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Dietary antiaging phytochemicals and mechanisms associated with prolonged survival

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Abstract

Aging is well-known an inevitable process that is influenced by genetic, lifestyle and environmental factors. However, the exact mechanisms underlying the aging process are not well understood. Increasing evidence shows that aging is highly associated with chronic increase in reactive oxygen species (ROS), accumulation of a low-grade proinflammatory phenotype and reduction in age-related autophagy, suggesting that these factors may play important roles in promoting aging. Indeed, reduction of ROS and low-grade inflammation and promotion of autophagy by calorie restriction or other dietary manipulation can extend lifespan in a wide spectrum of model organisms. Interestingly, recent studies show that some food-derived small molecules, also called phytochemicals, can extend lifespan in various animal species. In this paper, we review several recently identified potential antiaging phytochemicals that have been studied in cells, animals and humans and further highlight the cellular and molecular mechanisms underlying the antiaging actions by these molecules.

Keywords

Aging; Phytochemicals; Calorie restriction; Reactive oxygen species; Inflammation; Autophagy

1. Introduction

Aging is one of the most familiar yet least well-understood biological sciences. It is physiologically characterized as a progressive, generalized systematic dysfunction of almost all organs, giving rise to the escalated vulnerability to environmental challenges and resulting in increased risks of disease and death. Indeed, aging in humans is associated with a greatly increased incidence of a number of degenerative diseases including cardiovascular disease, Type 2 diabetes, cancer and Alzheimer's disease, and these chronic diseases

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account for more than 70% deaths among Americans 65 years of age and older [1]. Therefore, preventing or delaying the pathogenesis of these chronic diseases is an essential strategy to promote healthy aging. Interestingly, both aging and chronic diseases are highly associated with increased metabolic and oxidative stress, elevated chronic, low-grade inflammation, and accumulated DNA mutations as well as increased levels of its damage [2–4]. It is well established that calorie restriction delays age-associated organ disorders and increases lifespan in a wide range of species, suggesting that targeting nutrient-sensing and energy metabolism pathways may be an effective approach to delay aging process and age-related diseases.

Emerging studies showed that some phytochemicals have potential in reducing risk of chronic diseases, although they are not considered essential nutrients. Most phytochemicals are secondary plant metabolites which are present in a large variety of foods including fruit, vegetables, cereals, nuts and cocoa/chocolate as well as in beverages including juice, tea, coffee and wine. More than 1 g of phytochemicals per day is commonly ingested with the diet [5]. There are seven main categories of phytochemicals, including phenolic compounds, terpenes, betalains, organosulfides, indoles/glucosinolates/sulfur compounds, protein inhibitors and other organic acids [6] (Table 1). Phenolic compounds, also known as polyphenols, are the largest, most studied group. For example, tea flavan-3-ols (epigallocatechin gallate, EGCG), berry anthocyanins, soy isoflavones, and grape stilbenoids resveratrol are in this category [7–9]. Provitamin A carotenenes from carrots and pumpkins, limonene from oils of citrus and cherries, saponins from legumes belong to terpenes [6]. Although tocopherol (vitamin E) and omega-3 fatty acids are included in terpenes as phytochemicals [6] and may have antiaging potential [10,11], these chemicals are not be discussed in this article because these essential nutrients are well known and their biological functions have been previously reviewed. Many phytochemicals have been well studied for their abilities to prevent or treat chronic diseases, and there are several reviews in terms of the actions of phytochemicals in prevention and treatment of cancer [12,13], cardiovascular disease [14,15], obesity [16,17], diabetes [18], as well as neurological dysfunctions [19]. Recently, emerging evidence shows that some food-derived bioactive compounds have antiaging capabilities, although studies in this field are still relatively limited. In this review, we summarize several major potential antiaging phytochemicals or “phytonutrients” that have been studied in cells, animals and humans and further highlight the possible mechanisms in delaying aging process by these molecules.

2. Antiaging phytochemicals

2.1. Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic small molecule present in many plant-derived foods such as grapes, red wine [20], peanuts [21], various berries [22], cocoa [23], and hellebore [24]. About 20 years ago, there was a paradoxical epidemiological observation that French people who eat a diet high in saturated fat and drink red wine regularly have a lower incidence of coronary heart disease and longer lifespan, although it is well established that high intake of saturated fat is associated with high incidence of heart disease. Since the discovery that red wine contains significant amount of resveratrol, it has

drawn great interest in scientific community to explore its potential health benefits, leading to speculation that resveratrol might confer the health benefits of drinking red wine. For example, resveratrol in wine (up to 30 mg/L) [25] was found to inhibit the oxidation of low-density lipoproteins [26], a major step of the development of coronary heart diseases. In addition, a substantial body of evidence demonstrated that dietary resveratrol supplementation exerts beneficial effects on various other human chronic diseases and aging. It has been found that resveratrol can protect against diabetes, cancer and Alzheimer's disease [24,27,28]. In addition, resveratrol was reported to extend lifespan of yeast by 70% [9], a classic model in aging study. Further studies found that dietary intake of resveratrol can mimic calorie restriction in some ways and improve health and extend lifespan of *Caenorhabditis elegans* (*C. elegans*) [29], *Drosophila Melanogaster* (*Drosophila*) [30] and fish [31]. In concert with these findings, multiple studies using mouse models also demonstrated that dietary provision of moderate amount of resveratrol (0.01% or 0.04%) promoted health and increased lifespan of high-fat diet-induced obese male mice (C57B6) by 26 and 25%, respectively, when treatment started at about 1 year of age [32,33]. Consistently, resveratrol-treated obese mice displayed several physiological changes that are associated with healthier and longer life. Notably, resveratrol did not alter food intake or cause a significant reduction in body weight of obese mice, suggesting that the favorable effect of resveratrol on lifespan is not due to the secondary effect whereby it affects appetite or body weight. However, unlike high fat-fed mice, the same long-term resveratrol treatment (0.01% and 0.04%) did not extend lifespan of C57B6 mice fed a standard diet [33]. In addition, resveratrol (0.03%) also failed to exert such an effect in standard chow-diet-fed heterozygous mice [34,35]. Interestingly, it was also found that, even though the longevity was not increased in standard diet-fed mice, resveratrol treatment still exerted an array of other beneficial effects in mice that mimic some of physiological and transcriptional changes induced by calorie restriction, leading to overall healthier lives of these mice [33]. Based on these findings, it is very possible that resveratrol promotion of survival and extension of lifespan of the high-fat diet-fed mice is a secondary action whereby it favorably modulates high-fat feeding- or obesity-induced physiological and metabolic alterations in major organs and tissues, which could gradually lead to the pathogenesis of various chronic diseases. Indeed, resveratrol treatment reduces insulin resistance, oxidative stress, inflammation, vascular dysfunction, osteoporosis, cataracts, and the decline in motor coordination in high-fat diet-fed aged mice [33]. Specifically, resveratrol reduces the number of deaths caused by fatty changes in the liver combined with severe congestion and edema in the lungs [33], which are partially attributable to constant high-fat diet feeding. In humans, there is still no solid evidence that resveratrol intake can extend lifespan, but findings from several relatively short-term studies that resveratrol improved insulin resistance, blood flow, and various cardiovascular events, as well as decreased oxidative stress and inflammation may point to a promising antiaging action of this compound [36–39], given that cardiovascular disease is a major cause of age-related morbidity and mortality in humans. There are still several active clinical trials for assessing the potential beneficial effects of pure resveratrol or resveratrol containing products on various age-related health conditions, such as obesity, insulin resistance, impaired glucose tolerance, dyslipidaemia, inflammation, cognitive function, and Type 2 diabetes [40].

2.2. Epicatechin

(-)-epicatechin, a flavanol, can be found from various foods including apples, berries, chocolate, grapes, pears, and tea; however, cocoa bean has the highest levels of epicatechin (43,270 mg/kg fresh weight), far greater than that in the next highest epicatechin-containing product, green tea, which contains about 8,000 mg/kg [41–43]. Data from recent epidemiological studies indicate that people living on the San Blas Island, who are known to consume large amounts of cocoa beverage daily, have a considerable lower incidence of ischemic heart disease, stroke, diabetes, and a remarkably longer lifespan compared to those who live in the mainland of Panama [44,45]. Interestingly, these differences disappeared when people from San Blas Island migrated to Panama City where the amount of cocoa consumption was considerably reduced [46]. More recently, a study reported that dietary chocolate intake (4 kg chocolate/50 kg body weight) extended average life expectancy by as much as 4 years in humans [47]. While the underlying mechanism for this beneficial effect of dietary intake of cocoa products is unknown, recent human studies demonstrated that dietary intake of cocoa or chocolate can improve blood vessel function, insulin sensitivity, blood pressure, and inflammation [48], all of which could be associated with aging process. These data suggest that cocoa may exert health-promoting effects, although the specific cocoa components primarily responsible for these actions are not known.

It was recently reported that flavanol-rich cocoa beverage intake increased flow-mediated vasodilation in humans that was associated with increased plasma levels of nitric oxide, the major vasodilator in the circulation [49]. In line with this finding, another study found that intake of cocoa flavanols improved flow-mediated blood vessel dilatation in Type 2 diabetic patients [50], suggesting that the beneficial effects of cocoa on human vascular function may be at least partially attributable to flavanols such as epicatechin. While the various beneficial effects of cocoa epicatechin were primarily investigated using healthy humans or animals [44], there are only a few studies examining if epicatechin promotes lifespan.

It has been reported that long-term intake of cocoa polyphenolic extract increased lifespan in aged rats [51], and epicatechin was thought to partially contribute to this beneficial effect [52,53]. We recently reported that dietary intake of epicatechin (0.25% in drinking water) promoted survival of diabetic mice (50% mortality in diabetic control group vs. 8.4% in epicatechin group after 15 weeks of treatment). Interestingly, blood glucose, food intake and body weight gain were not altered by epicatechin treatment [54], suggesting that epicatechin promotion of mouse survival is not due to amelioration of hyperglycemia or modulation of calorie intake and metabolism, all of which are important factors affecting lifespan. Dietary intake of epicatechin (0.01–8 mmol/L in diet) also improved life span of *Drosophila* [54] and standard diet-fed aged mice (0.25% in drinking water, unpublished data). Consistently, it has been shown that dietary epicatechin extends lifespan of *C. elegans* [55]. We further found that extended lifespan by dietary epicatechin may be associated with reduced systematic inflammation markers and serum low-density lipoprotein cholesterol, increased hepatic antioxidant glutathione (GSH) concentration and total superoxide dismutase (SOD) activity, decreased circulating insulin-like growth factor-1 (IGF-1) and improved AMP-activated protein kinase- α (AMPK α) activity in the liver and skeletal muscle [54], all of which are established biomarkers that are associated with a longer and healthier lifespan.

Therefore, it is intriguing to speculate that epicatechin maybe a novel small food-derived molecule that is capable of prolonging healthy lifespan in humans.

2.3. Quercetin

Quercetin (3,3,4,5,7-pentahydroxyflavone) is a flavonoid abundant in many fruits and vegetables, including grapes, blueberries, cherries, onions, apples and broccoli [56,57]. It has been reported that dietary supplementation of quercetin at 200 mM extended the lifespan of *C. elegans* by 20%. Interestingly, recessive mutation of the gene *age-1* or *daf-2* (encoding for the IGF-1 receptor in *C. elegans*), two key players in the metabolic pathway to regulate the rate of aging, abolished the lifespan-extension effect of quercetin in *C. elegans* [58,59], suggesting that quercetin may prolong lifespan in the worms through inhibiting, either directly or indirectly, the *age-1* and *daf-2*-mediated pathways. A recent study reported that quercetin and its derivative quercetin caprylate can rejuvenate senescent fibroblasts and increase their lifespan via activating proteasome [60]. Quercetin is reported as the most potent reactive oxygen species (ROS) scavenger of ROS in the flavonoid family and quercetin has over 6 times the antioxidant capacity than the reference antioxidant trolox or vitamin C [61–63]. Moreover, quercetin possesses strong antiinflammatory capacity by inhibiting lipopolysaccharides-induced production of cytokines interleukin-1 α and tumor necrosis factor- α in immune cells [64,65]. These antioxidant and antiinflammatory properties may contribute to the antiaging effect of quercetin and its derivatives since chronic oxidative stress and inflammation are believed to play important roles in triggering the aging process (more detailed discussion on this topic is in second section of this paper).

2.4. Curcumin

Curcumin (diferuloylmethane) is a major curcuminoid that are present in 31 species of curcuma plants, including *Curcuma longa*, the rhizome of which provides the spice turmeric [66]. Curcumin is a lipophilic phenolic compound that constitutes as much as 5.4% of raw turmeric and is primarily responsible for the yellow color of turmeric [67]. Curcumin is comprised of curcumin I (94%), curcumin II (6%) and curcumin III (0.3%) [68]. Curcumin has been used for many centuries in traditional Chinese and Indian medicine as the treatment for several disorders including upset stomach, flatulence, dysentery, ulcers, jaundice, arthritis, sprains, wounds, acne and skin and eye infections [69]. Dietary intake of curcumin is considered safe, and it has been extensively studied for its potential beneficial effects on health. Accumulating evidence shows that curcumin supplementation extends lifespan of *Drosophila* [70–72] and *C. elegans* [73], and dietary intake (0.2%) of tetrahydrocurcumin, a metabolite of curcumin, significantly increased survival rate in mice [74]. Moreover, curcumin and piperine, an alkaloid present in black pepper, synergistically attenuated D-galactose-induced senescence in rats [75]. In addition, curcumin possesses antiinflammatory and antioxidant properties [76–78], which may also contribute to its potential antiaging effect.

2.5. Green tea extract and EGCG

Tea is the most consumed beverage in the world after water, and green tea extract is a rapidly growing dietary supplement in the United States because of its presumably

beneficial effects on cardiovascular disease [79], cancer [80], diabetes [81], obesity [82], and neurodegenerative disease [83]. The antiaging effect of green tea extract and its major bioactive component EGCG was not reported until recently, but all of these studies were performed with rodents or lower model organisms. Green tea extract reverses high-fat diet-induced mortality in *Drosophila* [84]. Consistently, EGCG increases lifespan of *C. elegans* maintained in normal or oxidative stress condition [85,86]. Green tea extract (80 mg/L containing 18.0% EGCG, 11.6% (–)-gallocatechin 3-*O*-gallate, 4.6% (–)-epicatechin 3-*O*-gallate, 15.0% (–)-epigallocatechin, 14.8% (+)-gallocatechin, 7.0% (–)-epicatechin, and 3.5% (+)-catechin), extends average lifespan of male C57BL/6 mice with normal diet from 801 days to 852 days [74]. Diet supplemented with the mix of 2% blueberry extract, 0.0115% EGCG and 0.3% pomegranate powder potentiated calorie restriction-induced longevity in aged male C57BL/6 mice [87]. However, the recent National Institute on Aging Interventions Testing Program reported that lifelong treatment of genetically heterogeneous male and female mice, beginning at 4 months of age, with 2% green tea extract, did not significantly extend their lifespan but diminished the risk of mid-life deaths in female mice [35]. Therefore, the antiaging effect of green tea extract and EGCG may depend on the mouse's genetic background and/or the presence of specific environmental factor such as nutritional stress. In addition, the treatment dose, duration and animal age at which the intervention begins may all affect the outcome. Studies assessing the antiaging action of green tea extracts in humans are scarce, but interestingly, a cohort study shows that routine green tea drinking significantly decreased mortality rate in Japanese women [88]. This result is somewhat consistent with the finding from above stated rodent study. However, further study is needed to determine whether green tea extract has a gender-specific effect on longevity.

2.6. Other phytochemicals/plant-extracts

Aged garlic extract, which contains s-allylcysteine, s-allylmercaptocysteine, allicin and diallosulfides, has been reported to increase lifespan and learning in mice [89]. Fisetin and butein (10 mM), two phytochemicals from fruits and Chinese lacquer tree, extended lifespan of the yeast *Saccharomyces cerevisiae* by 33% and 5%, respectively [9]. Phloridzin, a dihydrochalcone with high concentration in apple, was reported to increase lifespan of yeast through regulating SOD and Sir2, a nicotinamide adenine dinucleotide (NAD)⁺–dependent protein deacetylase [90]. Kaempferol, a flavonol present in ginkgo biloba, grapefruit, tea, broccoli and berries, also were found to extend lifespan of *C. elegans* by improving antioxidant potential and daf-16 translocation [91]. Glaucarubinone, a cytotoxic and antimalarial quassinoid known from different species of the plant family simaroubaceae, promotes mitochondria metabolism and extends lifespan of *C. elegans* [92]. Unlike many other natural compounds discussed in the paper, glaucarubinone appears to be a more potent antiaging agent, given that the effective doses are only 10–100 nM. Blueberry extracts were also found to increase longevity of *C. elegans* [93], which may be primarily ascribed to pro-anthocyanidins, a class of polyphenols or flavanols, as pure pro-anthocyanidins isolated from blueberry showed similar antiaging effect as whole blueberry extracts [93]. However, there have been no published studies, as to our knowledge, reporting the effect of these phytochemicals on aging in vertebrate animals.

3. Anti aging mechanisms of phytochemicals

3.1. ROS

While there is no unified mechanism underlying the aging process, a large body of evidence indicates that increased generation of ROS which are chemically reactive molecules with most of them containing oxygen and unpaired electrons is one of the major triggers of aging. Indeed, there is a strong correlation between chronological age and the levels of ROS generation and oxidative damage of tissues. ROS are primarily produced by mitochondrion during energy production (about 2% of total oxygen consumption was funneled to ROS) [94]. Access amount of ROS induces oxidation of fatty acids and proteins and causes oxidative damage of DNA that may lead to cellular senescence, functional alterations and pathological conditions [95,96]. Moreover, several age-related chronic diseases such as cardiovascular diseases, diabetes and cancer are associated with severe increases in oxidative stress [1,97]. Superoxide anion (O_2^-), the major form of ROS produced in mitochondrion, is quickly converted to hydrogen peroxide by two intracellular enzymes, SOD1 in cytosol and SOD2 in the matrix of mitochondria. Hydrogen peroxide is further deactivated to become water and oxygen by catalase or glutathione peroxidases (GPx) [98]. Endogenous antioxidant GSH and exogenous antioxidants including vitamins C and E are also important ROS scavengers. Cells maintain redox balance and thus its normal function through generation and destruction of ROS. However, this balance can be interrupted by environmental factors and aging that leads to an excessive bioavailability of ROS. In fact, mitochondrial integrity declines as a function of age [99], and ROS is increased but, GPx is decreased during aging [100,101]. Given that ROS-induced oxidative stress plays a key role in driving the aging process, reducing ROS is proposed as a leading strategy to delay aging and related degenerative diseases. Some food-derived phytochemicals may play a significant role in maintaining the ROS-antioxidant balance. There are three major mechanisms by which phytochemicals defend ROS, which are discussed in more detail below.

3.1.1. Phytochemicals can directly scavenge ROS—Many phytochemicals are found to have antioxidant activity capable of scavenging ROS, a property that may be primarily attributable to their phenolic hydroxyl groups. Indeed, the ROS scavenge capacity depends on the number and position of the hydroxyl group and substituent, as well as glycosylation of phytochemical molecules [102–104]. Generally, phytochemicals with more hydroxyl groups may have a stronger antioxidant capacity. For instance, kaempferol-3,7,4'-trimethylether, kaempferol-3,4'-dimethylether, kaempferol-7-neohesperidoside and kaempferol, which have one (in the five position), two, three and four free hydroxyl substitutions, respectively, have 0, 1.0, 1.6 and 2.7 times of trolox equivalent antioxidant capacity, respectively, as determined by the peroxyl radical absorbance capacity ($ORAC_{ROO\cdot}$) assay [105]. In addition, the 3',4' di-OH substitution is especially important to the antioxidant capacity, which is supported by findings that luteolin tetramethylether (without 3',4' di-OH structure), kaempferol (without 3',4' di-OH structure) and luteolin (with 3',4' di-OH structure) have 0, 2.67 and 3.57 times of trolox equivalent antioxidant capacity, respectively, although all these three compounds have four OH groups with the same basic structure flavones [105]. Moreover, substitution patterns in the B-ring and A-ring as well as the 2, 3-double bond (unsaturation) and the 4-oxo group in the C-ring also affect

antioxidant activity of phytochemicals [106,107]. Although how structural features confer the antioxidant capacity of phytochemicals is still not clear, it is generally believed that phytochemicals having at least one of these structural features such as 3',4'-*o*-dihydroxyl group, a 2,3-double bond in conjugation with a 4-keto moiety, or a 3-hydroxyl group have high cellular antioxidant activity [105–107].

3.1.2. Phytochemicals can enhance the production of antioxidants—GSH is a ubiquitous water-soluble antioxidant and also an essential cofactor for antioxidant enzymes. It exists in two forms: the sulfhydryl form or the reduced GSH and the oxidized glutathione disulfide (GSSG). GSH plays a very important role in ROS defense system as it has potent electron donating capacity. Age-associated increase in ROS accumulation is partially due to the attenuations of *de novo* GSH synthesis, GSH recycling and the GSH/GSSG ratio. Indeed, it was found that total GSH levels were reduced by 50% in the livers of aged rats [108]. In addition, both the expression and activity of hepatic glutathione reductase, a central enzyme regulating GSH/GSSG ratio by reducing GSSG back to GSH, are also declined in aging rats [109]. Similarly, SOD activity in the heart of rats is greatly reduced with aging from 101.9 units/mg protein at 4 months of age to 44.7 units/mg protein at 22 months old [110]. SOD is detoxification enzyme critical in counteracting cellular and molecular damage by converting superoxide to hydrogen peroxide, which can then be neutralized to water and oxygen by catalase. It has been shown that deletion of either cytosolic or mitochondrial SOD results in shortened lifespan in yeast [111], fruit flies [112] and mice [113,114], which is likely the result of increased oxidative stress. Consistently, over expression of peroxisomal catalase can lead to about 20% lifespan extension in mice [115]. These results provide evidence supporting the oxidative theory of aging and thereby strengthening the antioxidant system may be able to extend healthy lifespan in animals. Interestingly, our recently study showed that dietary epicatechin extension of mouse lifespan is associated with increased SOD activity in the liver of diabetic mice [54]. However, it is unclear from this study how epicatechin treatment increases hepatic SOD activity. Interestingly, studies showed that other proposed antiaging phytochemicals are also able to induce SOD activity in various species. Dietary intake of curcumin (2-mg/g medium) increased SOD activity by (32% in *Drosophila* [70]. In mice, curcumin treatment (8 mg/kg, ip injection for 5 days) can restore X-ray-reduced hepatic SOD and GSH contents [116]. Similarly, resveratrol (50 μ M) [117], EGCG (100 mg/kg, intradermal injection) [118] or quercetin (0.027% in the diet) [119] reversed oxidative stress-caused reduction of GSH and SOD levels in mice or rats. Combined, these results suggest that these antiaging compounds may facilitate healthy aging via promoting both the enzymatic (SOD) and non-enzymatic (GSH) antioxidant status, thereby attenuating the increased free radical formation and oxidative stress with aging, given that oxidative stress in aerobic cells is a major factor in aging process. However, there is possibility that maintenance of a better antioxidant capacity by these phytochemicals is a not a cause, but rather a consequence of their antiaging actions.

3.1.3. Phytochemicals regulate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway—Nrf2 is a transcriptional factor that plays a pivotal role in regulating various Phase II antioxidant enzymes and thereby detoxification process, including hemeoxygenase-1 (HO-1), NAD(P) H:quinoneoxidoreductase (NQO1) and GSH S-

transferase. Under normal condition, Nrf2 is associated with a Kelch-like ECH-associated protein1 (Keap-1) in cytoplasm. However, Nrf2 inducers such as ROS or electrophilic compounds cause dissociation of the Nrf2/Keap-1 complex by directly modifying the thiol group of Nrf2 and/or through kinase [*e.g.*, protein kinase C (PKC)]-dependent phosphorylation of Nrf2 Ser40 [120]. Dissociated Nrf2 then translocates into the nucleus where it induces the expression of several detoxifying enzymes, including SOD, GSH, NQO1 and HO-1, by binding to the antioxidant-response elements (AREs) in the promoter regions of the genes of these enzymes [121]. Thus, the Nrf2/ARE pathway plays an important role in transcriptional activation of various cellular defense mechanisms. Indeed, in Nrf2 gene-knockout mice, both basal and inducible levels of Phase II enzyme genes and proteins are greatly reduced that led to the increased sensitivity to chemical oxidants [122]. Therefore, search for agents that can activate Nrf2 may be an excellent strategy in protecting cells against oxidative stress and other environmental insults. The Keap1/Nrf2/ARE pathway can be activated by PKC, extracellular signal-regulated protein kinase (ERK), phosphatidylinositol-3-kinase (PI3K) and p38 MAP Kinase (MAPK) via phosphorylation of Nrf2 [123,124], suggesting that Nrf2 is not controlled only by a specific regulatory pathway. Some bioactive compounds, including sulforaphane [125,126], EGCG [127] and luteolin [128], have been shown to activate Nrf2 and induce HO1 and NQO1 production via the ERK- or PI3K-dependent mechanism. It was shown that activation of the Keap1/Nrf2/ARE pathway by curcumin is mediated via stimulation of the PKC [129] or MAPK [130] signaling pathway in human cells. A recent study reported that resveratrol promotes the survival of cerebellar granule neurons in rats by enhancing DNA-binding activity of Nrf2 and the expression of its downstream cytoprotective proteins NQO1 and SOD and also restoring the ethanol-reduced GSH by activation of the AMP-activated protein kinase (AMPK)/NAD-dependent deacetylase sirtuin-1 SIRT1 pathway [131,132]. Dietary intake of epicatechin was also shown to enhance Nrf2 protein expression and HO-1 accumulation in the nuclei of cortical neurons in mice [133]. This epicatechin action is not tissue specific, as it was also found that treatment of hepatocytes with epicatechin (10 μ M) activates PI3K/Akt and ERK signaling, leading to Nrf2 phosphorylation, nuclear translocation and transcriptional activity [134]. These results suggest that the observed antioxidant activity or reduction of oxidative stress by these compounds *in vivo* may not entirely or even primarily due to their direct scavenging ROS. Rather, targeting the Nrf2-mediated pathway by these phenolic compounds could play a more important role in mediating the antioxidant action *in vivo*, given that many of polyphenolic phytochemicals have a poor bioavailability, with achievable circulating concentrations typically being less than 5 μ M following dietary supplementation in both humans and animals [28,135]. However, the significant ROS scavenging activity can only be achieved *in vitro* at doses that are far beyond those attainable *in vivo* through dietary means.

3.2. Activation of AMPK and SIRT1 by phytochemicals

AMPK, an energy-sensing molecule highly conserved from yeast to mammals, is increasingly recognized as a master regulator of whole body energy homeostasis [136]. This heterotrimeric protein kinase, composed of a catalytic subunit (AMPK α) and two regulatory subunits (β and γ), senses low cellular energy levels by monitoring changes in the AMP:ATP ratio. AMP binding to the γ subunit induces a conformational change that allows

AMPK α to be phosphorylated at its threonine residue (Thr172) by the AMPK-activating protein kinase (LKB1). At whole body level, AMPK integrates stress responses, nutrient and hormonal signals to the control of food intake, energy expenditure and substrate utilization. At cellular level, activated AMPK inhibits hepatic gluconeogenesis [137], promotes fatty acid oxidation and insulin sensitivity [136] and regulates mitochondrial biogenesis [138]. To do so, AMPK α directly regulates the activity of acetyl-CoA carboxylase (ACC) and the peroxisome proliferator-activated receptor gamma coactivator-1- α (PGC-1 α) [139–141], two of the most important metabolic regulators. Specifically, AMPK α phosphorylates and inhibits the activity of ACC, the rate limiting enzyme for fatty acid synthesis [139,140]. Meanwhile, it phosphorylates and activates PGC-1 α , which controls the expression of many genes related to lipid oxidation and mitochondrial biogenesis [142,143]. Recently, several lines of evidence demonstrate that activation of AMPK increases lifespan and delays age-associated functional decline in various species [144–147]. Conversely, reduction of AMPK activity leads to age-associated dysfunction of skeletal muscle [148,149], blood vessel [150] and the liver [151,152]. Therefore, AMPK is emerging as an attractive target for developing strategies to extend healthy lifespan. Interestingly, all of above-discussed antiaging phytochemicals (resveratrol, EC, EGCG, quercetin and curcumin) have been found to activate AMPK. For instance, recent studies found that dietary resveratrol prevents Alzheimer's markers and increases lifespan of mice and *C. elegans* via activating the AMPK and SIRT1 pathways [9,32,153]. Resveratrol prevents renal lipotoxicity and inhibits mesangial cell glucotoxicity through activating AMPK-SIRT1-PGC1 α axis in *db/db* mice [154]. Results from a more recent study showed that the beneficial effects of resveratrol on metabolism including improved insulin sensitivity, glucose tolerance, mitochondrial biogenesis and physical endurance were lost in AMPK-deficient mice [155], further confirming the critical role of AMPK in mediating the various beneficial actions of this compound. Similarly, beneficial actions of quercetin [156,157], EGCG [158], curcumin [159,160] and epicatechin [54] were also associated with activation of the AMPK pathway, suggesting that activation of AMPK may serve as a central mechanism for many observed biological effects of phytochemicals, even though their chemical structures are somewhat different. However, how these compounds are able to activate AMPK is an intriguing question.

Sirtuins are enzymes that function as NAD⁺-dependent deacetylases and ribosyltransferases evolutionarily conserved from *Escherichia coli* to humans [161]. Sir2, one of sirtuins in *S. cerevisiae* yeast and *C. elegans*, mediate the life-extending effect of calorie restriction by increasing the ratio of NAD to its reduced form NADH [162,163]. In concert with these findings in microorganisms and invertebrate animals, the protein expression of SIRT1, homolog of the Sir2 gene in yeasts and worms and one of seven sirtuin members in vertebrate animals, is also increased in energy metabolism tissues in life-prolonged rats by calorie restriction [164]. Therefore, in addition to AMPK, SIRT1 also serves as a fuel-sensing molecule. Actually, recent studies show that these two enzymes share many common target molecules, suggesting that they may cross talk or on the same regulatory pathway in certain circumstances, which is not well understood. For instance, sirtuins promote deacetylation of several downstream molecules, including Ku70 [165] and p53 [166], two critical molecules for initiation of apoptosis, and LKB1 [167], a key molecule

upstream of AMPK. In this case, SIRT1 may be located at the upstream of AMPK. In addition, SIRT1 was shown to regulate the IGF-1 pathway through modulation of UCP2 expression [168,169]. SIRT1 also interacts with the forkhead/winged helix box gene, group O (FOXO), a crucial regulator of the IGF-1 signaling pathway. Specifically, SIRT1 was found to directly deacetylate and thereby activate FOXO1 [170] and FOXO3a [171], which play important roles in transducing insulin signaling downstream of serine/threonine protein kinase (Akt) [170]. In addition, Sir2 can directly bind to FOXO1-binding sites of some FOXO-targeted genes, such as antioxidant gene SOD, to enhance its expression through a deacetylase-activity-dependent manner in mammalian cells [170]. This finding suggest that, in addition to its function as a sensor of the energy status of cells, activation of sir2 and possibly other SIRTs may increase ability of scavenging ROS and therefore reduce oxidative stress.

Resveratrol was reported to directly activate SIRT1, which lead to improved mitochondrial biogenesis, energy metabolism, insulin sensitivity and survival of high-fat-fed mice [9]. However, whether these benefits of this compound are directly mediated via SIRT1 or through AMPK-mediated mechanism is still controversial. A recent study showed that mice fed a resveratrol (0.04%)-supplemented diet displayed the increased mitochondrial biogenesis and AMPK activation, whereas SIRT1-knockout mice displayed none of these benefits demonstrated in wild-type mice given resveratrol, suggesting that SIRT1 plays a critical role for AMPK activation and the subsequent beneficial effects of resveratrol on mitochondrial function [172]. However, results from some more recent studies indicated that resveratrol activates SIRT1 via stimulating the AMPK pathway, which is supported by observations that 1) resveratrol consistently increased SIRT1 and PGC-1 α activity in mice [155,173] and SIRT1 activates PGC-1 α , one of the major targets of AMPK signaling; 2) resveratrol cannot activate SIRT1 with native substrates *in vitro*, although it activates SIRT1 *in vivo* and deacetylate fluorophore-tagged substrates *in vitro* [174,175]; and 3) the abilities of resveratrol to activate SIRT1 and exert the beneficial metabolic effects in wild-type mice are lost in AMPK-deficient mice [155]. Interestingly, resveratrol treatment (oral gavage at 100 mg/kg body weight) was recently found to activate SIRT1 via cAMP phosphodiesterases-cAMP-Epac1-AMPK pathway both in C2C12 myotubes (50 μ M) and in mice [176].

While mechanistic studies on the antiaging effects of other phytochemicals are limited, a recent study shows that quercetin intake may improve exercise performance, skeletal muscle and brain function, which are associated with activation of the SIRT1/PGC-1 α pathway [156,157]. Similarly, EGCG was found to suppress oxidative stress that is mediated via the SIRT1/PGC-1 α pathway [158]. However, several other studies found that EGCG and quercetin did not activate SIRT1 but inhibit SIRT1 in cellular system [177–179]. This may be due to the instability of EGCG and quercetin because EGCG becomes oxidized and subsequently produce ROS in the medium and quercetin and its metabolite generates SIRT1-inhibitory metabolites [177]. Several other phytochemicals including piceatannol, fisetin and butein were reported to activate SIRT1 and subsequently lead to prolonged lifespan of *C. elegans* [9]. However, how they activate SIRT1 is unclear.

3.3. Growth hormone (GH)/IGF-1 pathway

The GH/IGF-1 axis, evolutionarily conserved from invertebrates to mammals, has been extensively investigated in the regulation of the aging process [180,181]. IGF-1 axis directly regulates its downstream molecules such as PI3K, AKT and FOXO. It also interacts with other aging-relevant signals including SIRT1, AMPK and mammalian target of rapamycin (mTOR) signaling [182]. While the detailed mechanisms by which IGF-I regulates lifespan is not completely clear, it is well recognized that circulating IGF-I levels are inversely associated with lifespan in mammals [181,183]. Our study indicated that serum IGF-I levels were reduced by epicatechin treatment in diabetic mice without alternation in hepatic IGF-1mRNA expression [54]. One possible interpretation is that epicatechin may affect the binding of IGF-I and its carrier IGF binding proteins (IGFBPs) because as much as 99% of IGF-I in circulation is bound to one of the six IGFBPs [184], which can prevent the proteolysis of IGF-I, and therefore extend its half-life [185]. Another possibility is that epicatechin reduces IGF-I level through activation of AMPK, which inhibits IGF-I signaling and its protein synthesis through the phosphorylation of insulin receptor substrate-1 [186]. Interestingly, curcumin [187], resveratrol [188] and EGCG [189] inhibit IGF-1/IGF-1 receptor axis, although the underlying mechanism for this action by these phytochemicals are unknown. Nevertheless, these studies provide further evidence that epicatechin, curcumin, resveratrol and EGCG may be food-derived antiaging agents, given the important role of IGF-1 in regulating lifespan of various model organisms.

3.4. Antiinflammation

A large body of epidemiologic studies has shown that low-grade chronic inflammation plays a crucial role in the process of aging and age-related diseases in older adults. Chronic proinflammatory markers including IL-6, C-reactive protein (CRP) and TNF- α are consistently elevated with age in the absence of acute infection or other physiological stress [190], which are largely the results of chronically elevated levels of ROS [191], endocrinosenescence (reduction of growth hormone and dehydroepiandrosterone) [192] and the gradual decline in immune function (accumulation of proinflammatory CD8+/CD28- T cells, the decrease of naive T cells (CD95-) and the marked shrinkage of T cell repertoire) [193,194]. Consequently, the sustained increases in these proinflammatory molecules impair the function and integrity of various tissues and organs and thus accelerate aging and aging-related chronic diseases, although this increase is still in the sub-acute range [195]. Interestingly, calorie restriction significantly attenuates the increase of these proinflammatory markers while extending lifespan [195,196], suggesting that antiinflammatory agents may have potential to extend healthy lifespan. Resveratrol [197], EGCG [198], curcumin [197], quercetin [199] and epicatechin [54] have been shown to lower proinflammatory markers and decrease the adhesion of monocytes to other cells via inhibiting nuclear factor (NF)-kappa (NF-kB), the master regulator of the transcription of various proinflammatory molecules. For instance, studies found that EGCG decreases the adhesion of monocytes to vascular endothelial cells both *in vivo* and *in vitro*, which is accompanied by attenuation of proinflammatory chemokines such as IL-8 and monocyte chemotactic protein-1 (MCP-1), and vascular adhesion molecule-1 and intercellular adhesion molecule-1 through inhibiting NF-kB [198]. Dietary intake of epicatechin can also

diminish serum proinflammatory markers CRP, IL-1 and MCP-1 in mice while promoting their health and survival rate [54].

3.5. Autophagy

Autophagy is an evolutionarily conserved mechanism of lysosomal proteolysis in eukaryotes in response to nutrient deprivation. Apart from providing the starving cell with energy from degraded self-components, autophagy removes harmful proteins and damaged mitochondria. Therefore, autophagy is an essential cellular homeostasis mechanism. Declined autophagy, occurring during aging and the pathogenesis of age-related chronic diseases, leads to increased ROS production, inflammation, cell death, neurodegeneration, cancer, compromised immune response, diabetes and elevated aging process [200]. On the contrary, increased autophagy leads to longevity as shown in calorie-restricted mice [201,202], which may be mediated through down-regulating mTOR, the central autophagy regulator [196], via regulating multiple upstream molecules including SIRT1 and PI3K/AKT [182,203]. For instance, SIRT1 deficiency results in elevated mTOR signaling, and activation of SIRT1 by resveratrol reduces mTOR activity, whereas inhibition of SIRT1 using nicotinamide enhances mTOR signaling [196]. These findings suggest that maintaining functional autophagy may delay aging. Interestingly, emerging evidence demonstrated that resveratrol [204], EGCG [205], epicatechin [206], quercetin [207] and curcumin [208,209] can induce autophagy in various types of cells via blocking mTOR activity. While the results from these *in vitro* studies may provide further evidence suggesting the antiaging action of these phytochemicals, it is presently unknown whether these compounds can activate autophagy *in vivo*.

4. Conclusions

Growing evidence shows that some phytochemicals (resveratrol, green tea extract, EGCG, epicatechin, quercetin and curcumin) present in the foods are antiaging molecules, and dietary intake of these compounds can promote health and extend lifespan in various animal models via multiple mechanisms, including reducing oxidative stress, suppressing low-grade chronic inflammation, inducing autophagy, as well as regulating several important molecules involved in promoting mitochondrial function and energy homeostasis (Fig. 1). Interestingly, it seems that these mechanisms mediating the proposed antiaging effects of phytochemicals are shared by calorie restriction, suggesting that nutritional intervention with these food components may be an alternative and more feasible strategy to promote healthy aging in humans. However, some of these compounds failed to extend lifespan of either normal diet-fed homozygous or genetically heterozygous mice. Therefore, it is possible that these agents may primarily act to correct the alterations of some metabolic pathways caused by chronically excessive calorie intake, thereby delaying the onset of age-related diseases that consequently lead to a healthier and longer lifespan. Alternatively, the antiaging potential of one or several of these molecules may depend on mouse genetic background, which may differ in energy regulatory mechanisms, resulting in different adaptation to nutritional or environmental stress and susceptibility to the pathogenesis of chronic diseases. If this is true, studies to determine these complex relationships and interactions among genetic background, nutrients and potential antiaging compounds may identify unique

modifier genes or metabolic pathways targeted by these phytochemicals and therefore could bring further insight into the mechanism of the antiaging action of these compounds.

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Abbreviations

ACC	acetyl-CoA carboxylase
AKT	serine/threonine protein kinase
AMPK	AMP-activated protein kinase
AREs	antioxidant-response elements
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
CRP	C-reactive protein
<i>Drosophila</i>	<i>Drosophila Melanogaster</i>
EGCG	epigallocatechin gallate
ERK	extracellular signal-regulated protein kinase
FOXO	forkhead/winged helix box gene, group O
GH	growth hormone
GPx	glutathione peroxidases
GSH	glutathione
GSSH	oxidized glutathione
HO-1	hemeoxygenase-1
IGF-1	insulin-like growth factor-1
IGFBPs	IGF binding proteins
IL	interleukin
LKB1	AMPK-activating protein kinase
Keap1	Kelch-like ECH-associated protein1
MAPK	p38 MAP Kinase
MCP-1	monocyte chemotactic protein-1
mTOR	mammalian target of rapamycin
NF-kB	nuclear factor (NF)-kappaB
NQO1	NAD(P)H:quinoneoxidoreductase
Nrf2	nuclear factor erythroid 2-related factor

ORAC	oxygen radical absorbance capacity
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator-1- α
PI3K	phosphatidylinositol-3-kinase
PKC	protein kinase C
ROS	reactive oxygen species
SOD	superoxide dismutase
TNFα	tumor necrosis factor- α

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Fig. 1.

Hypothetical cellular and molecular mechanisms of the antiaging effects of phytochemicals. Phytochemicals extend lifespan through reducing oxidative stress, suppressing low-grade chronic inflammation and inducing autophagy. As shown, there are cross talk and interaction at both cellular and molecular levels between these events.

Table 1

Classification of phytochemicals and their food sources

Category			Chemical	Food/Plant resources	
Phenolic compounds	Natural monophenols		Rosemarinol	Rosemary	
	Flavonoids (polyphenols)	Flavonols	Quercetin	Onions, tea, wine, apples	
			Kaempferol	Tea, strawberries, gooseberries	
			Fisetin	Tea, grape, onions	
			Myricetin	Grapes, red wine, berries	
		Flavanones	Naringenin	Citrus fruits	
		Flavones	Apigenin	Chamomile, celery, parsley	
			Luteolin	Beets, artichokes, celery	
		Flavan-3-ols	Catechin	white tea, green tea, black tea	
			(-)-Epicatechin	Tea, cocoa, grape	
			EGCG	green tea	
			Theaflavin	Black tea	
		Anthocyanins/Anthocyanidins	Pelargonidin	Bilberry, raspberry, strawberry	
			Delphinidin	Bilberry, blueberry, eggplant	
		Isoflavones	Genistein	Soy, red clover, peanuts	
		Chalconoids	Butein	Rhus verniciflua, dalbergia odorifera	
			Phlorizin	Pear, apple, cherry	
		Phenolic acids		Ellagic acid	Walnuts, strawberries, cranberries
				Curcumin	Turmeric, mustard
	Hydroxycinnamic acids		Caffeic acid	Burdock, hawthorn, artichoke	
	Lignans		Matairesinol	Flax seed, sesame seed, rye bran	
	Tyrosol esters		Tyrosol	olive oil	
	Stilbenoids		Resveratrol	Grape, wine, nuts	
	Alkylresorcinols			Wheat, rye and barley	
Terpenes	Carotenoids	Carotenes	Carotene	Carrots, pumpkins, maize,	
		Xanthophylls	Lutein	Spinach, turnip greens, lettuce	
	Monoterpene	Limonene		Oils of citrus, cherries, spearmint	
	Saponins			Soybeans, beans, other legumes	
	Lipids	Phytosterols	Sitosterol	Avocados, almonds, wheat germ	
	Triterpenoid	Glaucarubinone		Simaroubaceae plants	
Betalains	Betacyanins	Betanin		Beets, chard	
	Betaxanthins	Indicaxanthin		Beets, sicilian prickly pear	
Organosulfides	Polysulfides	Allyl methyl trisulfide		Garlic, onions, leeks	
Indoles, glucosinolates/sulfur compounds	Indole-3-carbinol			Cabbage, kale, brussels sprouts	
	Sulforaphane			Broccoli, cauliflower, brussels sprouts	
	Allicin			Garlic	

Category	Chemical	Food/Plant resources
Protein inhibitors	Protease inhibitors	Soy, legumes
Other organic acids	Oxalic acid	Orange, spinach, rhubarb
	Anacardic acid	Cashews, mangoes