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# Apigenin: a natural molecule at the intersection of sleep and aging

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NAD<sup>+</sup>, a pivotal coenzyme central to metabolism, exhibits a characteristic decline with age. In mice, NAD<sup>+</sup> levels can be elevated via treatment with apigenin, a natural flavonoid that inhibits the NAD<sup>+</sup>-consuming glycoprotein CD38. In animal models, apigenin positively impacts both sleep and longevity. For example, apigenin improves learning and memory in older mice, reduces tumor proliferation in a mouse xenograft model of triple-negative breast cancer, and induces sedative effects in mice and rats. Moreover, apigenin elongates survival in fly models of neurodegenerative disease and apigenin glycosides increase lifespan in worms. Apigenin's therapeutic potential is underscored by human clinical studies using chamomile extract, which contains apigenin as an active ingredient. Collectively, chamomile extract has been reported to alleviate anxiety, improve mood, and relieve pain. Furthermore, dietary apigenin intake positively correlates with sleep quality in a large cohort of adults. Apigenin's electron-rich flavonoid structure gives it strong bonding capacity to diverse molecular structures across receptors and enzymes. The effects of apigenin extend beyond CD38 inhibition, encompassing agonistic and antagonistic modulation of various targets, including GABA and inflammatory pathways. Cumulatively, a large body of evidence positions apigenin as a unique molecule capable of influencing both aging and sleep. Further studies are warranted to better understand apigenin's nuanced mechanisms and clinical potential.

## KEYWORDS

apigenin, sleep, aging, metabolism, NAD<sup>+</sup>, CD38

## 1 Introduction

One of the great early developments in the fields of biochemistry and metabolism was the discovery and characterization of nicotinamide adenine dinucleotide (NAD). The foundational work on this molecule occurred in multiple distinct stages (1). In 1906, Arthur Harden and William John Young proposed the existence of a “cozymase,” a chemical factor stable at high temperatures that increased the rate of the fermentation reaction in yeast (2). The involvement of a cofactor was an essential first insight, but they could not theorize on this factor's molecular structure and chemical behavior. In 1930, Hans von Euler and Karl Myrbäck determined that Harden and Young's cozymase contained an adenine

(the aromatic ring structure also present in DNA, ATP, Coenzyme A, and other essential molecules) (3), a reducing sugar group, and a phosphate group (4).

While the identification of NAD's structure allowed for theorizing on its mechanistic behavior, it was not until 1936 that Otto Warburg (the preeminent German scientist and Nobel laureate) and Walter Christian demonstrated that cozymase/NAD engaged in redox chemistry by transferring hydrides (5). NAD<sup>+</sup> (the oxidized form of NAD) serves as an oxidizing agent, and NADH (NAD<sup>+</sup> after receiving a hydride) functions as a reducing agent. It was subsequently demonstrated that NAD<sup>+</sup>/NADH plays a significant role in many diverse biochemical reactions that require electron exchange, a common motif across all living systems, including glycolysis, interconversions between pyruvate and lactate and pyruvate and acetyl-CoA,  $\beta$ -oxidation, the citric acid cycle, and oxidative phosphorylation (6). In addition, NAD<sup>+</sup> can be chemically modified to create other molecules of use, including phosphorylation of NAD<sup>+</sup> to NADP<sup>+</sup> by NAD<sup>+</sup> kinases (7), as well as conversion of NAD<sup>+</sup> to ADP-ribose (ADPR) and cyclic ADP-ribose (cADPR) by the glycoprotein and ecto-enzyme CD38 (8). The presence and functions of NAD<sup>+</sup> are conserved across all living cells, are associated with healthy and optimal function by biochemical and clinical measures, and both physical decline and aging correlate with lower NAD<sup>+</sup> levels in humans (9).

The enzyme CD38 is a significant consumer of NAD<sup>+</sup> and therefore, an essential regulator of its ambient concentrations and availability as a cofactor and substrate. CD38 was originally discovered in 1980 by Reinherz et al. (10). The finding was made as part of a larger study characterizing the surface of the T cell using monoclonal antibodies, which led to the identification of not only CD38 but also CD4, CD8, CD71/TFR-1, and others of varied functions, and CD38 was subsequently used as a marker of T cell identity (11, 12). Significant advancement in characterizing the biology of CD38 would come in 1992, when it was found to also be a glycoprotein cell surface marker on B cells, monocytes, bone marrow progenitors, and natural killer cells (13) and when experiments determined it to not only be a cell marker but a stimulator of activity in T and B cells (14).

As to what activity and its role in it, there soon came evidence that CD38 has dual enzymatic functions, catalyzing both the conversion of NAD<sup>+</sup> to cADPR as an ADP-ribosyl cyclase and the degradation of cADPR to ADPR as a cADPR-hydrolase (14, 15) (Figure 1). Under physiological conditions, 97% of the total output of CD38 is ADPR and the remaining 3% is the intermediate cADPR (16). Thus, CD38 requires a large amount of NAD<sup>+</sup> to generate a pool of cADPR. It was also shown that the locations and membrane orientation of CD38 vary widely; it is present in the plasma membrane facing both the extracellular milieu and the intracellular cytosol, in a soluble form in the cytosol, as well as in the nuclear and mitochondrial membranes, allowing its consumption

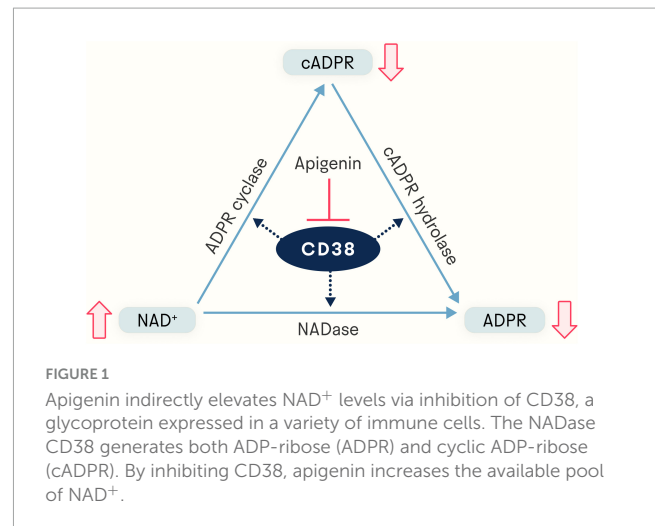


FIGURE 1

Apigenin indirectly elevates NAD<sup>+</sup> levels via inhibition of CD38, a glycoprotein expressed in a variety of immune cells. The NADase CD38 generates both ADP-ribose (ADPR) and cyclic ADP-ribose (cADPR). By inhibiting CD38, apigenin increases the available pool of NAD<sup>+</sup>.

of NAD<sup>+</sup> and production of cADPR and ADPR to influence extracellular and intracellular activities (17, 18).

While the quantity of cADPR produced is relatively small, it is still sufficient to contribute to cADPR's known role in calcium signaling (19). cADPR increases calcium-induced calcium release at lower cytosolic concentrations of Ca<sup>2+</sup> by targeting the Ca<sup>2+</sup> uptake mechanism of the endoplasmic reticulum (20, 21). cADPR activates ryanodine receptors to initiate the release of calcium stored in the endoplasmic reticulum (21, 22). ADPR, the dominant product of CD38's consumption of NAD<sup>+</sup>, is a strong synergistic agonist of the TRPM2 ion channel (23, 24). TRPM2, a calcium channel with connections to multiple age-related diseases (25, 26), is agonized by cADPR much more weakly than ADPR (24).

Given the many other more efficient means of regulating calcium signaling present in the cell, it has been theorized that the evolutionary role of CD38 is not to make cADPR and ADPR, but to deplete intracellular and extracellular NAD<sup>+</sup>. In this paradigm, cADPR and ADPR are just byproducts of NAD<sup>+</sup> depletion (17). This would make CD38 not an ADP-ribosyl cyclase *per se* but instead an NAD<sup>+</sup> glycohydrolase (27). Many studies have shown that high levels of CD38 and low levels of NAD<sup>+</sup> are associated with aging, immunity, metabolic health, and cancer development (17, 28).

Given the biological significance of NAD<sup>+</sup> and its regulator CD38, the discovery of the natural flavonoid 4,5,7-trihydroxyflavone (C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>), known as apigenin, and its characterization not only as an inhibitor of CD38 (thereby increasing levels of NAD<sup>+</sup> and decreasing levels of cADPR and ADPR) (Figure 1) but also as a broadly-acting molecule (29) with effects on sleep and aging holds therapeutic potential in humans. In this review, we provide an in-depth description of apigenin, diving into mechanistic evidence as well as its ability to impact health in animal models and humans.

## 2 Biosynthesis, biochemistry, and bioavailability

Apigenin is a flavone-class aglycone, a glycoside without its glycosyl group, of several natural glycosides. Flavones are one of

Abbreviations: 4CL, 4-coumarate CoA ligase; AD, Alzheimer's disease; ADPR, Adenosine diphosphate ribose (ADP-ribose); C4H, Cinnamate 4-hydroxylase; cADPR, Cyclic ADP-ribose; CHI, Chalcone isomerase; CHS, Chalcone synthase; CoA, Coenzyme A; FNS, Flavone synthase; FSP, Flavone synthesis pathway; GAD, Generalized anxiety disorder; GPP, General phenylpropanoid pathway; L-Phe, L-phenylalanine; L-Tyr, L-tyrosine; NAD, Nicotinamide adenine dinucleotide; PAL, Phenylalanine ammonia lyase; PD, Parkinson's disease; TAL, Tyrosine ammonia lyase.

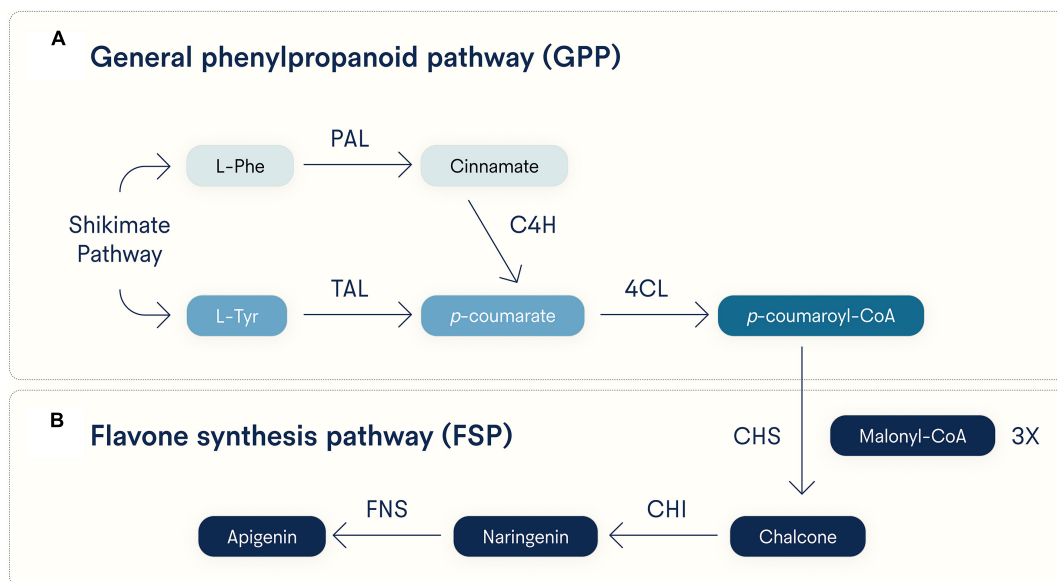


FIGURE 2

Major biosynthetic pathways for apigenin. Apigenin is synthesized in plants from either L-phenylalanine (L-Phe) or L-tyrosine (L-Tyr), both generated by the Shikimate pathway. Early and distinct reactive steps for each substrate converge on the last step of the (A) general phenylpropanoid pathway (GPP) and (B) a shared flavone synthesis pathway (FSP). Note that three equivalents of malonyl-CoA are incorporated into *p*-coumaroyl-CoA by CHS to generate chalcone. PAL, phenylalanine ammonia lyase; TAL, tyrosine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavone synthase (soluble FNS1 and membrane-bound FNS2 forms).

several sub-groups of flavonoids, the largest group of naturally occurring polyphenols that also include anthocyanidins, flavanols, flavanones, flavonols, and isoflavonoids (30, 31). Biosynthesis of apigenin only occurs in certain plants and, broadly speaking, flavonoids inhibit the transport of auxin, a morphogen-like plant hormone controlling growth and development (30).

As to its biosynthesis, apigenin can be generated via either of two converging pathways (Figure 2). One pathway starts with L-phenylalanine (L-Phe) and the other with L-tyrosine (L-Tyr), both products of the Shikimate pathway, a reactive process not found in animals but is used by plants to synthesize aromatic amino acids (32). Starting from L-Phe, it is converted to cinnamate via deamination by phenylalanine ammonia lyase (PAL), then oxidized by cinnamate 4-hydroxylase (C4H) to produce *p*-coumarate, which can also be made directly from L-tyrosine (L-Tyr) via deamination by tyrosine ammonia lyase (TAL) (33). The compound *p*-coumarate is then given a coenzyme A (CoA) at its carboxy group by 4-coumarate CoA ligase (4CL) to make *p*-coumaroyl-CoA. These two pathways toward *p*-coumaroyl-CoA synthesis comprise the general phenylpropanoid pathway (GPP, Figure 2A), and *p*-coumaroyl-CoA then enters the flavone synthesis pathway (FSP, Figure 2B) (33). In the FSP, chalcone synthase (CHS) uses three equivalents of malonyl-CoA to convert one *p*-coumaroyl-CoA to one chalcone. Chalcone isomerase (CHI) converts chalcone to naringenin, and finally, flavone synthase (either the soluble FNS1 or membrane-bound FNS2) oxidizes naringenin to apigenin (33).

The resulting product has a flavane nucleus of 15 carbon atoms (C6-C3-C6) and is considered a diphenyl-propanoid (34). The carbon atoms are arranged into two fused rings, the first of which is an oxygen-containing heterocycle and the second of which is a benzene ring constituting a phenylchromane nucleus,

and an additional phenyl group is attached to the phenylchromane base (34). The molecule's multiple rings, ketone group, and multiple hydroxyl groups create an electron-rich structure with a diverse assortment of binding affinities. Among other chemical feats, apigenin can scavenge hydroperoxide, chelate positive ions, and inhibit xanthine oxidase (35). Scavenging free radicals may be particularly important in apigenin's putative antiviral, anti-inflammatory, and antimutagenic properties (30, 36).

Of the flavonoids synthesized by plants, apigenin is one of the most commonly found across species. Apigenin is present, mainly in a glycosylated form, in several vegetables, herbs, beverages, and fruit, including parsley, celery, onions, chamomile, thyme, oregano, basil, tea, beer, wine, grapefruit, and oranges (37). Aside from the apigenin glycosides (the most common of which is apigenin-7-O-glucoside), there are also glucuronide, acetylated, and methyl ester forms, acylated varieties, as well as monomers, dimers, and larger polymers (34, 38). The apigenin glycoside and its by-products are more water soluble than unmodified apigenin, and the glycosidic form may have a higher bioavailability (36). As a polyphenol, apigenin is part of a class of molecules that are mainly absorbed in the small intestine; here, 5–10% of the total ingested quantity of apigenin is absorbed, mostly in monomeric or dimeric forms (39). A total of 90–95% of the remaining apigenin that is unabsorbed makes its way to the colon up to millimolar levels (40, 41). Metabolism of apigenin occurs in the liver and “phase one” metabolism utilizes cytochrome P450 (enzymes that metabolize many different drugs) and nicotinamide adenine dinucleotide phosphate and flavin-containing monooxygenase (29, 40, 42) to generate monohydroxy derivatives (41). “Phase two” (conjugative) metabolism conjugates these derivatives and requires UDP-glucuronosyltransferases (41), enteric and enterohepatic cycling

(43), glucuronidation and sulfation (29), and conversion into luteolin and sulfated and glucuronidated conjugates (44, 45).

In the intestinal lumen of the colon, apigenin is significantly metabolized by select species of microbiota adapted to utilize polyphenolic substrates (46, 47). This microbial activity converts polyphenols into smaller phenolic products (48, 49) that can be absorbed by the colon into circulation, and these metabolites may contribute to the health benefits ascribed to polyphenol intake, alongside the benefits of a given polyphenol (50, 51). Indeed, while 90–95% of ingested apigenin bypasses absorption in the small intestine and travels to the colon, animal studies have shown that less than 30% of the originally ingested quantity of apigenin is ultimately excreted in the feces, suggesting that a very significant portion of the total apigenin is metabolized by the microbiome into other molecules, which is indicative of physiological relevance (43, 52).

## 3 Apigenin and sleep

### 3.1 Animal models

Numerous studies across multiple animal models suggest that apigenin has connections with sleep and/or with its underlying neurobiology (Table 1). The invertebrate model *Drosophila melanogaster* can be used to study aspects of Parkinson's disease (PD), a neurodegenerative disease characterized by brain aggregates of alpha-synuclein and disrupted sleep (53). In a 2017 study, an alpha-synuclein-expressing transgenic *D. melanogaster* model of PD was used to assess the effects of dietary supplementation with apigenin on PD symptoms and biomarkers. Apigenin-supplemented animals demonstrated a reversal of many biochemical changes characteristic of PD, including alterations in glutathione-S-transferase activity and lipid peroxidation as well as levels of glutathione and oxidative stress (54). Findings germane to oxidative stress are intriguing given that, in flies, sleep deprivation causes early death via the accumulation of reactive oxygen species (55). Apigenin was also reported to decrease activity of monoamine oxidase and increase levels of dopamine (54). It would be interesting to know if this finding is reproducible in normal flies and in more complex rodent models. In rodents, supplementation with apigenin increased sedation or reduced locomotor activity in rodents (56, 57), factors that influence sleep latency. Similar experiments in mice also suggested that apigenin can protect against neurodegeneration while improving learning, memory, neurovascular activity, and levels of several key biomarkers, including Bdnf, Trkb, and Creb, all of whose levels are influenced by sleep quality (58).

### 3.2 Clinical studies

Clinical studies assessing apigenin's potential effects on sleep are promising, albeit limited (Table 2). Many of these studies utilize chamomile extract, which is often about 1% apigenin by mass (0.8–1.2%) and in which apigenin is a bioactive ingredient. It is difficult to be certain whether the effects observed from chamomile extract in these studies are solely due to apigenin, other

components of chamomile, or a combination of both. However, these results, combined with the animal data described above, suggest an important role for apigenin.

One study testing the effects of 270 mg of chamomile extract versus placebo twice per day for 28 days in patients with primary insomnia observed a trend toward improvement in daytime functioning, though it did not reach statistical significance (59). Another trial assessed the impact of chamomile extract versus placebo for eight weeks in patients with depression with or without anxiety. Subjects took a single 220 mg capsule daily in the first week and gradually increased this to five capsules (1100 mg) per day for the last four weeks. The authors observed significant reductions in depression and mood scores (60). While not measuring sleep directly, these mental health components are known contributors to sleep latency and quality, and vice versa (61). Additional studies have been conducted on multiple timescales assessing the potential of chamomile to treat anxiety. In one study, 1500 mg chamomile extract was given daily for eight weeks to two groups of patients, one with generalized anxiety disorder (GAD) and the other with GAD and comorbid depression. Chamomile extract reduced anxiety levels in both groups and attenuated depressive symptoms in the comorbid depression group (62). In a longer-term study, 500 mg chamomile extract was given three times daily for 12 weeks to patients with a primary diagnosis of moderate-to-severe GAD. Those whose GAD symptoms were ameliorated in response to treatment were then split into continued treatment versus placebo groups for 26 weeks. In addition to showing improved GAD symptoms during follow-up, chamomile-treated subjects also displayed significant reductions in body weight and mean arterial blood pressure (63).

Drinking chamomile tea after birth for two weeks also caused significant improvements in sleep efficiency and postnatal depression (64). These benefits largely disappeared four weeks post-test, though this could be due to broader changes in physiological activity weeks after birth, rather than a loss in efficacy of chamomile itself (65). Further, a topical formulation of chamomile containing chamazulene (an aromatic compound found in chamomile and related species) and apigenin was tested as a pain relief treatment in patients with migraine (66, 67), which can be a driver of poor sleep (67). Topical chamomile was found to significantly reduce pain, vomiting, nausea, phonophobia, and photophobia in patients with migraines (66). The analgesic properties may in part, be due to its reported ability to inhibit iNOS, PGE2, and COX2 (68). Further evidence comes from a recent cross-sectional study, which sought to identify associations between dietary levels of individual polyphenols and sleep quality. The flavonoid polyphenols apigenin and naringenin were both found to be significantly correlated with sleep quality. Specifically, a low level of dietary apigenin intake was associated with worse sleep quality (69). It would be interesting to better understand which of these effects are due to apigenin on its own or apigenin in conjunction with other molecules in chamomile.

### 3.3 Mechanistic evidence

Mechanistic studies suggest several mechanisms by which apigenin could increase sleep quality and quantity (Table 1).

TABLE 1 Animal studies where an intervention involving apigenin positively influenced sleep and/or aging.

Organism	Intervention	Effect(s)	Mechanistic Finding(s)	References
Worm	Liquid medium containing 200 or 400 $\mu\text{g}/\text{mL}$ apigenin glycosides until death	Extended lifespan and enhanced oxidative stress resistance	Reduced levels of reactive oxygen species Regulated the expression of several genes, including <i>daf-2</i> , <i>daf-16</i> , <i>sod-3</i> , <i>hsp-16.2</i> , <i>skn-1</i> , <i>gst-4</i> , <i>gcs-1</i> , <i>jnk-1</i> , and <i>sir-2.1</i> Promoted the translocation of <i>daf-16</i> and <i>skn-1</i> into the nucleus	(93)
	Diet containing 20, 40, or 80 $\mu\text{M}$ apigenin until death	Induced a mitohormetic response and prolonged life	Reduced ATP production by transiently inhibiting mitochondrial respiration and temporarily increasing reactive oxygen species, triggering increased mitohormesis and an adaptive increase in antioxidant capacity Lifespan extension by apigenin was mediated by <i>aat-2</i> , <i>daf-16</i> , and <i>skn-1</i>	(94)
Fly	Diet containing 10 $\mu\text{M}$ , 20 $\mu\text{M}$ , 40 $\mu\text{M}$ , or 80 $\mu\text{M}$ apigenin for 24 days	Elongated lifespan and improved biochemical parameters in Parkinson's disease flies	Increase in glutathione and dopamine levels Dose-dependent decreases in lipid peroxidation and activities of glutathione-S-transferase, monoamine oxidase, caspase-3, and caspase-9	(54)
	Treatment with 40 $\mu\text{M}$ or 80 $\mu\text{M}$ apigenin for seven days	Boosted lifespan and restored acetylcholinesterase activity in D-galactose-treated flies	Reduced oxidative stress Reduced opening of the mitochondrial permeability transition pore Lowered the activity of caspases 9 and 3	(92)
	Diet containing 25 $\mu\text{M}$ , 50 $\mu\text{M}$ , 75 $\mu\text{M}$ , or 100 $\mu\text{M}$ apigenin for 30 days	Preserved climbing ability and decreased oxidative stress in Alzheimer's disease flies	Decreased oxidative stress and inhibited acetylcholinesterase Prevented the formation of amyloid beta aggregates	(70)
Mouse	Intraperitoneal injection of 50 mg/kg apigenin three hours before lethal injection with lipopolysaccharide	Increases survival in mice treated with high doses of lipopolysaccharide	Inhibited production of proinflammatory cytokines $\text{IL-1}\beta$ , $\text{IL-8}$ , and $\text{Tnf}$ in lipopolysaccharide-stimulated mouse macrophages Non-canonical regulation of $\text{NF-}\kappa\text{B}$ activity <i>in vitro</i>	(95)
	Subcutaneous injection of 0.78 mg/kg apigenin for 10 days	Improved cardiometabolic health and thyroid parameters in mice with diabetes	Increased levels of insulin and thyroid hormone Reduced levels of glucose and cholesterol	(96)
	Oral administration of 1.5 mg/kg apigenin before 3 h of observation	Produced a mild sedative effect that was short-term	Locomotor activity was significantly lowered between 90 and 120 min after administration	(56)
	Oral administration of 10 mg/kg or 20 mg/kg apigenin daily for eight days	Improved learning and memory in mice with amyloid beta-induced amnesia	Protected neurovascular coupling, decreased neurovascular oxidative damage, and enhanced cerebral blood flow Blocked Acetylcholinesterase activity and increased acetylcholine levels Partially reversed the amyloid beta-driven decrease in <i>Bdnf</i> , <i>TrkB</i> , and phospho-CREB levels	(58)
	Intraperitoneal injection of 100 mg/kg apigenin for seven days	Elevated $\text{NAD}^+$ levels and enhanced various aspects of sugar and lipid metabolism in obese animals	Inhibited the $\text{NADase Cd38}$ and augmented hepatic $\text{NAD}^+$ levels Decreased global protein acetylation Improved glucose and lipid homeostasis	(97)
	Intraperitoneal injection of 20 or 40 mg/kg apigenin daily for 21 days	Lowered immobility time in depressive-like mice	Decreased the level of corticosterone while elevating the amount of <i>Bdnf</i>	(73)
	Intraperitoneal injection of 10 or 20 mg/kg apigenin daily for 20 days	Prevented behavioral and cognitive impairments in a mouse model of seizures	Increased levels of <i>Bdnf</i> , <i>Creb</i> , and phosphorylated <i>Creb</i> in the hippocampus Elevated level of serotonin Did not significantly affect seizure severity	(74)
	Oral administration of 0.5 mg/ml of apigenin daily for 42 days	Downregulated inflammatory genes and enhanced both learning and memory	Induced transcriptional changes linked to immunity, inflammation, and cytokine regulation Reduced inflammation and senescence in astrocytes	(101)
	Oral administration of 25 mg/kg of apigenin daily for 28 days	Restored heart function and inhibited cardiac inflammation in a mouse model of cardiomyopathy	In the heart, enhanced the transcription of genes related to the mitochondrial unfolded protein response	(99)
	Oral administration of five or 50 mg/kg apigenin for 56 days	Ameliorated atherosclerosis and improved metabolic parameters in a mouse model of atherosclerosis and non-alcoholic fatty liver disease	Reduced body weight and lowered plasma lipid levels Protected against the upregulation of inflammatory genes and pathways	(100)
	Intraperitoneal injection of 25 mg/kg apigenin daily for 28 days	Reduced tumor proliferation in a mouse model of triple-negative breast cancer	Induced widespread transcriptional changes associated with alternative splicing Elevated apoptosis in tumor cells	(102)

(Continued)

TABLE 1 (Continued)

Organism	Intervention	Effect(s)	Mechanistic Finding(s)	References
Rat	Intraperitoneal injection of 25 mg/kg 15 min before pharmacological tests	Induced a sedative effect characterized by reduced locomotor activity	Observed effect was not mediated by an interaction with GABA-benzodiazepine receptors	(57)
	Oral administration of 75 mg/kg apigenin for 14 days	Protected diabetic animals against myocardial infarction	Reduced activity of creatine kinase and lactate dehydrogenase Decreased cell death and oxidative stress Increased expression of myocardial Pparg	(98)
	Intraperitoneal injection of 10 or 20 mg/kg apigenin for 14 days	Improved behavioral and biochemical signatures in a rat model of Parkinson's disease	Decreased levels of Tnf- $\alpha$ , Il-6, and iNos1 Maintained mRNA levels of <i>Bdnf</i> and <i>Gdnf</i>	(77)

In *Drosophila*, dietary supplementation with apigenin decreased oxidative stress and acetylcholinesterase activity (70), reduced glutathione-S-transferase activity and lipid peroxidation, and increased glutathione levels, all of which are thought to support healthy sleep (71, 72). Apigenin supplementation in rodents decreased corticosterone levels and increased levels of Bdnf (73), CREB, phosphorylated CREB, and serotonin in the hippocampus (74, 75). These findings support the hypothesis of a pro-sleep role for apigenin, as the stress response is known to impair sleep (76). Moreover, BDNF, CREB, phosphorylated CREB (phospho-CREB), and serotonin have all been shown to be sleep-promoting, and BDNF, phospho-CREB, ACh, and TrkB were all found to be increased after apigenin treatment in a mouse model of amyloid beta (A $\beta$ )-driven neurodegenerative disease (58).

In rats, apigenin demonstrated GABAergic activity that was independent of GABA-benzodiazepine receptors and possibly mediated by the GABA<sub>A</sub> receptor (57). Apigenin also decreased levels of Tnf- $\alpha$ , Il-6, and iNos1 while maintaining elevated levels of Bdnf and glial Gdnf mRNA (77, 78). Findings involving BDNF are interesting given that this protein was reported to be significantly lower in insomniac patients with short sleep duration (79). Reports of apigenin ameliorating inflammatory markers are similarly intriguing, given a recent study showing that protracted sleep deprivation leads to severe inflammation in the mouse brain (80). While these studies suggest a prominent link between apigenin and sleep biomarkers, very little research has been done explaining how apigenin causally regulates sleep-relevant pathways. As such, future research is warranted to better understand the mechanistic relationship between apigenin and sleep health.

### 3.4 Sleep and aging

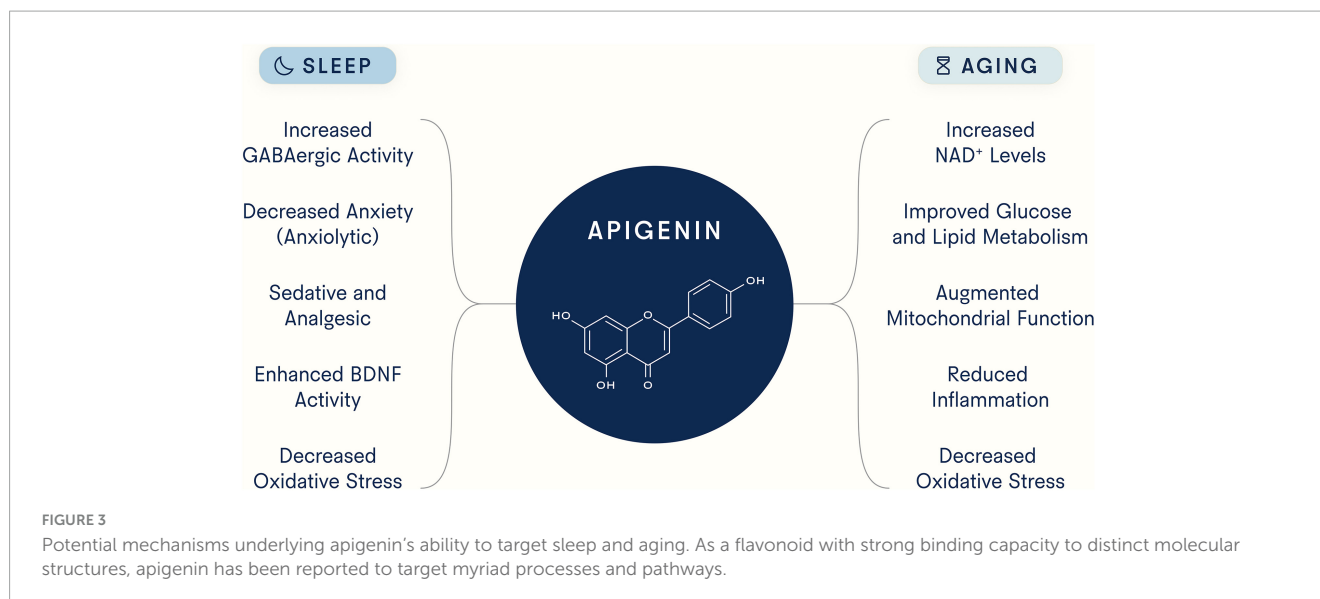
Given the evidence described above, it is important to note the close connection and interdependence between aging and sleep. Poor sleep can accelerate aging, as indicated by its alteration of age-associated epigenetic biomarkers (81) and its strong correlation with mortality risk (82) and healthspan (83). In turn, aging often leads to reduced sleep quality (84), producing a vicious cycle between the two. However, if poor sleep accelerates aging, which further worsens sleep, then improved sleep could improve aging, which would mitigate sleep's decline. Thus, the interdependence between sleep and aging may complicate our understanding of apigenin's effects. Longevity benefits reported in animal models could, for example, be due in part to improvements in sleep, a well-established reparative process. While this may be true, a closer look

at the mechanistic data reported suggests that apigenin can directly mitigate established hallmarks of aging. Apigenin's ability to act on a diversity of targets and processes makes it more likely that aging and sleep are largely being independently influenced.

At least some component of apigenin's efficacy may be due to apigenin-derived metabolites generated by the intestinal microbiome, wherein different products may each contribute to specific elements of apigenin's effects once entered into circulation. The microbiota has well-described roles in healthy sleep and aging, and dysbiosis is a known contributor to declines in both (85, 86). Indeed, dysbiosis is one of the 12 formally recognized hallmarks of aging (87). Changes in microbial composition could also at least partially explain the interdependence of sleep and aging, alongside other key factors like epigenetics and signaling molecules. Poor sleep quality and aging both alter distributions of microbial species, which alter the distributions of the end products of microbial chemistry and their resulting effects (88, 89). In addition, dietary supplementation with probiotics and targeted lifestyle modifications aimed at improving intestinal flora have

TABLE 2 Human clinical trials involving apigenin-containing chamomile.

Subject Status	Intervention	Clinical Finding(s)	References
Primary insomnia	540 mg chamomile extract	Trended toward an improvement in daytime functioning	(59)
Generalized anxiety disorder with or without comorbid depression	220–1100 mg chamomile extract	Improvements in depression and mood scores	(60)
Postnatal with poor sleep quality	Chamomile tea	Scores for symptoms of depression and physical-symptoms-related sleep inefficiency were lowered	(64)
Generalized anxiety disorder	1500 mg chamomile extract	Attenuation of anxiety symptoms	(63)
Migraine without aura	2 ml of topical chamomile oleogel	Reductions in nausea, pain, phonophobia, vomiting, and photophobia	(66)
Generalized anxiety disorder with or without comorbid depression	1500 mg chamomile extract	Amelioration of anxiety	(62)



been reported to improve sleep quality and mitigate immune and inflammatory aspects of the aging phenotype (90). While it's clear that sleep can influence aging and vice versa, additional research is needed to understand this relationship on a deeper level. Moreover, it would be intriguing to learn if apigenin's ability to influence aging (described in detail below) is due, at least in part, to effects on sleep.

## 4 Apigenin and aging

### 4.1 Animal models

Numerous research studies have examined the ability of apigenin to impact lifespan and/or age-related disease (Table 1). One model in *Drosophila* employs D-galactose, a compound that accelerates aging, shortens lifespan, and increases both oxidative stress and lipid peroxidation (91). In this model, apigenin partially reversed the lifespan-shortening effects of D-galactose (92). In a separate transgenic *Drosophila* model of Alzheimer's disease (AD) expressing Amyloid Beta-42 in neurons and producing AD-like amyloid beta aggregates, a daily diet containing apigenin for 30 days was shown to delay the loss of climbing typically seen in this model (70). Apigenin similarly increases survival in a fly model of PD (54) and, in *Caenorhabditis elegans*, dietary supplementation with apigenin or apigenin glycosides increases lifespan (93, 94).

Promising results have also been documented in vertebrate animal models. In mice, intraperitoneal injection of apigenin before lipopolysaccharide injection strongly blunts the pro-inflammatory and immunomodulatory effects of lipopolysaccharide that are otherwise lethal at high doses (95). In diabetic mice, subcutaneous injection of apigenin improved cardiometabolic health and thyroid function (96). Intraperitoneal injection of apigenin improved glucose and lipid homeostasis in obese mice (97). Further, a diet supplemented with apigenin restored cardiac function and reduced the risk for isoproterenol-induced myocardial infarction in rats with streptozotocin-induced diabetes (98). In a separate mouse model of cardiomyopathy, apigenin delivered via gavage improved heart function and suppressed cardiac inflammation (99). Similarly,

in a mouse model of atherosclerosis and non-alcoholic fatty liver disease, dietary apigenin reduced atherosclerosis, hepatic lipid accumulation, body weight, and plasma lipid levels (100). In addition, giving older mice apigenin in drinking water was shown to downregulate genes associated with immune activation and improve both learning and memory (101). In a recent study using a mouse xenograft model of triple-negative breast cancer, daily intraperitoneal injection of apigenin increased apoptosis in tumor cells and reduced tumor proliferation (102).

### 4.2 Clinical studies

Clinical studies have yet to directly investigate apigenin's ability to influence aging. Some studies have assessed apigenin more directly, though mostly in connection to sleep, depression, pain, and anxiety, as described previously (Table 2). While sleep and mental health quality are significant contributors to aging and health (84, 103), human studies assessing apigenin's potential effect on established biomarkers of aging, such as markers of cardiometabolic health, motor function, and cognition, are certainly needed. Some clinical studies have utilized other flavonoids chemically similar to apigenin, such as quercetin. With the exception of quercetin's two additional hydroxyl groups, quercetin and apigenin are chemically identical (104). Other research has employed wider varieties of non-flavonoid polyphenols, including phenolic acids, lignans, and stilbenes (105), and many molecules across the flavonoid and non-flavonoid polyphenol classes have been reported to target hallmarks of aging, including cellular senescence (104).

As described heretofore, one of apigenin's better-characterized functions is to increase levels of NAD<sup>+</sup> via inhibition of the NADase enzyme CD38. Specifically, mouse NAD<sup>+</sup> levels in the liver were nearly doubled in response to apigenin (97). The role of NAD<sup>+</sup> in health and longevity is beyond the scope of this review but has been comprehensively described elsewhere (106). Previous work has shown that NAD<sup>+</sup> levels decline with age (107), including in specific tissues such as the brain (108). This is due

in part to increased consumption and decreased production and salvage of NAD<sup>+</sup> (109, 110). Older humans with higher levels of NAD<sup>+</sup> relative to their age group tend to be healthier and exhibit fewer age-associated disease phenotypes (111). While NAD<sup>+</sup> levels often decline with age in human skeletal muscle, exercise-trained older individuals display NAD<sup>+</sup> levels comparable to younger subjects (112).

Clinical studies indicate that NAD<sup>+</sup> levels can be modulated by behavior change and/or supplementation. In humans and mice, exercise of moderate intensity and resistance training appears to increase levels of circulating NAD<sup>+</sup>, NADH, and NAMPT (113, 114), providing one of several possible biochemical explanations linking exercise to healthy aging. Direct supplementation in humans with NAD<sup>+</sup> precursors may also offer benefits. Analogs of nicotinic acid, an NAD<sup>+</sup> precursor also known as niacin or vitamin B3, improved mitochondrial function in skeletal muscle in patients with diabetes (115). Nicotinamide mononucleotide, a precursor to NAD<sup>+</sup>, was reported to be safe and well-tolerated (116), increase NAD<sup>+</sup> levels (117), augment insulin sensitivity in muscle (118), enhance energy (119), and reduce blood pressure (120). Nicotinamide mononucleotide's effects were found to be more mixed on grip strength (117, 121) and walking ability (121, 122). Nicotinamide riboside, another NAD<sup>+</sup> precursor reported to be safe and well-tolerated (123), was similarly shown to increase levels of NAD<sup>+</sup> (124). Nicotinamide riboside was also reported to decrease inflammation and enacted transcriptional changes pertinent to metabolism and mitochondrial function in healthy and overweight individuals (125). While exciting, larger, longer-term clinical trials are needed to better understand the safety and efficacy of direct NAD<sup>+</sup> precursors like nicotinamide mononucleotide and nicotinamide riboside.

### 4.3 Mechanistic evidence

While apigenin may be positively influencing aging by elevating NAD<sup>+</sup> levels, it could also have other systemic pro-longevity effects (Table 1). Apigenin mitigated D-galactose-driven accelerated aging in *Drosophila* by attenuating oxidative stress, reducing the opening of the mitochondrial permeability transition pore, and reducing the activity of caspases 9 and 3 (92). Concomitant with slowing disease progression in a transgenic fly model of AD, apigenin decreases oxidative stress, lowers acetylcholinesterase activity, and inhibits the formation of amyloid beta-42 aggregates (70). Long-lived worms treated with apigenin glycosides exhibited reduced reactive oxygen species levels and altered expression of numerous genes, including *daf-2*, *daf-16*, *sod-3*, *hsp-16.2*, *skn-1*, *gst-4*, *gcs-1*, *jnk-1*, and *sir-2.1*. Apigenin glycosides also promoted the translocation of *daf-16* and *skn-1* into the nucleus (93). In a more recent worm study, apigenin was shown to prolong lifespan by transiently inhibiting mitochondrial respiration and temporarily increasing reactive oxygen species levels. This, in turn, triggered a mitohormetic response which led to an overall increased capacity to deal with oxidative stress. Life extension also depended on the genes *aak-2*, *daf-16*, and *skn-1* (94). Such findings are interesting given that, in a mouse model of myocardial injury, the protective effects of apigenin were dependent on the mitochondrial unfolded protein response (99).

In lipopolysaccharide-stimulated mouse macrophages, apigenin inhibits the production of the proinflammatory cytokines Il-1 $\beta$ , Il-8, and Tnf. This flavonoid also engages in non-canonical regulation of NF- $\kappa$ B transcription factor activity through hypophosphorylation of Ser536 in the p65 subunit and the inactivation of the lipopolysaccharide-stimulated IKK complex (95). Apigenin has also been reported to improve glucose and lipid homeostasis in mice by increasing levels of insulin and thyroid hormone and reducing levels of glucose, glucose-metabolizing liver enzymes, and cholesterol (96). Further, *Cd38* knockout mice fed a high-fat diet have higher levels of NAD<sup>+</sup>, are less likely to develop obesity and metabolic syndrome, and have increased survival compared to wild-type animals (97). In contrast, *Parp1* knockout mice show worse survival on a high-fat diet. This may be due to the role *Parp1* plays in DNA repair and genomic stability (97). Additionally, in *Ldlr* and *Nlrp3* knockout mice fed a high-fat diet, apigenin appeared to reverse the cardiac and hepatic symptoms of the *Ldlr*<sup>-/-</sup> genotype in an inflammasome-dependent manner, as the apparent benefits of apigenin were abrogated in the double knockout, and treatment of liver cells cultured *in vitro* demonstrated consistent findings (100).

Apigenin's cognitive effects may be mediated by specific cell types. Older mice given apigenin in drinking water experienced glial cell-associated transcriptional changes across immunity, inflammation, and cytokine regulation into expression profiles that were characteristic of younger animals, and they also exhibited reduced inflammation and cellular senescence in astrocytes (101). In a recent study using immuno-deficient mice implanted with human tumor cell xenografts, apigenin appeared to induce transcriptome-wide reprogramming of cancer-associated alternative splicing in an RNA binding protein (hnRNPA2)-associated manner and induce switching of cancer-associated to non-cancer-associated alternative spliced isoforms (102). Studies in rats also allude to potential mechanisms of action for apigenin. Work by Ogura et al showed that, in a rat model of diabetic kidney disease, apigenin ameliorated renal injuries, pro-inflammatory gene expression, and tubular cell damage. In the kidney, apigenin also elevated the NAD<sup>+</sup>/NADH ratio and enhanced the activity of Sirt3 (126). In rats, protein expression of the nuclear receptor Pparg in the myocardium was elevated by apigenin (98).

## 5 Conclusion and future directions

Although compelling evidence suggests that apigenin exhibits both pro-longevity and pro-sleep properties (Figure 3), many important questions remain. First, given the binding promiscuity of flavonoid polyphenols and apigenin's broad systemic effects, additional work is needed to determine (1) whether there are additional targets of apigenin, (2) optimal dosage and safety over longer treatment periods and in different patient populations, and (3) the biological effects of all of apigenin's known chemical derivatives and the extent to which each contributes to the health benefits described here. There are many chemical forms of apigenin, each with unique features and products, that could affect biology in different ways, and to date there has not been extensive work aimed at comparing them. For example, Elkhedir et al. specifically used apigenin 6-C-arabinoside-8-C-glucoside and



apigenin 6,8-di-C-glucoside, the predominant derivatives in green pepper (93). While this study found animals treated with these compounds experienced multiple longevity benefits, further work is needed to determine which of these benefits are unique to apigenin derivatives versus apigenin itself. In addition, the therapeutic effects of apigenin could potentially be enhanced by improving its bioavailability, given its low absorption rate in the small intestine. However, the potential benefits of increased absorption of apigenin in the small intestine must be weighed against the reduced availability of apigenin in the large intestine for microbial conversion to smaller phenolic metabolites, which, as stated earlier, are also absorbed into the circulation and could exert their own effects on sleep and aging.

Further, not all mechanisms of increasing NAD<sup>+</sup> levels are similarly beneficial or effective. For example, elevating NAD<sup>+</sup> levels by inhibiting CD38 - an immune cell glycoprotein - may be more desirable than elevating NAD<sup>+</sup> levels by inhibiting PARP1 - an enzyme that responds to DNA damage and promotes DNA repair. Systematic comparisons and risk/benefit analyses of different interventions that boost NAD<sup>+</sup> levels would be valuable. More clinical studies are also needed to assess the ability of apigenin on its own - as opposed to apigenin in chamomile extract - to impact sleep-relevant parameters. Lastly, additional research is warranted to illuminate apigenin's mechanisms of action.

## Author contributions

DK: Conceptualization, Visualization, Writing—original draft, Writing—review and editing. AJ: Conceptualization, Visualization, Writing—original draft, Writing—review and editing.

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