# Dietary antioxidants and health effects: what are their optimal intakes?

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## Abstract

A well-balanced diet provides many compounds with antioxidant properties, such as vitamins, minerals, provitamins (e.g., *β*-carotene), and phytochemicals (e.g., carotenoids, polyphenols, organosulfur compounds). In addition to direct antioxidants, foods indirectly support the endogenous defense system, by providing substrates for the synthesis of glutathione, antioxidant defense enzymes, metal-binding proteins, or modulators of redox-dependent signaling pathways. Epidemiological studies indicate that higher intakes and circulating concentrations of vitamins C, E, carotenoids, and flavonoids reflect a lower risk of chronic diseases and all-cause mortality, suggesting the importance of optimal intakes of these substances. However, unlike antioxidant micronutrients, phytochemicals have no defined recommended intake levels. A diet should be based on consuming various plant foods (fruit, vegetables, legumes, whole grains, seeds, nuts), antioxidant-rich beverages, and a moderate intake of animal food products to fully exploit the health-promoting effects of dietary antioxidants.

**Key words:** antioxidants, health benefits, diet, nutrients, phytochemicals

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## Introduction

Reactive oxygen and nitrogen species are continually generated within cells through enzymatic and non-enzymatic reactions in mitochondria, peroxisomes, endoplasmic reticulum, and other cellular components, or received from exogenous sources. While low and moderate levels of reactive species are necessary for cellular functioning, gene transcription, immune cell activation, biosynthesis of macromolecules, and metabolic regulation, excess reactive species can damage biomolecules (1). The antioxidative defense system was developed during human evolution to prevent reactive species' uncontrolled formation and subsequent detrimental effects (2). In addition to enzymes, important components of the antioxidant defence network are non-enzymatic antioxidants and metal-binding proteins. However, for optimal functioning, support from exogenous antioxidants is required (2, 3). These compounds exert protective effects by inhibiting the production or scavenging of reactive species, by chain-breaking oxidative reactions, reactive metal chelating, restoration of endogenous antioxidants, and damaged biomolecules (4).

In addition to micronutrients (e.g., vitamins C, E, copper, zinc, and selenium), foods contain many phytochemicals, such as carotenoids, polyphenols, and organosulfur compounds with health-promoting properties (Table I). In addition to direct antioxidants, food supplies many ingredients that indirectly support the endogenous antioxidant system through synthesizing glutathione and antioxidant defense enzymes, metal-binding proteins, albumin, and other components (5).

Unlike for micronutrients, there are no established dietary reference values (DRVs) for non-nutritive bioactive compounds. DRVs for micronutrients for the European include population reference intakes (PRIs), the average requirements (ARs), and adequate intakes (AIs) as estimates of the daily amounts that are necessary to meet the needs of healthy populations (9). Compared to the well-chemically characterized nutrients with well-established metabolic roles, phytochemicals are chemically complex. Moreover, most of them are converted into active or inactive metabolites during digestion, absorption, and metabolism, making their quantification and contribution to specific health effects challenging to understand (10).

**Table I** Dietary antioxidants and major food sources (6-8)

**Tabela I** Dijetarni antioksidansi i njihovi glavni izvori (6-8)

| Antioxidants | | Food sources |
|---|---|---|
| Vitamins | Vitamin C | Citrus, strawberries, tomatoes, cruciferous vegetables, white potatoes |
| | Vitamin E | Plant-based oils, nuts, seeds, green vegetables, mangoes, avocados |
| Minerals | Copper | Organ meats, shellfish, fish, nuts, seeds, whole grains, chocolate |
| | Iron | Meats, poultry, seafood, fortified grains, nuts, seeds, legumes, green vegetables |
| | Selenium | Brazil nuts, seafood, cereals, poultry, red meat, eggs |
| | Manganese | Shellfish, nuts (hazelnuts, pecans), grains, legumes, species |
| | Zinc | Meats, poultry, seafood, legumes, nuts, seeds, whole grains |
| Carotenoids | $\alpha$-carotene | Pumpkin, carrot, mixed vegetables, tomatoes, tangerines |
| | $\beta$-carotene | Carrot, pumpkin, spinach, kale, cantaloupe |
| | $\beta$-cryptoxanthin | Pumpkin, papayas, red peppers, orange juice, yellow corn |
| | Lycopene | Tomato, watermelon, pink grapefruit, baked beans |
| | Lutein + zeaxanthin | Dark green leafy vegetables, yellow corn, avocado, egg yolk |
| Phenolic acids | Hydroxybenzoic acids | Strawberry, raspberry, grape juice, pomegranate juice |
| | Hydroxycinnamic acids | Fruits, vegetables (potato, lettuce, spinach), tea, coffee, cider |
| Flavonoids | Flavonols | Vegetables, cereals, fruits, spices, herbs, red wine, tea, cocoa |
| | Flavones | Celery, olives, spices (oregano, rosemary, dry parsley, thyme) |
| | Isoflavones | Grape seed/skin, soybean, soy products |
| | Flavanones | Citrus fruits and juices, spices and herbs (peppermint) |
| | Anthocyanins | Berries, cherries, plums, red wine |
| | Flavanols | Fruits, wine, tea, chocolate |
| Lignans | | Seeds, whole grain, bran, beans, berries, vegetables, tea, coffee, wine |
| Stilbenes | | Grapes, peanuts, red wine |
| Organosulfur compounds | Isothiocyanates, indoles, allylic sulfur compounds | Onion, garlic, broccoli, cabbage, cauliflower, etc. |

## Dietary antioxidants

### *Vitamins*

Vitamin C, as a cofactor of enzymes, is necessary for synthesizing collagen, L-carnitine, some peptide hormones, and neurotransmitters. In addition, vitamin C is a free-radical scavenger, a modulator of oxidative stress pathways, and an enhancer of other non-enzymatic endogenous and exogenous antioxidants (11). Plasma ascorbate concentrations of less than 20 µmol/L are accompanied by non-specific signs of vitamin C deficiency, such as fatigue symptoms (12). A vitamin C intake of 60-100 mg/day is required for adequate vitamin C status (50 µmol/L). However, higher intakes (200-400 mg/day) lead to ascorbate plasma saturation (70-80 µmol/L) (13). At doses of 500 mg and higher, which are significantly higher than could be obtained through a regular diet, the bioavailability of vitamin C declines due to decreased absorption and increased urinary excretion rates (14). Recommended vitamin C intakes for non-smoking men and women are 110 mg/day and 95 mg/day, respectively. Pregnant and lactating women have additional needs in vitamin C intake (Table II) (13). Smokers and overweight/obese adults also require higher dietary intakes to maintain adequate vitamin C levels (15). From a health-promoting aspect, there is evidence that each additional vitamin C intake of 50–100 mg/day reduces the risk of all-cause mortality, cardiovascular disease, some malignancies and other chronic diseases. However, there are no well-documented benefits for additional vitamin C intake in saturated individuals. Moreover, high-dose vitamin C supplements could be associated with breast cancer and kidney stones (16, 17).

**Table II** Dietary reference intakes for antioxidant micronutrients

**Tabela II** Dijetarni referentni unosi za antioksidativne mikronutrijente

| | Age | Vitamin C (mg/day) | Vitamin E (mg/day) | Zinc (mg/day) | Iron (mg/day) | Copper (mg/day) | Manganese (mg/day) | Selenium (µg/day) |
|---|---|---|---|---|---|---|---|---|
| Infants | 7-11 months | 20 | **5** | 2.9 | 11 | **0.4** | **0.02-0.5** | **15** |
| Children | 1-3 years | 20 | **6-9** | 4.3 | 7 | **0.7** | **0.5** | **15** |
| | 4-6 years | 30 | **9** | 5.5 | 7 | **1** | **1** | **20** |
| Male | 7-10 years | 45 | **9** | 7.4 | 11 | **1** | **1.5** | **35** |
| | 11-14 years | 70 | **13** | 10.7 | 11 | **1.3** | **2** | **55** |
| | 15-17 years | 100 | **13** | 14.2 | 11 | **1.3** | **3** | **70** |
| | ≥ 18 years | 110 | **13** | 9.4-16.3[a] | 11 | **1.6** | **3** | **70** |
| Female | 11-14 years | 70 | **11** | 10.7 | 13 | **1.1** | **2** | **55** |
| | 15-17 years | 90 | **11** | 11.9 | 13 | **1.1** | **3** | **70** |
| | ≥ 18 years | 95 | **11** | 7.5-12.7[a] | 16 (11[b]) | **1.3** | **3** | **70** |
| | Pregnant women | 105 | **11** | +1.6 | 16 | **1.5** | **3** | **70** |
| | Lactating women | 155 | **11** | +2.9 | 16 | **1.5** | **3** | **85** |

This table (taken from the DRV Finder, see www.efsa.europa.eu) presents Population Reference Intakes (PRIs) in ordinary type and Adequate Intakes (AIs) in **bold** type.

The AIs for vitamin E refer to $\alpha$-tocopherol only, which is the physiologically active form. Other tocopherols and tocotrienols do not contribute to the vitamin E requirement.

[a] PRIs for zinc are provided for four levels of phytate intake (LPI): 300, 600, 900 and 1,200 mg/day

[b] for postmenopausal women

Ova tabela (preuzeta iz DRV Finder-a, videti www.efsa.europa.eu) predstavlja populacione referentne unose (PRI) predstavljene uobičajenim i adekvatne unose (AI) predstavljene **podebljanim** brojevima.

AI za vitamin E se odnosi samo na $\alpha$-tokoferol, koji je fiziološki aktivan oblik. Drugi tokoferoli i tokotrienoli ne doprinose potrebama za vitaminom E.

[a] PRI za cink je definisan za četiri nivoa unosa fitata (LPI): 300, 600, 900 i 1200 mg/dan.

[b] za žene u postmenopauzi

### ***Vitamin* E**

Among different tocopherols and tocotrienols, referred to as vitamin E, $\alpha$-tocopherol is the predominant form in tissues. It exhibits the most efficient scavenging activity against lipid peroxyl radicals formed by lipid peroxidation of polyunsaturated fatty acids (PUFAs) (18). In addition to being a direct antioxidant, this liposoluble compound, through epigenetic mechanisms, can prevent lipid peroxidation of cell membranes, lipoproteins, and other lipid-containing biomolecules (19). Since vitamin E intake should be correlated with the intake of PUFAs, based on the typical dietary intake of PUFAs, the estimated requirements for vitamin E range between 12 and 20 mg (20). In Europe, the adequate intake (AI) for $\alpha$-tocopherol is set at 11 mg/day for women and 13 mg/day for men (21) (Table II).

Considering the oxidative-modifications hypothesis of atherosclerosis, vitamin E has been recognized as a promising modulator of cardiovascular disease (CVD). Although circulating $\alpha$-tocopherol levels are inversely related to CVD mortality risk, there is no conclusive evidence for the health-protective effect of higher vitamin E intake (22, 23). Furthermore, vitamin E supplementation could be associated with adverse cardiovascular outcomes (23). Therefore, based on the current evidence data, the focus should be on the intake of vitamin E-rich foods rather than high-dose vitamin E supplements (19). However, higher than recommended vitamin E doses should benefit individuals with low dietary intake or depletion of vitamin E and other micronutrients due to malnutrition or pathologic conditions (19, 24).

***Minerals***

In addition to other functions, iron and trace elements (e.g., copper, zinc, manganese, and selenium) also have essential roles in redox homeostasis. These minerals are cofactors of antioxidant enzymes, which present the first line of defense against reactive species (25). Iron and copper, as constituents of the inner membrane complexes involved in the electron transport chain, have essential roles in mitochondrial metabolism (26). In addition to glutathione peroxidases, thioredoxin reductases, and other selenoproteins, which protect against oxidative stress, selenium is also needed for some enzymes that help repair proteins from oxidative damage (5). Suboptimal selenium status, primarily due to inadequate selenium contents in soil, has been associated with the risk of several diseases, including cancer, cardiovascular, immune, and metabolic diseases (27, 28). Therefore, selenium biofortification of plants and livestock is recognized as a beneficial strategy for increasing selenium dietary intake (29).

A balanced diet is sufficient to supply recommended mineral intake for most individuals (Table II). However, the risk of low intakes is more likely in specific groups, including older people, pregnant women, vegetarians or vegans, and people with chronic diseases (30).

***Carotenoids***

The most abundant and well-studied carotenoids in foods are provitamin A precursors ($\alpha$-carotene, $\beta$-carotene, and $\beta$-cryptoxanthin), and lycopene, lutein, and zeaxanthin, which have no provitamin A activity. The primary dietary sources of these compounds are fruit and vegetables (Table I). Egg yolk, milk products, seafood, and food additives also contribute to carotenoid intakes, but to a lesser extent (31). Health-protective effects of carotenoids are attributed to their antioxidant, anti-inflammatory, anti-diabetic, anti-tumor, and anti-aging activities (32).

Serum/plasma and skin carotenoid levels are recognized as reliable biomarkers of fruit and vegetable intake (33, 34). While plasma total carotenoid concentration of <1000 nmol/L is recognized as a risk factor, concentrations ≥ 2500 nmol/L have protective attributes (35). Epidemiological data support increasing intake of carotenoids as nutritional strategies for preventing cardiovascular and malignant disease and promoting longevity (22). Therefore, the general population is advised to consume green-yellowish, yellow, orange, or pink-red foods to supply various carotenoids. However, a higher intake of some carotenoids could be beneficial for specific health purposes. For example, $\beta$-carotene and lutein are associated with cardiometabolic health, lycopene with anti-cancer effects, and lutein and zeaxanthin with protective effects on vision and eye health (36).

In the absence of dietary reference value for carotenoids, some recommendations are provided on the assumption that 4.8 mg of $\beta$-carotene provides 800 µg of vitamin A. Additionally, epidemiological data suggest that the optimal plasma level of $\beta$-carotene (0.4 µmol/L) corresponds to 2–4 mg of dietary $\beta$-carotene intake daily (37). The intake recommendation for lutein (10–20 mg/day) is based on the risk of yellowing of the skin, and for lycopene (5.7–15 mg/day) on its antioxidant activity (38).

***Polyphenols***

Phenolic compounds are a diverse group of phytochemicals that can be categorized into several classes and subclasses based on chemical structures. The main classes include flavonoids and non-flavonoids (phenolic acids, stilbenes, and lignans). Flavonoids are classified into flavones, flavanones, flavonols, flavan-3-ols, anthocyanins, and isoflavones (39). The estimated average intake of polyphenols through diet is about 1 g/day, making them the most predominant class of dietary antioxidants in human nutrition (40).

Total intakes, the dominant types, and food sources of polyphenols are related to specific diet patterns. In France, fruit and vegetable intake supplies about 30% of daily polyphenols, while coffee, tea, fruit juices, and cereals are other contributors (41). Phenolic acids account for about 50%, while flavonoids (including anthocyanins) and tannins present about 25% of polyphenols (42). In Spain, coffee and fruit are the greatest contributors to polyphenols intake. However, the most discriminatory elements of the Mediterranean polyphenol profile pattern compared to others are olives and olive oil (43). The primary sources of polyphenols in a Sicilian cohort's diet were nuts, cherries, and beverages (coffee, red wine, and tea) (44).

Due to their antioxidative, anti-inflammatory, and many other biological activities, long-term intake of polyphenols is related to protection from cardiovascular disease, type II diabetes, neurodegenerative diseases, and some cancers (45). However, recent epidemiological studies indicated that a higher intake of some specific polyphenol compounds, especially flavonoids, may be more beneficial than total polyphenols intake (46). While flavones and flavonols are associated with preventing coronary heart diseases, anthocyanins and flavan-3-ols decrease the overall risk of CVD (47).

***Organosulfur compounds***

Organosulfur-containing compounds, excluding sulfur-containing amino acids, are characteristic of some foods, such as *Allium* and *Brassica* vegetables. In addition to contributing to unique flavors, these phytochemicals exert many biological activities (48). While *Allium* species provide precursors of S-alk(en)yl-L-cysteine sulphoxides**,** which transform into highly reactive thiosulfinates, thiosulfonates, and sulfides, *Brassica* vegetables contain glucosinolates, which are precursors of isothiocyanates, indole-3-carbinols, and other compounds. Among these metabolites, the most extensively studied are allicin (diallythiosulfinate), a major active garlic compound, and sulforaphane, found in broccoli, cabbage, and other cruciferous vegetables (49). In addition to direct radical scavenger activities, organosulfur compounds are potent inducers of the Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway (50). Besides well-established chemopreventive and anticancer properties, these compounds, as natural hydrogen sulfide ($H_2S$) donors, have recently attracted attention as potential cardioprotective agents (51). Cruciferous and allium vegetable intakes were related to a lower risk of age-related atherosclerotic vascular disease mortality (52). It is also reported that increasing total vegetable intake is inversely associated with subclinical atherosclerosis parameters, highlighting the vascular protective effects of cruciferous vegetables (53).

***Antioxidant capacity of foods***

Considering that foods contain various nutritive and non-nutritive antioxidants and their cumulative, additive or synergistic protective effects, several approaches have been proposed for evaluating the antioxidant potential of foods. Based on the antioxidant food database, there are significant differences in antioxidant capacity among different foods (54). There is evidence that selecting foods based on dietary total antioxidant capacity (TAC) has affected dietary antioxidant intake and modulation of low-grade inflammation but had no influence on blood dietary antioxidant levels (55). Recently, it has been demonstrated that dietary TAC correlates well with quality diet scores and may be an indicator of healthy eating patterns (56).

Based on TAC values and serving sizes, fruit and vegetables can be divided into low-, medium- and high-antioxidant groups. For example, black and red color fruit and vegetables, which are rich in anthocyanins, are ranked as high-antioxidant foods. Higher L-ascorbic acid content, total phenolics, and carotenoids are attributed to medium-antioxidant foods and chlorophyll content (e.g., leaf green vegetables) within the low-antioxidant category of foods (57).

Despite the recommendation for eating at least 400 g of fruit and vegetables per day (58) or daily consumption of multiple portions of a variety of fruits and vegetables (59), low consumption of fruit and vegetables is recognized as a global problem, affecting high- and low-income countries (60). Therefore, the “eat by color” approach could be a valuable supportive strategy for fruit and vegetable intake recommendations for health-promotion benefits (61).

As shown in Table I, fruit and vegetables are primary sources of vitamin C and phytochemicals (polyphenols, carotenoids), while vegetable oils, seeds, and nuts are the best sources of vitamin E. In addition to plant sources, selenium and zinc are present in animal food products, including seafood, red meat, poultry, and eggs. Having in mind these facts, the composite dietary antioxidant index (CDAI) summarizes the intake of the following dietary antioxidants: vitamins (A, C, E), zinc, selenium, and carotenoids) (62). Apart from reflecting fruit and vegetable intakes, CDAI can discriminate between antioxidant-rich and antioxidant-poor dietary patterns. Recently, there have been several reports stating that higher CDAI levels are inversely associated with risks of hypertension (63), low bone mass and osteoporosis (64, 65), post-stroke depression (66), handgrip strength (67), and mortality rates (68).

## Conclusion

A higher intake of antioxidants through foods, rather than isolated in dietary supplements, is associated with many health protective effects and longevity. In addition to antioxidant vitamins and minerals, foods provide many non-nutritive antioxidant compounds. However, their profiles and biological activities vary depending on the types of food. Therefore, a well-balanced diet should be based on the consumption of a variety of plant foods (fruit, vegetables, legumes, whole grains, seeds, nuts) and antioxidant-rich beverages, followed by moderate consumption of animal products to fully exploit their health-protective effects.

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# Dijetarni antioksidansi i zdravstveni efekti: koliki je optimalan unos?

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**Kratak sadržaj**

Dobro balansirana dijeta je izvor brojnih antioksidanasa, kao što su vitamini, minerali, provitamini (npr. *β*-karoten), i fitohemikalija (npr. karotenoida, polifenola, organosumpornih jedinjenja). Osim direktnih antioksidanasa, namirnice obezbeđuju organizmu mnoge sastojke koji indirektno podržavaju endogeni antioksidativni zaštitni sistem, kao što su supstrati neophodni za sintezu glutationa, enzima antioksidativne zaštite, metalo-vezujućih proteina ili modulatori redoks osetljivih signalnih puteva. Epidemiološke studije ukazuju na inverznu povezanost dijetarnih unosa i/ili serumskih koncentracija vitamina C, E, karotenoida i flavonoida sa rizikom od brojnih hroničnih nezaraznih bolesti i ukupnog mortaliteta, ukazujući na značaj njihovog optimalnog unosa. Suprotno antioksidativnim mikronutrijentima, za fitohemikalije nisu utvrđene vrednosti preporučenog dnevnog unosa. Ishrana treba da se zasniva na konzumiranju raznovrsnih biljnih namirnica (voće, povrće, mahunarke, integralne žitarice, semenke, orašasti plodovi), napitaka bogatih antioksidansima, uz umereno konzumiranje namirnica animalnog porekla, kako bi se u potpunosti iskoristili protektivni efekti dijetarnih antioksidanasa.

**Ključne reči:** antioksidansi, zdravstveni efekti, ishrana, nutrijenti, fitohemikalije

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