/m2). After 16 weeks of the dietary intervention, the prevalence of MetS was decreased from 66 to 21% in the children who consumed the Mediterranean diet as compared to no change in those consuming a standard diet. Although the results must be substantiated in a large-scale trial, this study suggests a healthy diet containing

many antioxidants is therapeutic against MetS. Further, the Mediterranean diet led to an increased intake of some key antioxidants, i.e., zinc, selenium, vitamins C and E, and flavonoids. One question that remains to be explored is the magnitude of the contribution from dietary antioxidants to the reduced incidence of MetS, which was not gauged in this study. It seems that dietary antioxidants may not play a considerable role as anticipated. In this context, de la Iglesia, et al.[52] reported that dietary therapy targeting weight loss would improve many key components of MetS as obesity is the most crucial component of MetS. If this were true, increased antioxidant intake might not be able to prevent or treat MetS. For example, Manning, et al.[53] found in a one-year intervention trial with 151 adults with MetS that lipoic acid (600 mg/d), vitamin E (100 IU/d) or a combination did not improve insulin resistance or the components of MetS. Similarly, quercetin, a common dietary flavonoid displaying potent antioxidant activity, was found to increase HDL cholesterol and decrease waist circumference in adults without signs of MetS but failed to improve oxidative stress biomarkers, e.g., circulating oxLDL and urinary $iPF_2$.[54] In the other flavonoid study with patients with MetS, EGCG-rich green tea or extract elevated glutathione in whole blood and TAC in plasma.[55] As the authors failed to assess biomarkers of radical-induced oxidized products, the increase in antioxidant defense should not be extrapolated to a reduction in systematic oxidative stress and MetS incidence.

In a large-scale prospective SU.VI.MAX trial, French adults (n = 5220; age, 49.4) free of MetS were randomly assigned to receive a supplement containing a combination of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, and 100 µg selenium and 20 mg zinc per day) or a placebo.[56] At the end of the 7.5-year follow-up, the antioxidant supplementation did not affect the risk of MetS, but baseline serum concentration of β-carotene and vitamin C were negatively associated with the risk of MetS. Interestingly, baseline serum vitamin E and selenium were not associated with the risk of MetS, and zinc was associated with increased risk. While there was lack of adverse effect from the supplementation, the trial raised a question about suitability of antioxidant supplementation in populations with adequate intake of dietary antioxidants. Most importantly, the study implicates that the effectiveness of antioxidant supplementation in the prevention of MetS may only be manifested in people with enhanced oxidative stress status or those consuming poor diets. Overall evidence at this time tends to suggest that dietary antioxidants may neither prevent MetS nor treat its components through their antioxidant actions.

## Conclusion

Reactive oxidant species are constantly produced in the human body. Even with a very short half-life, a small proportion of reactive oxidant species can escape from the antioxidant defense network composing enzymatic and small-molecule antioxidants to induce oxidative damages. Particularly, chronic, uncontrolled production of reactive oxidant species overwhelms the antioxidant defense capacity, which is called oxidative stress, leading to the development and progression of many chronic diseases, including metabolic disorders, CVD, certain cancers, cognitive decline, and others. Thus, it is a rational hypothesis that boosting antioxidant defense capacity would be preventive or therapeutic against radical-related disorders/diseases. A simple way to strengthen the antioxidant defense is consuming nutrients that can either scavenge reactive oxidant species or stimulate synthesis of endogenous antioxidants.

In order to assess clinical significance of dietary antioxidants and to test study hypotheses, determining biomarkers of either antioxidant defense, oxidized products or both must be undertaken. MetS is a disorder with a cluster of metabolic abnormalities including dyslipidemia, hyperglycemia, hypertension, and abdominal obesity, which all are risk factors for CVD. The association of MetS with enhanced oxidative stress, evidenced by increased oxidized products and inadequate antioxidant defense, paves the way for using dietary antioxidants in its prevention and treatment. However, the limited data generated from human studies appear to not support the notion that antioxidants can decrease risk for MetS or reduce the number of MetS components through their antioxidant actions. If the hypothesis that reactive oxidant species would contribute to development and progression of MetS were true, therapeutic efficacy of antioxidants should have been realized. The null results noted in studies can be attributed to a challenge in design of antioxidant interventions to alleviate oxidative stress and prevent/treat disease as many factors may confound likelihood of study success, e.g., optimal antioxidant combinations and doses, as well as the best physiological and biochemical conditions to intervene in MetS. Importantly, studies must include not only relevant clinical outcomes but also measures of antioxidant defense and radical-oxidized products in order to validate the efficacy of antioxidants.

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