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Polyphenols and Aging

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Abstract

Age-associated changes within an individual are inherently complex and occur at multiple levels of organismal function. The overall decline in function of various tissues is known to play a key role in both aging and the complex etiology of certain age-associated diseases such as Alzheimer's disease (AD) and cancer. Continuing research highlights the dynamic capacity of polyphenols to protect against age-associated disorders through a variety of important mechanisms. Numerous lines of evidence suggest that dietary polyphenols such as resveratrol, (−)-epigallocatechin-3-gallate (EGCG), and curcumin have the capacity to mitigate age-associated cellular damage induced via metabolic production of reactive oxygen species (ROS). However, recently acquired evidence also demonstrates a likely role for these polyphenols as anticancer agents capable of preventing formation of new vasculature in neoplastic tissues. Polyphenols have also been shown to possess other anticancer properties such as specific cell-signaling actions that may stimulate the activity of the regulatory protein SIRT1. Additionally, polyphenolic compounds have demonstrated their inhibitory effects against chronic vascular inflammation associated with atherosclerosis. These increasingly well-documented results have begun to provide a basis for considering the use of polyphenols in the development of novel therapies for certain human diseases. And while the mechanisms by which these effects occur are yet to be fully understood, it is evident that further investigation may yield a potential use for polyphenols as pharmacological interventions against specific age-associated diseases.

Keywords

Polyphenols; aging; SIRT1; angiogenesis; cancer; Alzheimer's disease; neuroprotection

Introduction

Aging is a highly complex process marked by succeeding events that promote alterations in the normal functioning of an individual organism over time [1–5]. This change can be observed at all levels of organismal function beginning with fine scale alterations of cellular protein production and changing configurations of cellular macrofeatures such as the presence and distribution of signal receptors [5–6]. Aging changes occur at larger scales also and include a generalized decrease in the efficiency of function in entire organs or systems which may in turn contribute to the development of disease [7]. Importantly, various alterations in the physiochemical environments of cells found in certain tissues such as the hippocampus have

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been shown to play a major role in the etiology of disease states like Parkinson's disease and Alzheimer's disease (AD) [8–9]. However, none of these potential changes are guaranteed events but rather age-associated risk factors in which the true etiology is usually multi-factorial and inherently complex. These risk factors associated with aging, such as atherosclerosis, AD, and cancer, have been shown to result from fundamental biological processes [9].

Recently acquired evidence has pointed to a series of phenomena that are thought to contribute to the aging process. Chief among these findings is that the primary contribution to aging is a result of oxidative stress experienced during normal metabolism [10]. Oxidative stress that results from normal metabolism is often exacerbated by a wide variety of environmental insults including metabolic processing of ingested food products [11], exposure to environmental toxins [12], and infection [13]. While appropriate function of an organism requires metabolic reorganization of various chemical building blocks, there is also a detrimental effect that results from the accumulated byproducts of those processes. The highly reactive byproducts generated during metabolism, such as Reactive Oxygen Species (ROS) and to a lesser extent Reactive Nitrogen Species (RNS), have the capacity to rapidly oxidize, and thus damage, many classes of molecules [14]. Cellular machinery exists to deal with limited exposure to these types of reactive compounds and they may even benefit cell survival to a certain degree [15]. But it is the accumulated damage to important cellular components and structures that partially contribute to senescence at the cellular level. ROS have been shown to greatly contribute to age-related changes at the subcellular level through the destruction of all types of organic molecules including proteins, lipids, carbohydrates, and DNA [14,16]. Because the formation of ROS primarily occurs as a direct result of energy production processes like electron transport, nearly all tissues of the body will inevitably be exposed to the damaging effects. However, the effects of ROS can be particularly evident in certain tissues such as brain. Because the brain utilizes as much as 20% more oxygen than other tissues also undergoing mitochondrial respiration, the potential for ROS exposure is concomitantly increased. In addition, brain tissue-specific reactions such as those involving the enzyme monoamine oxidase also contribute to comparatively high levels of ROS like H_2O_2 [16]. Taken together these findings indicate that there are tissue-specific effects due to ROS that must be accounted for in terms of how the total organism is affected by the repeated stress that results from oxidative damage. Regardless of location or specific contribution to disease, oxidative stress probably constitutes the most important overall mechanism for cellular aging.

While oxidative stress plays an important role in the aging process, chronic inflammation has also been implicated as a major contributing factor for cellular senescence and is known to be important in the etiology of many diseases including cardiovascular disease, arthritis, autoimmune dysfunction, pulmonary dysfunction, Alzheimer‘s disease and cancer [17–20]. Because the diseases linked to chronic inflammation are highly varied, much attention has been placed on the role of inflammation as it relates to aging. And since the passage of time is a necessity for any condition to be termed ‘chronic’, there is an intrinsic relationship between the damaging effects of long-term inflammation and aging itself. One of the most important approaches to addressing the relationship between chronic inflammation and disease states centers around the study of pivotal proteins that mediate the action of multiple types of cells [21]. One such important protein is NF-κB which is implicated as a key transcription factor in the development of tumors, tumor metastasis, angiogenesis (an essential component for tumor growth), and chronic inflammation [21–22]. Such proteins have been targeted as ideal candidates for anticancer therapies due to the pivotal role they play in the development of disease [21].

Another intrinsic age-related state observed in numerous organisms pertains to the buildup of toxins within cells and organs [23–24]. The source of such toxins is highly variable and constitutes a significant factor in the etiology of some age-associated diseases. In the case of

AD these toxins may arise from the sequential action of β-and γ-secretase which modify endogenous proteins like amyloid precursor protein (APP) [16]. Modification of APP by these enzymes contributes to the formation of beta amyloid (Aβ) peptides known to play a role in the etiology of AD [25]. Another example in relation to AD involves the build-up of Ca^{2+} in aged neurons which is considered a possible factor in the development of neuronal dysfunction and cellular death [26]. And while a great deal of analysis has contributed to our understanding of the role of accumulated toxins in relation to disease, it is important to emphasize the interrelatedness of the previously discussed factors such as oxidative stress and chronic inflammation. There is a clear relationship that has emerged which indicates each may give rise to or enhance the effect of the others.

In terms of cardiovascular disease, analysis has revealed a similar convergence of factors involving oxidative stress, chronic inflammation, and toxin build-up. And even though the etiology of cardiovascular disease involves different combinations of genetic and environmental factors from those found in AD and cancer, the clinical features display a high degree of continuity. One noteworthy and well-documented exception to the traditional etiology of cardiovascular disease is that of the “French paradox”. In certain populations it has been observed that high fat diets usually leading to cardiovascular disease have diminished health risks when polyphenolic consumption is also high [27]. While these epidemiological studies presented compelling health benefits for polyphenol consumption, there was little information regarding the molecular basis for such actions. It should also be emphasized that in order to achieve a wide range of health benefits, polyphenols must act at multiple levels of biological regulation. For example *in vivo* evidence shows that attenuation of myocardial reperfusion injury is achieved with treatment by (−)-epigallocatechin-3-gallate (EGCG) [28–29]. These effects are mediated through decreased activity of the transcription factor NF-κB. By downregulating this key protein, EGCG is able to modify and thus diminish the normally pro-inflammatory response elicited by activation of NF-κB signaling pathways during reperfusion [30]. In addition the anti-inflammatory responses produced by EGCG reduced the *in vitro* activation of STAT-1, another important transcription factor in the regulation of cell response to inflammatory cytokines [31]. When taken together, the deactivation of these two pro-inflammatory cell-signaling pathways represent an important mechanistic explanation for the anti-inflammatory action of EGCG. EGCG has also been shown to have *in vitro* effects against the production of inducible nitric oxide synthase (iNOS), an important ROS-generating enzyme. iNOS is produced as part of a normal inflammatory response in the presence of aggravating bacterial endotoxin or cytokines [32]. The production of iNOS and subsequent increased toxic build up of NO, a powerful ROS, results in oxidative damage that accumulates with age. However, the oxidative damage associated with such inflammation is inhibited by EGCG due to the reduced activity of iNOS and the decreased production of large quantities of NO within susceptible tissues such as the heart and brain [32].

The relationship between the potential health benefits of polyphenols and the biological processes associated with cancer have also been extensively investigated. Polyphenols have been pursued as a possible line of therapeutic interventions against many diseases including cancer because of their ability to modulate master regulatory molecules involved in various disease states in the same way that NF-κB is subject to the effects of polyphenols in the cardiovascular system. Polyphenols thus have the capacity to modulate the associated biological states of oxidative stress and chronic inflammation that are found to contribute to the etiology of some cancers via their regulatory effects on such master molecules. For example, Benitez et al. have suggested that the polyphenol resveratrol may exert antiproliferative and apoptotic effects by mediating the inhibition of NF-κB during *in vitro* studies of human prostate cancer cells [33]. Furthermore, using an *in vivo* model of prostate cancer, recent investigations have shown that oral administration of green tea polyphenols to mice decreased the production of NF-κB among other regulatory molecules [34]. Because downregulation of NF-κB is known

to trigger apoptotic and antiproliferative effects, the evidence for the effectiveness of polyphenols in the prevention of cancer is increasingly apparent. NF-κB is known to initiate tissue-specific stress responses throughout the body and it is clear that it plays a pivotal role in generating an appropriate response to molecular insults under normal conditions. At the same time it would be difficult to overstate its importance as a mediator of numerous pathological states. In addition polyphenols have a role in suppression of inflammation related to cancer development. Studies using mouse models with UVB-induced skin tumors have shown that green tea polyphenols such as EGCG were capable of reducing the inflammation markers cyclooxygenase-2 and prostaglandin-E2 associated with tumor development [35]. These results further widen our understanding of the effective capacity of polyphenols in their ability to render health benefits and indicate the need for additional analysis.

In terms of our ability to effectively address diseases and risk-factors associated with aging, much attention has been given to the therapeutic effects of dietary polyphenols which have been shown to mitigate age-associated phenomena such as oxidative stress, chronic inflammation, and toxin accumulation [36–38]. Because there is a multitude of diseases and disorders resulting from such factors of aging, the need to achieve greater understanding of the role that polyphenols might have in treating them is critical to understanding common conditions associated with aging. Polyphenols are a class of phytoalexins found in the tissues of a widespread range of plants. As antioxidants, polyphenols are normally produced by plants for their antibiotic and antifungal properties [39]. While there may be as many as 10,000 naturally occurring polyphenols, only a few plants containing an abundance of these compounds are common sources of food in the human diet. These include the skin of red grapes, peanuts, green tea, black tea, and certain plant-derived products like curcumin, the main component of turmeric [40]. Even though most research has tended to focus on the major polyphenol present in a given plant or food product, recent indications are that there may be greater complexity among the polyphenols present in these sources than once thought [41]. This new finding may point to the possibility that there are numerous derivatives of major polyphenolic compounds whose properties are more suitable for addressing different disease states in humans. Or perhaps that a combination of these polyphenols given in specific proportions may hold therapeutic benefits not yet realized. Nevertheless the ability of polyphenolic compounds to attenuate the effects of a wide variety of human disease models is well documented. However, the development of new and effective treatments depends strongly on a greater understanding of the bioavailability and metabolism of polyphenols [41].

Among the examples of polyphenolic compounds well-studied for their antioxidant properties are resveratrol (3–4′-5-trihydroxy stilbene), curcumin, quercetin, and (−)-epigallocatechin-3-gallate (EGCG). In addition to these common polyphenols there exists a wide range of derivatives and related compounds also having antioxidant or anti-inflammatory effects. The purpose of this review will be to consider the relationship among these major polyphenols and their ability to combat risk factors associated with aging.

Bioavailability of Polyphenols

Bioavailability is a key factor in the description of polyphenols and is an essential aspect for understanding the role they might play in addressing human disease. But the role of polyphenols in the etiology and treatment of disease is hardly definitive and some have challenged the very nature of polyphenolic mechanisms of action within the body [42]. And though the mechanism of action is disputed by certain investigators, there remains a clear recognition based on aforementioned *in vitro*, *in vivo*, and epidemiologic studies that suggest polyphenols possess beneficial health effects. However, in order to maintain proper perspective on the emerging potential benefits to human health there must be continued recognition of the true implication of *in vitro* versus *in vivo* studies.

While some data exists pertaining to the bioavailability of certain polyphenols, other examples remain unanalyzed for their metabolic properties. This situation is often complicated by a wide range of additional factors. For example, all polyphenols possess tissue-specific effects, and differences in age-related absorptive capacity. They also display variable formation of conjugates by the liver and modification by the small intestine that may be affected by factors such as age [43]. In terms of application to human health one must also consider dose size as a central aspect to understanding how these compounds might be beneficial [43]. However, not all studies regarding the use of polyphenols address these factors. While some attempts have been made on the part of investigators to make reasonable assessments about *in vivo* performance of polyphenols based on *in vitro* studies, there has not been a sustained effort to systematically characterize the true extent of all confounding factors and the degree to which they influence polyphenol metabolism [44]. The fact remains that certain investigative approaches have been limited to *in vitro* analyses while others have sought to use appropriate organismal models for these studies. The need to better understand overall aspects of polyphenol metabolism is essential and must be addressed in detail if polyphenols are to be considered as a group of therapeutic agents.

In terms of the metabolism and bioavailability of resveratrol, it has been noted that resveratrol interacts well with Phase I and II enzymes which catalyze oxidation and conjugation reactions to improve water solubility while at the same time decreasing the *in vivo* half-life of resveratrol to 8–14 minutes and thus reducing its bioavailability [45]. Other *in vivo* studies have shown that resveratrol is detectable in blood plasma up to 72h after oral administration [46] and have lead to the possibility that resveratrol may be held in reserve via binding to specific blood proteins such as albumin [47]. These data suggest a complicated interplay between the biological modifications of parent compounds in order to increase solubility while at the same time allowing for the sequestration of polyphenols by constitutively produced molecules in the blood. It must therefore be understood that the evaluation of resveratrol as a possible therapeutic agent requires detailed analysis regarding these confounding factors.

In further consideration of the effects of such compounds investigators have discovered that tissue-specific delivery of polyphenols gives rise to alternative outcomes in terms of cellular response. Additional recent evidence shows that EGCG may be involved in the down-regulation of NO production in 4T1 murine mammary carcinoma cells under *in vitro* conditions and thus may indicate a means for limiting metastasis [48–49]. Due to the poor prognosis of patients having undergone metastatic transformation the search for therapies to alleviate such complications is extremely important. And this work has shown that 4T1 carcinoma cells exposed to EGCG display decreased expression of eNOS, leading to the conclusion that EGCG down-regulates NO production and thus contributes to the decreased metastatic capacity of these cancerous cells. It is important to note that EGCG also possesses the ability to block the migration of 4T1 cells by reducing cellular cGMP levels [49]. Since cGMP is known to contribute to the migratory capacity of these cells then the cellular decrease in cGMP levels by administration of EGCG may well represent a therapeutic target for the prevention of metastasis [49–50]. Taken together with the above results, these studies show that EGCG is capable of exerting varying effects. While EGCG may stimulate NO production in the endothelium thus contributing to the prevention of atherosclerosis, it also has the capacity to decrease NO production in 4T1 carcinoma cells thus preventing metastasis. The seemingly selective action of EGCG against abnormal cells and disease processes are important attributes and constitute compelling evidence to continue seeking new treatment possibilities to exploit these properties. And even though the variations in tissue-specific effects of polyphenols are not completely understood, it is evident that the variable properties of polyphenols stem, in part, from tissue-specific biological modification and sequestration. As such, the individual driving forces for polyphenol bioavailability must be considered and at the same time the clinical significance should not be underestimated.

As noted above, polyphenols have been demonstrated to be powerful antioxidants, metal chelators, and cell signaling molecules. Nevertheless the *in vitro* studies on the cellular effects of these substances have often focused on the unmodified parent compound as present in the extract source without regard to potential physiological modifications. However, recent evidence shows that striking differences may occur in terms of the biological actions of major polyphenols once they are metabolized [42,51]. While many polyphenols have been studied extensively and their biologically relevant analogues recognized, there is a marked lack of systematic analysis concerning the metabolic modifications that occur upon ingestion. However, quercetin is a well-studied example of a polyphenolic compound that may give an indication as to the metabolic fate of other polyphenols. Quercetin is believed to display pharmacological properties with great potential to address diseases and risk-factors associated with aging [52–53]. Quercetin is prevalent in fruits and vegetables in a glycosylated form but is metabolized at the point of absorption across the small intestine. As much as 93% of quercetin found in the blood is modified to quercetin 3-sulfate, quercetin 3-glucuronide, and 3-methylquercetin 3-glucuronide [51]. Importantly no glycosylated or free quercetin was observed in blood serum. When exposed to these metabolites of quercetin, *in vitro* analysis of human umbilical artery smooth muscle cells (HUASMCs) failed to elicit the same anti-inflammatory response as when they were exposed to the unmodified parent compound quercetin. This study may have confirmed the results of many investigations showing the beneficial effects of quercetin against the development of atherosclerosis via inhibition of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule- 1 (VCAM-1). However, it also showed that the development of clinically applicable therapeutic interventions must take into account the physiologically relevant conjugates of polyphenols like quercetin [51]. This is evidenced by the fact that while quercetin reduced the TNF-α mediated induction of ICAM and VCAM, metabolically prevalent quercetin conjugates were not able to downregulate the pro-inflammatory ICAMs and VCAMs produced by HUASMCs exposed to either TNF-α or LPS. This result demonstrated the need to expand the focus of research efforts to address the metabolic alterations of polyphenolic compounds that can potentially determine their overall efficacy against various risk factors of aging. And no less important is the need to experimentally address the physiologically relevant concentrations of quercetin and other polyphenols that can be achieved through diet [51,54]. The outcome of these experiments may determine the role that polyphenols have over the ability of cells to migrate through endothelia, an important stage of the inflammatory response. The results of these experiments have established an important means to better understand the biologically relevant forms of dietary polyphenols and their potential benefits for reducing diseases and other risk-factors associated with aging. In addition they have suggested that the need to address the metabolic alterations that occur in all *in vivo* models is essential to the potential production of therapeutic polyphenolic preparations.

Polyphenols Display Neuroprotective Effects

One of the most important aspects of current polyphenol research is the focus on the neuroprotective capacity that is a characteristic feature of this broad family of compounds [55–56]. Because many diseases of aging can be directly linked to repeated oxidative stress and chronic inflammation [57], therapies that diminish such effects have become an important tool in seeking more effective treatments for diseases such as AD [58]. New findings have also shown that polyphenols function as neuroprotective compounds at multiple levels including those that do not involve antioxidant properties [59]. In general, polyphenols have the capacity to chelate metal ions and to directly quench free radical species that contribute to oxidative damage [60]. While both of these physiochemical properties are known to contribute to the overall neuroprotective effects of polyphenols, recent research involving *in vitro* models show that polyphenols may have the capacity to activate or inhibit various cellular signaling pathways involving NF-κB, SIRT1, MAPK's, heat shock proteins (HSP), and numerous

additional regulatory molecules. Each of these signaling molecules or cascades play an important role in basic functions ranging from senescence, apoptosis, and the activation or production of transcription factors [16,61–62].

This new information has added a certain level of complexity to the relationship between polyphenols and human metabolism and has helped create greater understanding of how they might have such a wide range of purported human health benefits. But it also helps to account for the difficulties encountered in elucidating the exact mechanisms through which they work. Only recently it has been established through the use of autoradiography that specific polyphenol binding sites for resveratrol exist throughout the brains of rats, though it is unclear based on the techniques utilized what the molecular nature of those receptors might be. Moreover, the greatest concentration of these receptors was centered in the choroid plexus and subfornical organ of the brain [63–64] and interestingly, the primary protein produced by the choroid plexus is transthyretin (TTR) which comprises 25% of all CSF fluid and is the main binding protein for Aβ proteins in CSF. Since the deposition of Aβ proteins contributes to the formation of neuronal plaques, a hallmark physiological feature of AD, activation of TTR production could be a mechanism for the neuroprotective effects of resveratrol [65]. Additionally Stein, et al. have shown that deactivation of TTR during *in vivo* experiments with mouse AD models contributes to increased levels of Aβ oligomers and neuronal loss due to apoptosis in the hippocampus [66]. Thus the relationship between the location of specific polyphenolic receptors that bind resveratrol in the hippocampus and the proteins produced there indicate that cell signaling cascades probably play a vital role in the neuroprotective capacity of polyphenols by ultimately activating suppressive binding proteins like TTR.

Numerous epidemiological analyses have established a clear relationship between the neuroprotective effects of polyphenols and the decreased risk of age-associated neurological dysfunction such as that found in AD and Parkinson's Disease [65]. But the nature of those protective effects is not purely limited to the antioxidant properties of polyphenolic compounds. Recent evidence derived from *in vitro* cellular models now suggests that polyphenols such as resveratrol and EGCG not only possess the capacity to directly scavenge free radical species but to also regulate the cytotoxic effects of Aβ oligomers and fibrils via phosphorylation of phosphokinase C (PKC) [9,65,67]. By contributing to the phosphorylation of PKC, EGCG and resveratrol activate the transmembrane protein α-secretase. α-secretase catalyzes the formation of a soluble, non-amyloidogenic (non plaque-forming) protein from the amyloid precursor protein (APP) which is specifically located in the membrane of neuronal cells. Via this pathway, soluble APP is formed and thus does not allow for the formation of neuritic plaques, a hallmark feature of AD. An alternative pathway to the modification of APP involves two additional enzymes, β- and γ-secretase, that sequentially process the APP protein leading to the formation of insoluble, amyloidogenic oligomers or fibrils. Under these conditions neuritic plaques are formed indicating that this is an important factor in understanding the etiology of AD [25,68]. This information, derived from *in vitro* models, indicates that polyphenol derived therapies might be used to exert control over the ratio of APP molecules entering into each pathway and may suggest avenues for the development of new treatments that diminish the risk of developing AD as a function of the aging process.

Polyphenols Activate Important Cell Regulatory Proteins

Understanding the role that polyphenols play in regulating processes associated with cellular stress and aging requires a close analysis of the changing molecular and physiochemical environments of the various *in vitro* and *in vivo* models of human disease (Table1). While many studies have reported on the variety of beneficial effects of polyphenols there is also evidence to suggest that there are age-related differences in the effectiveness of these compounds in the treatment of model dysfunctions [69]. In addition, *in vitro* analyses by many investigators have

shown that compounds like resveratrol have the capacity to significantly affect the cellular environment even at low concentrations while others suggest that polyphenols may possess cytotoxic effects under certain conditions [70]. Because there are tissue specific and temporally associated changes in cell functioning it stands to reason that the effects of polyphenol metabolism might also be affected. Thus the use of polyphenols as therapies against age-associated risk factors must account for such complexities.

As mentioned above, the role of polyphenols like resveratrol as regulators of age-associated phenomena is not entirely limited to their radical scavenging properties. Instead resveratrol has also been shown to be a potent activator of the protein SIRT1 under *in vitro* conditions [71–72]. SIRT1 plays a crucial role in many cell signaling pathways and functions as a NAD^+-dependent protein deacetylase that targets acetylated lysine residues in substrate proteins. As the human analogue of the well-characterized Sir2 gene found in yeast, its overexpression has been shown to extend the lifespan of lower eukaryotes like yeast and worms [62]. Evidence acquired from the use of *in vitro* studies shows that SIRT1 is capable of deacetylating a wide variety of proteins including p53, NF-κB, HSF-1, FOXO1, 3, and 4, and PGC-1α which directly activates those proteins [61,73]. Because resveratrol amplifies the ability of SIRT1 to mediate the activation of such an array of important biomolecules, it therefore exerts influence over replicative senescence, inflammation, apoptosis, protein homeostasis, stress resistance, and metabolism [61]. This type of extensive influence over the lifecycle of the cell has contributed to the generous amount of attention received by resveratrol and other polyphenolic compounds and constitutes a significant opportunity for the development of therapies against the effects of aging. Interestingly recent research has pointed out that many of the investigations on resveratrol have focused on its ability to promote cell survival under conditions of induced environmental stress or disease [62,74]. However there is great clinical interest in the potential effects on the replicative capacity of normal cells in the presence of SIRT1 activators like resveratrol. To that extent Michishita et al. conducted an *in vitro* study of two cell types that included all seven of the known human SIRT proteins in order to evaluate their effects on the replicative lifespan of normal human fibroblasts and prostate epithelial cells [74]. This investigation failed to show a link between overexpression of SIRT1 enzymes and an increase in the survival of either cell type. However, additional *in vitro* studies by Huang et al., in which human diploid fibroblasts overexpressing SIRT1 were exposed to stressful conditions, did show an antagonistic relationship between SIRT1 overexpression and cell senescence as evidenced by cell proliferation and activation of ERK/S6K1 signaling [62]. These seemingly inconsistent results may be resolved through analysis of the conditions under which the experiments took place or may perhaps be attributable to type-specific characteristics of the cell lines that were used [62]. In addition, it is possible that there are cellular regulatory mechanisms that specifically diminish the effects of overproduction of SIRT1 under normal conditions while allowing it to persist under stressful conditions. These results that indicate SIRT1 is capable of having selectively beneficial effects against cellular stress further emphasize the need to understand the molecular nature of SIRT1 and its role as a mediator of molecular stress responses. Either way these provocative results demand further investigation into the role of sirtuins in the activation of cell survival mechanisms. The variations in cell response due to the timing, location, and microenvironment of the tissues involved, or even localized cellular stress conditions may be exploited as a target for further study of the variable effects of polyphenol activated sirtuins.

Much evidence has been gathered to suggest that calorie restriction (CR) is an effective method for the extension of organismal lifespan and has been shown to be an effective approach for alleviating common diseases of aging such as cancer, neurodegenerative disorders, and Type 2 diabetes [75–76]. However recent evidence has begun to challenge this theory and lead to a deeper understanding of the role that CR plays along with protein restriction in modulating metabolic processes associated with aging in *in vivo* models [77–78]. Whatever the molecular

process at work may be, reduction of caloric intake to meet minimal nutritional requirements is probably not a viable option for clinical approaches to treating disease. But it is still an important notion in the development of new treatments because of the known advantages cited across numerous studies. Because CR is a theoretically effective but practically unlikely therapy, investigators have sought to develop pharmacological interventions that might mimic a state of CR within an organism. In addition to the above findings for SIRT1, recent *in vivo* investigations have also shown that polyphenols like resveratrol allosterically activate sirtuin deacetylases in a fashion similar to the effects seen with CR [75]. In fact Smith et al. have used a Causal Network Modeling approach to show that SRT501 (a proprietary formulation of resveratrol) produced a molecular profile similar to that observed under calorie-restricted conditions in both genetically and diet-induced obese mice [75]. Perhaps most importantly, these studies show that the effects of the polyphenol resveratrol and CR are both mediated by the sirtuin protein deacetylases and thus supports previous work showing sirtuin-mediated efficacy in the improvement of metabolic profiles of mammals, rodents, and humans [75,79].

In addition to its ability to elicit a metabolic state similar to calorie restriction, resveratrol has also been shown to have anti-inflammatory effects [24,80–81]. Inflammatory responses that result from normal functions, as well as disease and infection, comprise one of the main causes of aging and senescence for all organisms. Chronic inflammation has been implicated in numerous diseases such as cardiovascular disease, cancer, autoimmune dysfunction, and neurodegenerative disorders [16–19]. Due to the significance of chronic inflammation in the etiology of a wide variety of diseases, much effort has been focused on medicinal interventions to offset some of its deleterious effects. Resveratrol has been experimentally shown in *in vivo* models to down-regulate the production of proinflammatory signaling molecules such as TNF-α, NF-κB, and epidermal growth factor (EGF) [75,82–83]. Each of these molecules is well-studied and known to operate at different levels of inflammatory signaling cascades. NF-κB is a transcription factor that plays a role in the activation of many important genes related to the inflammatory response while TNF-α has been demonstrated to be a key player in the genesis of diseases such as atherosclerosis which arerelated to chronic inflammation [19]. In addition, EGF is another signaling molecule upregulated during an inflammatory response and has been shown to contribute to the Human Papillomavirus (HPV)-induced immortalization of cervical cells [84]. Evidence suggests that the polyphenol EGCG is capable of exerting antitumor effects by blocking the EGF receptor under *in vitro* conditions in cervical cells and thus decreases the EGF-dependent EGFR signaling that contributes to the activation of MAPK [84].

Polyphenols and Cardiovascular Function

The dietary consumption of polyphenolic compounds, especially the phytochemical EGCG, may possess a significant ability to alter the chemical environment of different types of cells. These alterations are important in the study of many diseases and the purported health benefits of polyphenols like EGCG are especially relevant in terms of understanding the multi-factorial contributions from different tissues necessary to maintain cardiovascular health [85–86]. In fact epidemiological evidence also exists to further support the claim that diets rich in polyphenols lead to a relative improvement in aging across large groups of individuals [87]. Ultimately, cardiovascular health depends on a wide array of endogenous and exogenous influences such that the inherent age-associated risks result from both normal functioning (e.g., oxidative stress and inflammation) and environmental insults [88]. Thus it is important to seek out therapies for age-associated risk factors whereby interventions occur at the earliest levels of cell to cell communication in order to develop the most effective novel treatments. The underlying reason for the study of the effects of compounds like EGCG on various models of human disease extends from their known ability to activate cell signaling cascades that typically enhance comprehensive anti-inflammatory and anti-cancer responses. Recent research has also

demonstrated that there are tissue specific responses to polyphenols that must be considered in terms of the development of pharmacological interventions [89]. And though the nature of these differential effects is not well understood, the details of the molecular action of polyphenols are essential for deeper understanding of their physiological role throughout the body. By comparing different studies the elucidation of signal cascades can help to resolve our understanding of those tissue-specific responses for both *in vivo* and *in vitro* treatments of age-associated disease models with polyphenols.

The free radical compound nitric oxide (NO) is a well known mammalian cell signaling molecule produced biosynthetically from the reaction of arginine and oxygen via tissue-specific nitric oxide synthase (NOS) enzymes. It is also produced from the reduction of nitrate. Regardless of the source, NO is produced by one of three variants of the NOS enzyme and plays a key role in proper cardiovascular function, homeostasis, and neuronal signaling. In terms of cardiovascular health the most important of the NOS enzymes is endothelial NOS (eNOS). eNOS is a calcium dependent, redox sensitive, and constitutively produced enzyme responsible for the regulated production of NO in endothelial tissues. Within endothelial cells, such as those that line the cardiovascular tissues of the body, NO is produced and rapidly diffuses to the surrounding smooth muscle cells and leads to vasodilation [90]. Importantly the biological production of NO has also been shown to negatively influence platelet aggregation as well as leukocyte adhesion on the interior walls of the vascular system, both of which are factors in the development of atherosclerosis [91].

Recent research has pointed to the efficacy of cell signaling molecules like NO in their ability to mediate the physiological effects of red wines and green tea on endothelial cells of the vascular system under *in vitro* conditions [92–93]. This research has shown that NO can be used to block the release of exocytotic vesicles called Weibel-Palade bodies in stimulated human umbilical vein endothelial cells (HUVECs). The exocytosis of these bodies is normally mediated by molecules like thrombin upon injury or damage to the vascular endothelial surface. Release of the contents, such as the cell adhesion molecule P-selectin, represents a fundamental event in the initiation of a normal inflammatory response within the cardiovascular system [93]. By stimulating the release of molecules like P-selectin, both mechanical damage to the vasculature and physiochemical alterations within endothelial cells, may elicit this normal inflammatory response. Once released P-selectin interacts with leukocyte P-selectin glycoprotein ligand-1 (PSGL-1) and contributes to the process of leukocyte rolling. The inflammatory responses that occur in HUVECs allow for the production of additional cell adhesion molecules that ultimately permit leukocyte adhesion to the endothelial cells of vascular walls. Together these events play an important role in the etiology of atherosclerosis [93].

While the accumulation of white blood cells signifies the ability of the body to identify and repair injured vascular tissue, the concomitant persistence of inflammation in localized areas ceases to yield normal health benefits when that exposure is chronic. One of the most relevant diseases of aging that arises from the chronic inflammation of blood vessels is atherosclerosis [94]. Even though the etiology of atherosclerosis is fundamentally complex and multi-factorial, therapies that alleviate chronic inflammation have become important targets for resolving issues associated with disorders of this type. Recent work by Yamakuchi et al. has shown that the polyphenol EGCG is capable of inhibiting the HUVEC exocytosis of Weibel-Palade bodies under *in vitro* conditions and in a dose-dependent manner [93]. In so doing, this study showed that EGCG is capable of attenuating the externalization of P-selectin from Weibel-Palade bodies in the endothelial cells of the vascular system. This result indicated that EGCG has the potential to reduce aggregation of the inflammatory leukocytes that directly contribute to atherosclerosis. In order to contribute to the functional evidence that EGCG prevents such aggregations, the same investigators showed that EGCG was capable of reducing thrombin-

induced leukocyte adherence which may lend further evidence to the possibility that EGCG might be used in an important therapeutic approach for addressing the pathology of cardiovascular diseases related to chronic inflammation [93]. Interestingly, this study showed that neither epicatechin (EC), epicatechin gallate (ECG), nor epigallocatechin (EGC) were capable of delivering the same benefits [93]. The biological pathway by which the effects of EGCG are achieved is not completely understood. However, evidence suggests that EGCG is capable of increasing phosphorylation of eNOS as well as signaling molecules like Akt, without affecting the total concentration of these species. This ability to promote phosphorylation would indicate that EGCG directly stimulates the activity of eNOS and leads to a concurrent increase in NO production under such *in vitro* conditions. This increased NO production is then directly responsible for the decreased exocytotic secretions from Weibel-Palade bodies that are necessary for an inflammatory response.

Polyphenols Exhibit Anticancer Properties

Cancer is one of the most pervasive causes of death in western society and constitutes a significant threat to public health. As such, scientific research that addresses the age-associated change in the potential for cancer risk has been extensively elaborated [95]. And through much scientific investigation into the complex etiology of various cancers important advances have been made in understanding the nature of environmental factors such as diet in relation to tumor development [96–97].

In terms of its direct relevance to human health, perhaps the most important area of polyphenol research pertains to the anticancer properties of examples frequently found in common dietary sources [96–98]. This body of evidence illustrates numerous examples whereby the utilization of polyphenols may hold potentially powerful therapeutic interventions against a host of cancer-related phenomena. The role of polyphenols as anti-tumorigenic agents is complex and research has indicated that their anticancer properties are exerted at multiple levels including inhibition of gene expression [99], inhibition of angiogenesis [100], inhibition of metastasis [100–101], and suppression of cell proliferation [102] among many others.

While the anticancer properties of major polyphenols like resveratrol, EGCG, and quercetin have been well established, the molecular nature of their antiproliferative effects may be quite different. Recent *in vitro* investigations have demonstrated that EGCG modulates the effects of vascular endothelial growth factor (VEGF), in part by phosphorylating the VEGF receptor [99]. VEGF, which can activate up to 350 genes in stimulated HUVECs, is an important factor in activating downstream signaling pathways leading to antiapoptosis, angiogenesis, and activation of NF-κB [99]. The genetic modulations in HUVEC gene expression that occur under the influence of EGCG administered in the presence of VEGF indicate that cholesterol biosynthesis is upregulated, microtubule activities are modified, and inhibition of cell proliferation occurs. Similar effects on the inhibition of gene expression have been noted in various *in vitro* models of disease including proliferation of prostate cancer, cervical cancer, and epidermal carcinomas [99].

A second major anti-tumorigenic attribute among polyphenols is the inhibition of angiogenesis. Angiogenesis has long been recognized as a fundamental event in the development of invasive tumors since a vascular network to support delivery of blood is a necessary structural element required for the progression of disease [103]. As a result of the promising possibility that angiogenesis might be a target for pharmacological interventions, evidence gathered to that end has established the presence of a host of proangiogenic and antiangiogenic molecules that carefully regulate the production of new blood vessels. A number of these molecules and their receptors have been targeted for therapeutic reasons, including their early appearance within the processes of cell signaling that lead to new vessel production [104]. VEGF, as mentioned

above, plays a major role in the initiation of angiogenesis by activating a large number of genes to support vascularization of cancerous tissues and thus represents a popular target for therapeutic inhibition [104]. The demonstrable *in vitro* inhibition of VEGF-mediated angiogenesis by the polyphenol EGCG, which targets the vascular endothelial growth factor receptor (VEGFR), showed promising results in terms of suppression of a wide variety of tumors [100,105]. Moreover, many additional non-polyphenolic drugs are currently being designed to target VEGF and its receptor but the benefits associated with suppression of this signaling system are not entirely clear. Bergers and Hanahan (2008) have recently noted the unfortunate result that efforts to exploit antiangiogenesis as a means of tumor suppression by attenuating the VEGF signaling cascade have fallen short of their originally perceived possibilities [106]. However these studies did not include the potential long-term effects of polyphenols in attenuating the VEGFR in tumors. This may constitute the need for a new direction in polyphenol research to assess the long-term effects of exposure as it relates to therapeutic interventions. Since data now show that only initial treatments with VEGF/VEGFR blockers may lead to growth arrest or even tumor shrinkage, the sustained clinical benefits may be in doubt. The authors speculate that adaptive resistance (evasion) and intrinsic non-responsiveness represent two possible reasons for the lack of enduring efficacy seen with these treatments [106].

Summary

The substantial number of naturally occurring polyphenols represents both an enticing possibility and an inherently significant check against finding new treatments for major risk factors associated with aging. Understanding the mechanisms, modes of action, tissue specificity, timing and scope of application of polyphenols in various disease processes has proven to be a challenging but achievable goal. And as the scientific literature produces evidence in favor of the preventive and therapeutic benefits, public interest also expands. The increasing realization that polyphenol-rich diets may directly benefit human health fuels continued research interest in these important compounds.

To understand the significance of dietary polyphenols in terms of their potential benefit to human health, one must appreciate the scope of actions they are capable of performing. Polyphenols have often been generically referred to as “antioxidants” for their ability to react with and quench ROS produced during metabolic processes. The prevalence of ROS can increase rapidly under oxidative conditions giving rise to an even more significant threat to cellular structures and functions. During times of environmental stress or chronic inflammation they become especially relevant and may direct cellular actions at key points in the development of age-associated diseases such as cancer, atherosclerosis, and AD. Polyphenols are also often credited with having metal sequestering capabilities which may prevent the cytotoxic effects of heavy metal accumulation over time. Even with these important functions at work, comparatively well characterized polyphenols like resveratrol and EGCG have been shown to display other important biological actions.

One of the most intriguing properties of the green tea polyphenol EGCG is its capacity to elicit different responses based on tissue-specific cues. As noted above it has been demonstrated to promote the release of NO in the endothelial tissues of blood vessels. This event prevents the release of vesicles that normally become a contributing factor in inflammatory responses and are known to play a role in the etiology of atherosclerosis. But the ability of EGCG to increase NO production inhibits this sequence of events. On the other hand EGCG has also been demonstrated to possess anti-metastatic effects against cancer cells, due to fact that it decreases NO production in 4T1 murine mammary cancer cells. These selectively beneficial effects represent a considerable reason to continue research into the use of these compounds for therapeutic purposes. Due to the mounting evidence that the biological actions of EGCG are

mediated by alterations such as phosphorylation of downstream signaling molecules there is now greater certainty that polyphenols are capable of triggering existing cellular defense mechanisms and cascades. Further understanding of such mechanisms through the study of both *in vitro* and *in vivo* models is a central feature in expanding the clinical applications of polyphenols.

Finally, consideration of the anticancer properties of polyphenols shows that their effects may be displayed at various stages in the process of tumor development. Evidence suggests that some common dietary polyphenols may contribute to the decreased proliferation of cells, tumor shrinkage, decreased angiogenesis within neoplastic growths, and modulation of signaling systems to trigger programmed cell death. The effectiveness of polyphenols against tumor development represents one of the most important future applications of this knowledge. And while a complete understanding of their beneficial effects may not yet be fully realized, the established results of numerous studies points to a need for further investigations that can expand our understanding of the dynamic role these dietary substances play in the attenuation of certain risk factors associated with aging.

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Table 1

The attenuation of age-associated diseases by polyphenols

Polyphenol	Effect	Result
Resveratrol	activation of SIRT1 leading to deacetylation of p53, NF-κB, HSF-1, FOXO1/3/4, and PGC-1α	influences replicative senescence, inflammation, apoptosis, stress resistance, and metabolism
	activation of SIRT1 may mimic calorie restriction	increased lifespan
	binds receptors in the brain to stimulate production of transthyretin (TTR)	TTR sequesters Aβ fibrils important in etiology of Alzheimer's disease
EGCG	upregulates NO production in endothelial cells	may prevent inflammation associated with atherosclerosis
	down-regulates cGMP and NO production in carcinoma cells	prevention of metastasis
	blocks EGF receptor in cervical cancer cells	prevention of tumor growth
Quercetin	down-regulates production of TNF-α	anti-inflammatory properties reduce development of atherosclerosis