Nutraceuticals for Promoting Longevity

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Abstract: Objective: To summarize the main findings on nutraceuticals that slow aging processes by delaying and even preventing the development of multiple chronic diseases and improve productivity and quality of life in the elderly.

Methods: Literature search of the relevant papers known to the authors was conducted.

Results: The most robust environmental manipulation for extending lifespan is caloric restriction without malnutrition. Some nutraceuticals can mimic caloric restriction effects. This review will focus on the nutraceuticals that impact insulin-like growth factor 1 receptor signaling and sirtuin activity in mediating longevity and healthspan.

Conclusion: Aging is considered to be synonymous with the appearance of major diseases and an overall decline in physical and mental performance. Caloric restriction is well established as a strategy to extend lifespan without malnutrition. A variety of nutraceuticals were reported to mimic the effect of caloric restriction by modulating the activity of insulin-like growth factor 1 receptor signaling and sirtuin activity and consequently promote longevity and healthspan.

Keywords: Nutraceuticals, longevity, caloric restriction, insulin-like growth factor 1 receptor (IGF1R), silent mating type information regulation 2 homology 1 (SIRT1).

1. INTRODUCTION

Caloric restriction (CR) without malnutrition is the most robust environmental manipulation known to increase active and healthy lifespan in diverse species. CR exhibited beneficial effects in reducing the incidence of age-related diseases such as obesity, diabetes mellitus, hypertension, cardiovascular disease, and cancer. Thus, CR may be a physiological strategy for extending lifespan through a slower cell growth and metabolism and a decreased accumulation of senescent cells; however, it is recommended only for selected segments of an adult human population.

Studies using model organisms and in human centenarians implied molecular mechanisms of longevity such as reducing the activity of mammalian target of rapamycin (mTOR) and insulin-like growth factor 1 receptor (IGF1R) signaling. It was observed from CR and genetic manipulation studies that the silent mating type information regulation 2 homolog 1 (sirtuin1 or SIRT1) activity also modulated longevity and healthspan. These pathways were modulated also by some nutritional substances, nutraceuticals. As CR is neither a practical nor an advisable practice for all human populations, nutraceuticals are being investigated as an alternative. Their impact on IGF1R signaling and SIRT1 activity in mediating longevity and healthspan is described below and summarized in Table 1.

2. INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR (IGF1R) AND LONGEVITY

IGF1R is conserved evolutionarily from worms to humans [1] and activated by insulin and insulin-like growth factor 1 (IGF1). IGF1 is mainly produced in the liver by stimulation of growth hormone (GH) secreted from somatotrophs in the anterior pituitary [2]. Circulating IGF1 binds to insulin/IGF1 receptors on the cell membrane of organs and primarily promotes tissue growth at an early developmental stage. IGF1R, through insulin receptor substrate 1 (IRS1), IRS2, phosphoinositide 3-kinase (PI3K),...
Table 1. Effect of nutraceuticals on longevity and healthspan through IGF1R signaling and SIRT1 activity modulation. Listed are the classification, chemical structures and sources of natural compounds along with experimental models and studies confirming their effects on longevity.

<table>
<thead>
<tr>
<th>POLYPHENOLS</th>
<th>Compound Name</th>
<th>Structure</th>
<th>Sources</th>
<th>Experimental Model</th>
<th>Effect on Longevity</th>
<th>Refs.</th>
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<tbody>
<tr>
<td>POLYPHENOLS</td>
<td>Quercetin</td>
<td><img src="image1" alt="Quercetin structure" /></td>
<td>Spices, vegetables, and fruits</td>
<td>Worms</td>
<td>Extended lifespan by 21%</td>
<td>[39]</td>
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<td>POLYPHENOLS</td>
<td>Fisetin</td>
<td><img src="image2" alt="Fisetin structure" /></td>
<td>Fruits and vegetables</td>
<td>Mice</td>
<td>Extended the median and maximal lifespan</td>
<td>[120-125]</td>
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<tr>
<td>POLYPHENOLS</td>
<td>Myricetin</td>
<td><img src="image3" alt="Myricetin structure" /></td>
<td>Fruits, (berries), vegetables, tea</td>
<td>C.elegans</td>
<td>Improved healthspan and lifespan.</td>
<td>[126-127]</td>
</tr>
<tr>
<td>POLYPHENOLS</td>
<td>Epicatechin</td>
<td><img src="image4" alt="Epicatechin structure" /></td>
<td>Cacao and tea</td>
<td>Obese diabetic mice</td>
<td>Decreased mortality from 50% in control to 8% in epicatechin treated mice</td>
<td>[41]</td>
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<tr>
<td>POLYPHENOLS</td>
<td>Luteolin</td>
<td><img src="image5" alt="Luteolin structure" /></td>
<td>Reseda luteola, broccoli, parsley, olive oil, celery</td>
<td>Human monocytic cells (THP-1)</td>
<td>Induced expression of SIRT1, SIRT3, SIRT6, FOX03a</td>
<td>[125]</td>
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<tr>
<td>POLYPHENOLS</td>
<td>Aspalathin</td>
<td><img src="image6" alt="Aspalathin structure" /></td>
<td>Rooibos tea leaves</td>
<td>Worms</td>
<td>Extended lifespan by 20% to 25% under high glucose feeding</td>
<td>[38]</td>
</tr>
<tr>
<td>POLYPHENOLS</td>
<td>Butein</td>
<td><img src="image7" alt="Butein structure" /></td>
<td>Citruses, vegetables (tomatoes, bean sprouts, potatoes)</td>
<td>S. cerevisiae</td>
<td>Increased the average lifespan by 31% and prolonged maximal lifespan</td>
<td>[110]  [128-132]</td>
</tr>
<tr>
<td>Compound Name</td>
<td>Structure</td>
<td>Sources</td>
<td>Experimental Model</td>
<td>Effect on Longevity</td>
<td>Refs.</td>
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<tr>
<td><strong>4,4’-dimethoxychalcone (DMC)</strong></td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Angelica keiskei koidzumi</td>
<td><em>S. cerevisiae</em></td>
<td>Extended lifespan</td>
<td>[133-135]</td>
<td></td>
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<tr>
<td><strong>Resveratrol</strong></td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Grapes, blueberries, and red wine</td>
<td>Mice</td>
<td>Increased lifespan by 9% in old female mice SIRT1 activator</td>
<td>[42-44] [111-114]</td>
<td></td>
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<tr>
<td><strong>Curcumin</strong></td>
<td><img src="image3.png" alt="Structure" /></td>
<td>Ginger, Fam: Zingiberaceae.</td>
<td><em>C. elegans</em>, <em>D. melanogaster</em>, mice; HUVECs</td>
<td>Increased lifespan; attenuated senescence in human cells</td>
<td>[109] [136-140]</td>
<td></td>
</tr>
<tr>
<td><strong>Berberine</strong></td>
<td><img src="image4.png" alt="Structure" /></td>
<td>Fam: Berberidaceae</td>
<td><em>Drosophila</em>; Human cells (L-02)</td>
<td>Prolonged lifespan under the high-temperature conditions; Increased SIRT1 expression and cell viability (19%)</td>
<td>[141-142] [143-144]</td>
<td></td>
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<tr>
<td><strong>Lycopene</strong></td>
<td><img src="image5.png" alt="Structure" /></td>
<td>Tomatoes</td>
<td>Human (Clinical trial)</td>
<td>Reduction in plasma IGF-1 and IGFBP-3 levels, and inhibit activation of IGF-1R</td>
<td>[45-50]</td>
<td></td>
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<tr>
<td><strong>Crocin</strong></td>
<td><img src="image6.png" alt="Structure" /></td>
<td>Crocus sativus (saffron spice)</td>
<td>Mice</td>
<td>Antioxidant protection of ovaries</td>
<td>[145]</td>
<td></td>
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<tr>
<td><strong>Corticosterone</strong></td>
<td><img src="image7.png" alt="Structure" /></td>
<td>Steroid hormone</td>
<td>Human</td>
<td>Decreased plasma levels of IGF-1 and reduced IGF-1R levels</td>
<td>[51]</td>
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Table 1. Contd…
and protein kinase B (AKT) [3] activates mTOR. Moreover, the IGF1R pathway modulates gene expression through transcription factors such as forkhead box O (FOXOs) [4], which control the expression of genes implicated in age-related processes (oxidative stress, apoptosis, cell-cycle) [5]. Initial studies in C. elegans using reduced functionality have identified gene mutations such as age-1 and daf-2 that resulted in a profound extension of lifespan [1, 6]. Subsequent cloning studies identified the daf-2 gene as being homologous to both mammalian insulin receptor (IR) and IGF1R that have been conserved in yeast, worms, fruit fly, mice and humans, and are causally linked to aging and longevity across species [7].

IGF1, the major energy sensing pathway, exerts pleiotropic effects via several intracellular signaling pathways promoting growth mediated by IGF1R. Circulating levels of IGF1 and IGF binding protein-3 (IGFBP-3) vary among individuals and are affected by genetic, environmental or lifestyle factors. A positive correlation was found between bioavailable levels of IGF1 and increased risk of age-related diseases including cancer [8]. Moreover, decreased IGF1R signaling pathway activity has been observed as a result of either genetic or environmental manipulation, and was found to be accompanied by the extension of lifespan in worms, flies, mice and likely humans [9]. Mutations in the IGF1R gene in mice and humans have also been implicated in longevity signaling. For example, female mice harboring loss-of-function mutations in the igf1 gene in the liver are smaller than controls but live 16% longer [10]. Furthermore, mice that are heterozygous for a mutated allele of the IGF1R (Igfr1+/-) are ~10% smaller than wild-type mice, expressing ~50% of wild-type IGF1R levels; they exhibit a significant reduction in IGF1-induced intracellular signaling and become insulin-resistant with age [11, 12]. Nevertheless, female Igfr1+/- mice have a 33% longer mean lifespan than wild-type littermates.

Several studies have identified IGF1R alterations in humans and correlated them with human longevity. The G/A polymorphism in IGF1R was associated with long-living Italian individuals compared to other Italians, resulting in reduced IGF1 plasma levels [13]. A combination of IGF1R/Asp-IRS2/Val-UCP2 allele was also associated with longevity, as was reported in another study involving Italian people [14]. Genetic alterations in IGF1R affecting longevity were not limited to Italian people. Other studies conducted on Ashkenazi Jews revealed a genetic variation in IGF1R among centenarians. Two heterozygous IGF1R gene mutations, Ala37Thr and Arg407His, were found to be more frequent in a cohort of Ashkenazi Jewish centenarians than in control individuals [15]. These mutations suppressed IGF1 signaling and decreased transcription of target genes [15]. Female carriers of these mutations have high IGF1 levels and slightly short stature, implying that they are IGF1-resistant [16]. These studies provide evidence that reduced IGF1 signaling can positively affect the human lifespan.

Longevity in several animal species, including worms, fruit flies and mice, was associated with increased resistance to stress [17, 18]. Cellular stress can be caused by diverse extracellular or intracellular stimuli such as heat, hypoxia, irradiation, glucose deprivation and the accumulation of reactive oxygen species (ROS) [19]. To avoid organelle and DNA damage, as well as aggregation of unfolded proteins, the cell’s stress response is through activation of survival mechanisms such as autophagy or apoptosis induction [19]. Various stress stimuli inhibit mTOR, a central signal transducer that promotes cell growth, metabolism and survival in conditions of nutrient abundance. Inhibiting mTOR by rapamycin and CR decreased IGF1 signaling stimulated autophagy and induced improved response to cellular stress and increased lifespan [9, 19, 20].

The reduction in IGF1 signaling could upregulate the expression of FOXO transcription factors (tumor suppressor) and antioxidant genes to elicit stress resistance [18, 21]. Resistance to cellular stress was accompanied by extended lifespan in mice. As in the case of IGF1R, also FOXO mutations were reported in humans and were implicated in human longevity. For example, an association between single nucleotide polymorphisms (SNPs) in FOXO3A gene and longevity was confirmed in several independent studies involving human participants [22-25]. Interestingly, a SNP rs2802292 upregulated FOXO3A expression was associated with human longevity. The SNP rs2802292 is located in the
FOXO3A enhancer region, which can lead to the upregulation of FOXO3A expression. The presence of the SNP rs2802292 can partially explain the differences in FOXO3A association with longevity between genders, since its activity in females may be modulated by estrogens through estrogen receptor response elements located within the rs2802292-encompassing region [26].

3. DIETARY INTERVENTIONS LIMIT IGF1R SIGNALING AND PROMOTE LONGEVITY

Caloric restriction (CR) refers to dietary intervention with an overall 20–40% reduction in total caloric intake. CR is the most studied and reproducible non-genetic intervention known to extend the healthspan and/or lifespan in organisms, ranging from unicellular yeast to monkeys. It started with a simple experiment where a dietary intake reduction extended the lifespan of rats [27], providing a foundation to study experimentally the relationship between nutrition and the biology of aging. Animals fed a calorie-restricted diet are less likely to suffer from common age-related conditions such as obesity, neurodegenerative disorders, autoimmune disorders and neoplastic lesions [28]. No data exist that correlate the human lifespan and calorie-restricted diet, however, several reports support protection from age-related diseases [29, 30]. Since feeding studies are difficult to conduct in humans, the best information is from CR studies in nonhuman primates. Two studies in rhesus monkeys revealed disparate results; one showed that a CR diet extended lifespan [31], the other found no effects of CR on longevity [32].

Despite differences in which CR is carried out in diverse organisms, CR is the most robust environmental manipulation known to increase active and healthy lifespan by modulating nutrient-signaling pathways, including mTOR, insulin/IGF1-like signaling and SIRT1 [33].

Dietary factors may influence IGF1 signaling by affecting IGF1 bioavailability, IGF1R activity or expression levels. CR decreases serum IGF1 concentrations and consequently signaling mediated by the IGF1R pathway. Factors determining IGF1 circulating levels include genetic variation affecting expression levels of IGFI and IGFBPs [34, 35], as well as exogenous factors (e.g., dietary habits or other lifestyle factors). In the cases of chronic or acute CR, serum levels of IGF1 are strongly reduced [36, 37].

4. NATURAL PRODUCTS AS A CALORIES RESTRICTION (CR) MIMETIC

Many compounds isolated from plants and fungi prolong lifespan and prevent age-related diseases in model organisms. They modulate the same cellular and physiological pathways as CR, including those involving insulin and IGF1, mTOR and SIRT1 functions. Modulating these age-related pathways results in the activation of various cellular processes such as autophagy, DNA repair and ROS neutralization to delay aging and prevent chronic diseases by improving bodily functions and stress resistance. Various plant and fungal molecules described below modulate the insulin/IGF1/FOXO pathway in worms, fruit flies and rodents (Table 1).

Aspalathin, a dihydrochalcone glucoside compound isolated from rooibos tea leaves extends the lifespan of worms by 20–25% under high glucose feeding [38]. Its life extension effect is associated with reduced cellular ROS accumulation and activation of daf-16, the ortholog of FOXO in worms (Table 1).

Quercetin, a major flavonoid polyphenol in the human diet, found in spices, vegetables and fruits, also extends the lifespan of worms [39]. It increases mean and median lifespan by 18% and 21%, respectively, while maximal lifespan is not affected [39]. Quercetin does not increase lifespan in worms lacking daf-2 gene, which encodes an ortholog of the human IGF1R [39] (Table 1).

Inositol, a small carbohydrate compound found in fruits, beans and nuts, extends the lifespan of male and female fruit flies by 17% and 13%, respectively [40]. Its life extension effect is attributed to dfoxo (the ortholog of FOXO in fruit flies) pathway activation. Inositol also improves the climbing ability of flies, thereby improving healthspan. Pinitol, an inositol-related compound, also extended lifespan similarly [40] (Table 1).

Epicatechin, a flavone compound isolated from cacao and tea, extends the lifespan of obese diabetic mice (50% mortality was observed in untreated mice vs 8% in epicatechin-treated mice) [41]. It reduces systemic inflammation and improves muscle stress output and antioxidant levels in the liver. Notably, it reduces blood IGF1 levels, suggesting reduced activation of the IGF1 signaling pathway (Table 1).

Resveratrol (RSV), a phenolic compound found in grapes, blueberries and red wine, increases the lifespan of mice [42], enhances insulin sensitivity and reduces blood IGF1 levels and consequently reduces insulin/IGF1 pathway signaling. It reduces age-related degeneration in cognitive function, blood vessels and bones, but fails to extend lifespan in mice in the absence of a high-calorie diet or high-fat diet (HFD) [43, 44] (Table 1).

Lycopene, a carotenoid found in tomatoes, increased serum IGFBP-3 and decreased serum IGF1/IGFBP-3 ratio in lycopene-fed animals [45]. Consumption of tomato products and/or intake of lycopene was inversely associated with IGF1 (or the ratio of IGF1/IGFBP-3) and positively associated with IGFBP-3. Moreover, lycopene inhibited the activation of IGF1R (i.e., reduced tyrosine phosphorylation of IGF1R) [46]. In a clinical trial of lycopene supplementation, a reduction in plasma IGF1 and IGFBP-3 levels was observed [47]. In cross-sectional epidemiologic studies, a high consumption of cooked tomatoes was associated with low serum IGF1 levels [48], and a high lycopene intake was associated with high IGFBP-3 levels [49]. The Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) revealed that dietary interventions with carotenoids, fruits and vegetables may affect the IGF1 system positively or negatively. For example, statistically significant positive associations were observed between serum concentrations of α-carotene and lutein/zeaxanthin and intake of fruits with serum IGFBP-3 concentrations in women, but not in men [50] (Table 1).

Corticosterone, which usually increased during CR, decreased plasma IGF1 concentrations and also reduced...
IGF1R levels and consequently the inhibition of several downstream signaling pathways [51] (Table 1).

**Butyrate**, a short-chain fatty acid (SCFAs), is a fermentation product of dietary fiber and polysaccharides produced by the gut microbiota of the large intestine. Butyrate is converted into the ketone body β-hydroxy butyrate (BHB), which enhances the mean lifespan of worms by ~20% [52]. BHB levels in the blood also increase during fasting, intense exercise, or CR, and serve as a source of energy independent of glucose, possibly delaying aging by increasing stress resistance [53, 54]. Based on these observations, some authors proposed that the life-extension effects of CR may be mediated at least in part by BHB [53, 54] (Table 1).

**Senolytic compounds** are a new class of compounds that target cellular senescence, a process in which damaged cells persist and become toxic to cells around them. Cellular senescence drives multiple age-related diseases. Dasatinib, a tyrosine kinase inhibitor that blocks the Bcr-Abl and Src kinase family [55], interferes with ephrin dependence receptor ligands (EFNB)-dependent suppression of apoptosis [56]. They reduce viability and cause cell death to senescent human cells. Quercetin, a natural flavonoid, inhibits PI3K and other kinases [57, 58] and induces apoptosis of senescent cells without affecting normal cells [59]. A mixture of dasatinib and quercetin reduced the number of senescent cells in old mice [59] and increased mouse lifespan compared to controls [60] (Table 1). Furthermore, in an open-label pilot study in humans, treatment with dasatinib and quercetin was successful in alleviating physical dysfunction in idiopathic pulmonary fibrosis (IPF).

Interestingly, a recent study showed that targeting the IGF1R with a monoclonal antibody was associated with a reduction in inflammation and increased lifespan by 9% in old female mice [61]. However, it remains to be seen whether this intervention targeting IGF1R may be viable in humans. Finally, CR not only reduces the bioavailability of IGF1 but is also causing the activation of sirtuin proteins such as SIRT1 and both directly and indirectly to promote longevity [62].

### 5. SIRTUINS AND LONGEVITY

Sirtuins are nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylases (HDACs) [63] involved in the modulation of structure and function of cellular proteins. Their protein targets are not only histones but many other proteins responsible for genome integrity [64], inflammation, antioxidative defense [65], apoptosis [66], metabolism [67], aging [68] and circadian rhythm [69].

Sirtuins are highly conserved proteins present in all eukaryotes and the majority of prokaryotes [70]. The number of sirtuin family members increases with the complexity of the organism's biological organization. Yeasts have only one sirtuin (Sir2) while C. elegans and D. melanogaster have four (Sir 2.1-Sir 2.4) and five sirtuins (dSirt1, dSirt2, dSirt4, dSirt6, and dSirt7), respectively [71]. In mammals, including humans, the sirtuin family is constituted of seven proteins (SIRT1-SIRT7) [70] with different subcellular distributions and catalytic activity. SIRT2 is located in cytoplasm but migrates to the nucleus during G2/M transition to deacetylate histone H4 and regulate chromatin condensation [72]. SIRT1, SIRT6, and SIRT7 are mostly found in the nucleus. SIRT7 is the only sirtuin present in nucleoli, and as previously shown by Michishita and colleagues [73], along with SIRT6, but not SIRT1, was associated with condensed mitotic chromosomes. Nucleocytoplasmic shuttling is a feature of SIRT1 and depends on post-translational modifications of this protein [63]. Sirtuins (SIRT3, SIRT4, and SIRT5) reside in the mitochondrial matrix in human cells [74].

All SIRTs are deacetylases that cleave NAD+ into nicotinamide (NAM), which is followed by the removal of the acetyl group from N-epsilon lysine residue of the target protein and formation of 2′-O-acetyl-ADP-ribose (2′-OAADPr). SIRT1-3 exhibit strong deacetylase activity, while SIRT4 predominantly catalyzes ADP-ribosylation [75]. SIRT5 can modify target proteins by demalonylation, desuccinylation and deglutaryl ation [76-78]. Similar to SIRT4, SIRT6 shows ADP-ribosylation activity [79].

The catalytic activity of sirtuins depends on NAD+ availability or NAD+/NADH ratio. This relation links sirtuin enzymatic activity to the cell metabolism and antioxidiant defense making them important stress sensors [80]. Indirectly, sirtuin activity depends on processes that influence NAD+ level in cells such as exercise, CR and low-glucose availability [81]. The activity of nicotinamide phosphoribosyltransferase (NAMPT), an enzyme essential for NAD+ synthesis, regulates levels of key NAD+ intermediates NAM and NMN (nicotinamide mononucleotide), as well as the activity of NAD+ consuming enzymes like Poly(ADP-ribose) polymerases (PARPS), all of which also modulate sirtuin activity [82-84]. The presence of sirtuins in almost all living systems and main cellular compartments indicates that lysine acetylation of proteins is a highly conserved and common regulatory mechanism of homeostatic processes in nature.

### 6. SIRTUINS AND THE AGING MECHANISM

Sirtuins play an important role in delaying cellular senescence through the repression of telomere shortening and the prevention of genomic instability, one of the most prominent hallmarks of the aging process. Sasaki and colleagues [85] reported that cellular senescence was accompanied by a decrease in SIRT1 protein, but not mRNA level, in embryonic mice and human lung fibroblasts. In contrast, Stamatovic and colleagues [86] revealed a decline in SIRT1 mRNA and protein levels followed by lower enzyme activity in brain micro-vessels of both aged mice and humans. The age-related decrease in SIRT1 and SIRT3 expression was found in the heart tissue of women but not in men's hearts [65]. Thus, age-related changes in sirtuin expression might be cell-, tissue- or even sex-specific [87-89]. SIRT1 and SIRT6 overexpression suppressed both replicative senescence with telomere attrition and stress-induced premature senescence of cells without telomere involvement [71]. Increased human telomerase (hTERT) expression as a result of SIRT1 upregulation of FOXO3A protein, prolonged the lifespan of the human umbilical cord fibroblast (HUC-F2) cells [90]. SIRT6 deficiency also caused telomere dysfunction, followed by chromosomal fusions and premature development of cellular aging.
phenotype [91]. SIRT1 deacetylation of FOXO4 induces cell cycle arrest while deacetylation of p53 prevents stress-induced cellular senescence [92, 93]. Nuclear sirtuins (SIRT1, SIRT6, and SIRT7) interact with histones and stabilize chromatin structure [94]. SIRT1 deacetylates histones H4, H3, H1 at specific lysine sites and promotes heterochromatin formation and transcriptional silencing [95].

In lower organisms, the SIRT1 protein extended the lifespan of yeasts by suppressing genomic instability [96]. Later studies confirmed that sirtuins modulate the lifespans of worms, flies and mice [97-99]. Among seven sirtuins in mammals, only SIRT1, SIRT6 and SIRT7 were related to the extension of lifespan. Overexpression of SIRT1 protein in mice hypothalamus increased lifespan by 9% in males and by 16% in females [100]. Sirt7-/- mice had increased perinatal lethality and reduced lifespan [101], while SIRT6-deficient mice (Sirt6-/-) developed abnormalities, severe metabolic defects and died at about four weeks after birth [102]. A recent study has shown that SIRT6 homozygous inactivating mutation leads to severe congenital anomalies and perinatal death in human fetuses [103]. The key molecular mechanisms through which sirtuins affect an organism’s lifespan include the reduced activity of mTOR and IGF1R signaling. Under stress conditions, SIRT1 modulates mTOR through interaction with the TSC1-TSC2 inhibitory complex and reduces protein synthesis and cell growth [104]. Sirtuins such as SIRT1 and SIRT2 were reported to activate FOXO proteins [63]. Overexpression of SIRT6 in mice was accompanied by a decreased level of IGFl and increased lifespan [99]: this pathway has a significant role in human lifespan extension as was indicated above.

It is believed that the increased expression of sirtuins, due to increased stress mediates the beneficial effects of CR on health and lifespan [71]. Sirtuins could be activated by two types of stress: metabolic/energy stress as a result of nutrient/calorie restriction and genotoxic stress [64]. CR upregulates SIRT1 expression in rodent and human metabolically active tissues such as kidney, liver, skeletal muscle and brain [105-107]. One of the major molecular pathways involved in sirtuin’s modulation of lifespan is the upregulation of 5′ adenosine monophosphate activated protein kinase (AMPK) and the increase of NAD+ levels. SIRT1 and AMPK comprise a reciprocal positive regulating loop. AMPK indirectly activates SIRT1 by upregulating NAMPT gene expression leading to increased NAD+ availability, while SIRT1 activates AMPK by deacetylating liver kinase B1 (LKB1), an AMPK activator [108].

7. NUTRACEUTICALS THAT ACTIVATE THE SIRTUIN PATHWAY

More than 14000 sirtuin-activating compounds (STACs) [71] including natural products (nutraceuticals) and synthetic compounds have been identified.

Resveratrol (RSV) is the most prominent SIRT1 activator [109]. Other phenolic compounds such as fisetin, butein and quercetin also activate sirtuins in different organisms [110]. Resveratrol activation of SIRT1 is an upstream effect through AMPK signaling [111]. Therefore, AMPK knock-out mice are resistant to the metabolic effect of dietary RSV [112]. Moreover, Park and colleagues have shown that in vivo RSV indirectly activates SIRT1 through the cAMP-Epacl-AMPK-Sirt pathway [113]. RSV competitively inhibits cAMP-degrading phosphodiesterases, thus increasing cAMP levels, AMPK signaling, NAD+ levels and activation of SIRT1. RSV has limited therapeutic potential in the treatment of age-related disorders. In humans, 75% of orally administered RSV is absorbed by oral and intestinal mucosa but the availability of this polyphenol is less than 1% as a result of rapid detoxification in the liver and a short half-life [114]. Furthermore, RSV increases the activity of phase II detoxifying enzymes in the liver and thus further induces its own degradation [115]. Interestingly, Giovannini and colleagues have demonstrated the synergistic effect of RSV and eight additional compounds (berberine, tyrosol, quercetin, catechin, ferulic acid, curcumin, nicosamide and malvidin) that increased SIRT1 expression in Human Cervical Carcinoma (HeLa) cells [114]. The main molecular pathway by which these compounds affect SIRT1 expression involves the stimulation of AMPK and inhibition of mTOR activity [114]. Although the beneficial effect of CR on longevity has been appreciated in a number of species, the safety of this dietary regime limits its utilization to some groups of healthy adults [116]. In contrast, nutraceutical mimetics may serve as an alternative. A clinical study on 48 healthy subjects reported that the administration of 500 mg/day of RSV had the same effect on SIRT1 activity as CR (1000 cal/day). Both RSV and CR treatment increased SIRT1 serum levels to 5.5- and 3.6- fold, respectively [117]. Results of two independent studies imply a protective effect of an RSV-rich diet on the cardiac system indicating that RSV may play a role in primary prevention of cardiovascular disease [118, 119] (Table 1).

Fisetin, a flavonoid found in fruits and vegetables has a senotherapeutic effect in mouse and human tissues. In their recent study, Yousef zadeh and colleagues showed that a fisetin-enriched diet reduced senescence markers in mouse tissues and reduced age-related pathology as a result of restored tissue homeostasis [120]. Furthermore, fisetin decreased levels of senescence-associated β-galactosidase activity in human adipose tissue, indicating the translational potential of fisetin treatment. Moreover, fisetin showed a neuroprotective role by decreasing both inflammation and oxidative stress [121, 122]. The anti-inflammatory effect of fisetin was exerted through the downregulation of inflammatory genes (IL-1β and TNF-α) [123] and was related to increased SIRT1 expression and activity [124]. In nerve cells, fisetin upregulated activating transcription factor 4 (ATF4) and NF-E2-related factor 2 (Nrf2) in a dose- and time-dependent manner, increased GSH level and reduced oxidative stress [122]. An in vitro study on human monocytic cells (THP-1) revealed that fisetin and flavone, luteolin independently reduced ROS production by decreasing expression of p47phox (a cytosolic protein, a component of NADPH oxidases) and increasing the expression of SIRT1, SIRT3, SIRT6 and FOXO3A [125] (Table 1).

Myricetin is another flavonoid found in fruits, vegetables and tea that affected worms’ longevity (C. elegans) in a sirtuin dose-dependent manner [126]. Namely, mutations in SIRT1 homolog (Sir-2.1) abolished the myricetin extension of lifespan in wild-type C. elegans. In
mammals, myricetin induced oxidative phosphorylation in skeletal muscle and brown adipose tissue by increasing SIRT1 deacetylation of PGC-1α [126]. Akindehin and colleagues reported an in vivo effect of myricetin treatment on the expression of mitochondrial sirtuins (SIRT3 and SIRT5) resulting in a bodyweight reduction in mice by 11% [127] (Table 1).

Butein, a chalcone found in citrus and vegetables like tomatoes, bean sprouts and potatoes, was reported as an activator of SIRT1 [128]. It prolonged the lifespan of D. melanogaster by 31% [110] but no such effect was found in higher organisms. Nevertheless, butein may indirectly extend the human lifespan by its beneficial effect on health [129]. Preclinical studies have shown that butein has enormous potential as an anti-cancer agent [130]; it also has antihypertensive [131], anti-inflammatory and antioxidant [128], anti-adipogenic effects [132] (Table 1). These positive effects of butein require clinical confirmation.

4,4′-dimethoxychalcone (DMC) is a chalcone found in Angelica keiskei koidzumi, a Japanese plant, which prolongs the survival of several species such as yeasts, worms and flies, and decreases senescence in human cell cultures by the induction of autophagy [133]. Unlike RSV and CR [134], DMC affects autophagy in a SIRT1-independent manner [133, 135] (Table 1).

Curcumin, a polyphenol found in ginger and Curcuma longa plants, is a well-established nutraceutical examined extensively as an anti-aging, anti-cancer and anti-inflammatory agent [109]. In vivo studies showed that curcumin treatment mediated lifespan extension in C. elegans and D. melanogaster by increasing antioxidant capacity and inducing the expression of age-related genes [136]. Mice that were fed with a curcumin metabolite, tetrahydrocurcumin (ThC), had a significantly longer average lifespan (by 12%) compared to the control group [137], but only if the ThC diet started at a young age. Curcumin attenuates senescence mainly through its ability to reduce ROS production by increasing the activity of antioxidant enzymes [136]. Moreover, curcumin pretreatment of human umbilical vein endothelial cells (HUVECs) decreased the levels of aging parameters (senescence-associated β-galactosidase and p21) by increasing the expression and activity of SIRT1 [138]. Inhibition of SIRT1 diminished the protective role of curcumin. Anti-cancer and cardioprotective activities of curcumin are related to the upregulation of the SIRT1 mRNA by 34a and consequently increasing levels of SIRT1 mRNA [146].

Compounds that activate sirtuins may either increase NAD+ synthesis or its bioavailability. Thus, therapies that maintain high NAD+ levels may slow down aging, promote longevity and mitigate age-associated diseases [81]. An increase in NAD+ level can be achieved either by the administration of NAD+ precursors (NAD+-boosters), increasing NAMPT expression or reduction of NAD+ consumption [147]. Supplementation of key NAD+ intermediates, such as nicotinamide riboside (NR) and NMN, can improve age-related conditions [84]. Treatment of aged cerebrovascular endothelial cells (CMVECs) with NMN in rats improved their angiogenic ability and decreased oxidative stress counteracting the adverse effects of aging [148]. Pre-treatment with pharmacological SIRT1 inhibitor prevented these effects. Gomes and colleagues reported that NMN increased NAD+ level and restored mitochondrial function in the muscles of elderly mice to that of a young mouse in a SIRT1-dependent manner [149].

Supplementation with the amino acid tryptophan (Trp) or all three forms of vitamin B3 (NAM, niacin and NR) and possibly with nicotinic acid riboside (NaR), increase NAD+ synthesis through different anabolic pathways [150]; however, not all NAD+ precursor supplements are equally tolerated and thus suitable as supplements for slowing aging and promoting longevity [151]. Dietary intake of Trp or less than 20 mg/day of niacin for adults meet the baseline requirements for NAD+ [150]. Treatment of diabetic patients with high doses of NAM was hepatotoxic [152]. Trammell and colleagues reported that NR is more orally bioavailable than NAM and has no side effects after administration to mice and human participants [153]. Results from a recent clinical study involving 24 healthy male subjects implied that oral administration of 1000 mg/day of NR was well tolerated and lowered systolic blood pressure and aortic stiffness [154]. No improvement in insulin sensitivity or blood glucose control was attributed to the good health of the participants. In a study involving 40 obese and insulin-resistant men, Dollerup and colleagues reported no effects of NR (1000 mg x 2, 12 weeks) on glucose tolerance and function of β-cells of pancreas suggesting that NMN and NRPT (nicotinamide riboside and pterostilbene) could be considered in future clinical trials [155]. In 2016, the first

**Crocin** is a natural carotenoid found in Crocus sativus (saffron spice); it exhibited antioxidative activity in cyclophosphamide (CPM)-treated ovaries by increasing SOD2 and PGC-1α levels. SIRT1 level decreased as a result of a re-established redox balance [145] (Table 1).

A combination of polyphenols could have a positive synergistic effect on deacetylase activity of sirtuins. Giovannini and colleagues [114] demonstrated the stimulating effect of RSV and eight additional compounds (berberine, tyrosol, quercetin, catechin, ferulic acid, curcumin, niclosamide and malvidin) on the expression of SIRT1 in HeLa cells. The authors showed that the combined use of berberine and tyrosol or ferulic acid and quercetin or tyrosol and ferulic acid had a synergistic effect, as they increased SIRT1 expression for more than the sum of the two substances administered separately. A mixture of total flavonoids from Chinese hickory (Carya cathayensis Sarg.) prevented senescence of HUVEC cells by decreasing miR-19.1% and decreased apoptosis [143].

Berberine, an alkaloid found in berberine prolonged Drosophila lifespan by attenuating an aging-accelerating effect of temperature [141]. Berberine has multiple targets in cells but its anti-aging impact is mainly through the AMPK activation [142]. Berberine affected SIRT1 expression in human cells (L-02) causing increased cell viability (by 19.1%) and decreased apoptosis [143]. In vivo berberine activated AMPK/SIRT1/PGC-1α pathway and significantly improved cognitive and muscular function of 24-month-old rats [144] (Table 1).
A human clinical study for assessment of NMN effect started in Japan [156], but no data are yet available.

An alternative approach to elevate NAD+ in cells is by increasing the activity of nuclear, cytoplasmic, and mitochondrial isoforms of NAMPT (NAMPT1, NAMPT2, and NAMPT3), the key enzymes for NAD+ synthesis in main cellular compartments. This approach should be applied with caution since an increased expression of NAMPT has been measured in different cancers including breast, prostate, melanomas, etc. [157].

CONCLUSION

Delaying aging processes by reducing diseases may be an attractive strategy for promoting longevity. Several signaling pathways were implicated in the modulation of age-related diseases, the most understood are the IGF1 and SIRT1 pathways, which are modulated by nutrition. CR, which extends lifespan across species, mediates its function in part by modulating IGF1 and SIRT1 expression and function. CR reduces the bioavailability of IGF1 and also causes the activation of SIRT1, both directly and indirectly promoting longevity.

Nutraceuticals that are CR mimetics have the potential to promote longevity by modulating IGF1 and SIRT1 expression and function (Fig. 1). An increasing number of nutraceuticals were reported to modulate IGF1 and SIRT1. Many nutraceuticals promote longevity in rodents and there are limited data in non-rodent primates. Translation of animal data to humans is challenging, also because of human studies’ limitations that include ethical study design, regulatory limitations and costs.

LIST OF ABBREVIATIONS

- 2’-OAADPr = 2’-O-acetyl-ADP-Ribose
- AKT = Protein Kinase B
- AMPK = 5’ Adenosine Monophosphate-Activated Protein Kinase
- ATF4 = Activating Transcription Factor 4
- BHB = β-Hydroxy Butyrate
- CPM = Cyclophosphamide
- CR = Caloric Restriction
- DMC = 4,4′-Dimethoxychalcone
- EFNB = Ephrin Dependence Receptor Ligands
- FOXO = Forkhead Box
- HDAC = Histone Deacetylases
- HFD = High-calorie Diet or High-fat Diet
- hTERT = Human Telomerase
- HUC-F2 = Human Umbilical Cord Fibroblast
- HUVEC = Human Umbilical Vein Endothelial Cells
- IGF1 = Insulin-like Growth Factor 1
- IGF1R = Insulin-like Growth Factor 1 Receptor
- IGFBP-3 = IGF Binding protein-3
- IPF = Idiopathic Pulmonary Fibrosis
- IR = Insulin Receptor
- IRS1 = Insulin Receptor Substrate 1
- IRS2 = Insulin Receptor Substrate 2
- LKB1 = Liver Kinase B1

Fig. (1). Effect of CR mimetics on the modulation of IGF1R signaling and SIRT1 activity in promoting longevity.
REFERENCES


[22] NAM = Nicotinamide Mononucleotide

[23] PI3K = Phosphoinositide 3-kinase

[24] ROS = Reactive Oxygen Species

[25] RSV = Resveratrol

[26] SCFA = Short-Chain Fatty Acid

[27] SIRT1 = Silent mating Type Information Regul ation 2 homolog 1

[28] SNP = Single Nuclear Polymorphism

[29] STACs = Sirtuin-Activating Compounds

[30] Thc = Tetrahydrocurcumin

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CONFLICT OF INTEREST

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