

RESEARCH MONOGRAPH . COMPOUND PROFILE

Spermidine

An endogenous polyamine present in all eukaryotic cells, distinguished among geroprotective candidates by physiological autophagy induction via EP300 acetyltransferase inhibition, eIF5A hypusination, and epidemiologically validated cardiovascular and cognitive protection in aging populations.

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ABSTRACT

Spermidine (N-(3-aminopropyl)butane-1,4-diamine) is a naturally occurring triamine polyamine present in all living cells and in the human diet, principally from wheat germ, soybeans, fermented foods, and aged cheeses. First observed as a crystalline component of human semen by Antonie van Leeuwenhoek in 1678, spermidine was structurally characterized in the early twentieth century and subsequently identified as a central metabolite in polyamine biosynthesis, formed by the transfer of an aminopropyl group from decarboxylated S-adenosylmethionine to putrescine by the enzyme spermidine synthase. The compound participates in multiple essential cellular processes including chromatin structure modulation, translational regulation through hypusination of eukaryotic translation initiation factor 5A (eIF5A), cell proliferation, and the induction of macroautophagy.

The geroprotective potential of spermidine was established in a series of investigations beginning with the Eisenberg et al. (2009) demonstration that exogenous spermidine extends chronological lifespan in yeast, nematodes, and flies through autophagy-dependent mechanisms [1]. The molecular basis for autophagy induction was subsequently characterized as inhibition of the acetyltransferase EP300 (p300), resulting in hypoacetylation of core autophagy proteins (ATG5, ATG7, ATG12, and LC3) and convergent deacetylation of cytoplasmic proteins that parallels the acetylproteome shifts produced by caloric restriction and by other caloric restriction mimetics [2, 3]. Spermidine thereby occupies a mechanistically distinct position among autophagy inducers: it acts through acetyltransferase inhibition rather than

through mTOR suppression (rapamycin), AMPK activation (metformin), or sirtuin activation (resveratrol).

The landmark Eisenberg et al. (2016) study in *Nature Medicine* demonstrated that oral spermidine supplementation extends lifespan in mice, reduces cardiac hypertrophy, preserves diastolic function in aged animals, delays progression to heart failure in salt-sensitive hypertensive rats, and enhances cardiac autophagy, mitophagy, and mitochondrial respiration in an ATG5-dependent manner [4]. Epidemiological analysis of the Bruneck Study cohort (n = 829, 20-year follow-up) within the same report identified a significant inverse association between dietary spermidine intake and cardiovascular mortality, all-cause mortality, and cancer-related mortality, with the highest-intake tertile exhibiting a risk reduction comparable to approximately 5.7 years of aging [4, 5]. These findings were extended by Kiechl et al. (2018), who confirmed the inverse relationship between dietary spermidine and mortality in a larger epidemiological analysis [5].

Clinical investigation of spermidine has advanced through several randomized controlled trials. The SmartAge trial (Wirth et al. 2018, 2022) evaluated spermidine-rich wheat germ extract supplementation in older adults with subjective cognitive decline; a 3-month pilot study reported modest memory improvement, while a 12-month Phase IIb trial did not demonstrate significant modification of memory performance or biomarkers at the studied dose [6, 7]. The POLYCAD trial (NCT05128331), a Danish randomized double-blind placebo-controlled study of 24 mg/day spermidine in 187 elderly patients with coronary artery disease, completed enrollment in 2025 and represents the first dedicated cardiovascular outcomes trial for spermidine [8]. Additional trials have examined metabolic responses to spermidine

supplementation (NCT05459961) and dose-escalation safety in aging populations.

Pharmacokinetic studies have revealed that dietary spermidine is rapidly absorbed from the intestinal lumen but is subject to extensive presystemic conversion to spermine, resulting in minimal elevation of circulating spermidine concentrations following oral supplementation at doses up to 40 mg/day [9, 10]. This observation suggests that the biological effects of oral spermidine may be mediated through local gastrointestinal and first-pass hepatic mechanisms, through polyamine interconversion in target tissues, or through modulation of gut microbiota-derived polyamine pools rather than through systemic plasma exposure.

Spermidine supplementation has demonstrated a favorable safety profile in all completed human studies. No serious adverse events attributable to the compound have been reported. The European Food Safety Authority authorized spermidine-rich wheat germ extract as a Novel Food in 2021 with a recommended upper intake of 6 mg/day of spermidine [11]. Higher doses (up to 40 mg/day of purified spermidine for 28 days) have been evaluated without significant adverse effects in healthy older men [10]. This monograph reviews the chemistry, biosynthesis, and dietary sources of spermidine; the molecular pharmacology of autophagy induction, eIF5A hypusination, and anti-inflammatory signaling; pharmacokinetics; the preclinical and clinical evidence base across cardiovascular, cognitive, and geroprotective applications; sourcing and quality verification; reconstitution and handling; stack-interaction considerations; adverse events and safety signal; and a comparative assessment of five geroprotective and autophagy-inducing compounds (rapamycin, resveratrol,

nicotinamide mononucleotide, urolithin A, and metformin)
against spermidine on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

IUPAC name: N-(3-aminopropyl)butane-1,4-diamine.

Common name: Spermidine.

Synonyms: 1,5,10-triazadecane; 4-azaoctamethylenediamine; N-(3-aminopropyl)-1,4-diaminobutane.

Chemical Abstracts Service registry numbers: 124-20-9 (free base). The trihydrochloride salt (CAS 334-50-9) is the most commonly supplied research-grade form.

PubChem CID: 1102.

ChemSpider ID: 1071.

KEGG compound ID: C00315.

DrugBank ID: DB03566.

Molecular formula: C₇H₁₉N₃ (free base); C₇H₂₂Cl₃N₃ (trihydrochloride salt).

Structural formula: NH₂(CH₂)₃NH(CH₂)₄NH₂. The compound is a linear aliphatic triamine consisting of a 1,4-diaminobutane (putrescine) moiety linked through one terminal amine to a 3-aminopropyl group.

1.2 Structural class and chemistry

Spermidine is a member of the polyamine class of organic polycations, which includes putrescine (a diamine), spermidine (a triamine), and spermine (a tetraamine). At physiological pH (7.4), all three nitrogen atoms of spermidine are protonated, conferring a net charge of approximately +3 and enabling electrostatic interactions with negatively charged macromolecules including DNA, RNA, phospholipids, and acidic proteins [12].

Molecular weight: 145.25 g/mol (free base); 254.63 g/mol (trihydrochloride salt).

Physical properties: Colorless to pale yellow hygroscopic liquid (free base) or white crystalline powder (trihydrochloride salt). The free base is miscible with water and has a strong amine odor. Boiling point of the free base is approximately 129 degrees Celsius at 2 mmHg. The trihydrochloride salt is freely soluble in water, sparingly soluble in ethanol, and insoluble in diethyl ether. Melting point of the trihydrochloride salt is approximately 250 to 254 degrees Celsius with decomposition.

Stereochemistry: Spermidine is achiral. The molecule contains no stereocenters. All commercial forms are therefore the single constitutional isomer without enantiomeric considerations.

1.3 Biosynthesis

Spermidine is biosynthesized in all eukaryotic and most prokaryotic cells through the polyamine biosynthetic pathway. The pathway begins with the decarboxylation of L-ornithine by ornithine decarboxylase (ODC; EC 4.1.1.17) to yield putrescine (1,4-diaminobutane). ODC is the rate-limiting enzyme of the pathway and is subject to tight transcriptional, translational, and post-translational regulation, including rapid proteasomal degradation mediated by the protein antizyme [13]. Putrescine is then converted to spermidine by spermidine synthase (SRM; EC 2.5.1.16), which transfers an aminopropyl group from decarboxylated S-adenosylmethionine (dcSAM) to one terminal amino group of putrescine. The dcSAM donor is produced from S-adenosylmethionine by S-adenosylmethionine decarboxylase (AMD1; EC 4.1.1.50), a second rate-limiting enzyme of the pathway. Spermidine can be further converted to spermine by spermine synthase (SMS; EC 2.5.1.22), which transfers a second aminopropyl group from dcSAM to the opposite terminal amino group. The interconversion between spermine and spermidine is mediated by spermidine/spermine N1-acetyltransferase (SAT1) and polyamine oxidase (PAOX), which acetylate and oxidize spermine back to spermidine through the catabolic arm of the polyamine pathway [14].

Intracellular spermidine concentrations in mammalian cells are in the range of 0.1 to 1.0 millimolar, maintained through the balance of biosynthesis, catabolism, and membrane transport. Tissue concentrations decline with age in multiple organs, and the age-associated decline in spermidine levels has been proposed as a contributing factor to the age-dependent decline in autophagy and the accumulation of dysfunctional mitochondria and protein aggregates [1, 4].

2. Abstract

Spermidine is an endogenous polyamine and caloric restriction mimetic that induces macroautophagy through inhibition of the acetyltransferase EP300, modulates translational fidelity through hypusination of eIF5A, and exerts cardioprotective, neuroprotective, and anti-inflammatory effects that have been validated in organisms ranging from yeast to humans [1, 2, 4]. The compound is present in the daily human diet at estimated intakes of 7 to 25 mg per day (population-dependent), with the highest concentrations found in wheat germ, soybeans, natto, aged cheeses, mushrooms, and whole grains [5, 15].

The geroprotective thesis for spermidine rests on three convergent lines of evidence. First, exogenous spermidine extends lifespan in yeast (*Saccharomyces cerevisiae*), nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), and mice, with lifespan extension abolished when essential autophagy genes are deleted or pharmacologically inhibited [1, 4]. Second, epidemiological studies in human populations have identified significant inverse associations between dietary spermidine intake and cardiovascular mortality, all-cause mortality, and cancer-related mortality, with effect sizes comparable to several years of reduced biological aging [4, 5]. Third, clinical trials have evaluated spermidine supplementation in older adults with subjective cognitive decline and in patients with coronary artery disease, producing preliminary evidence of tolerability and biological activity, though definitive efficacy data from large-scale randomized trials remain pending [6, 7, 8].

The molecular pharmacology of spermidine is multifactorial. The autophagy-inducing mechanism operates through EP300 acetyltransferase inhibition, which reduces acetylation of ATG5, ATG7, ATG12, and LC3 and thereby derepresses the autophagy initiation and elongation machinery [2, 3]. This mechanism converges with but is distinct from mTOR-dependent autophagy induction (rapamycin) and AMPK-dependent autophagy induction (metformin). A second major mechanism is the provision of the aminobutyl moiety for hypusination of eIF5A, a post-translational modification unique to this translation factor and essential for the translation of mRNAs encoding polyproline-rich proteins, mitochondrial biogenesis factors, and autophagy regulators [16, 17]. A third mechanism involves anti-inflammatory activ-

ity through suppression of NF-kappaB signaling and modulation of T-cell differentiation, contributing to the immunomodulatory and anti-inflammatory effects observed in cardiovascular and neurological disease models [18].

The compound is well tolerated in human studies at doses ranging from 1.2 to 40 mg per day for durations up to 12 months. No serious adverse events have been attributed to spermidine supplementation. The principal limitations of the current clinical evidence are the modest sample sizes of completed trials, the equivocal efficacy data from the SmartAge cognitive trial, and the absence of completed cardiovascular outcomes trials (the POLYCAD trial is ongoing). The preclinical evidence base, the epidemiological associations, and the established safety profile support continued investigation.

3. Discovery and Development History

3.1 Early observations and structural characterization

The polyamines were first observed in 1678 when Antonie van Leeuwenhoek described crystalline structures in human semen using his single-lens microscope [19]. These crystals were subsequently identified as spermine phosphate. The chemical characterization of the polyamines proceeded over the following two centuries: spermine was structurally elucidated by Rosenheim in 1924, and the triamine spermidine was isolated and characterized as a distinct compound by Dudley, Rosenheim, and Starling-Rosenheim in 1926, who demonstrated its presence in tissues and in biological fluids distinct from semen [19, 20].

3.2 Biosynthetic pathway elucidation

The biosynthetic pathway from ornithine to putrescine to spermidine to spermine was elucidated in the 1950s through 1970s through the work of multiple laboratories. Tabor and Tabor (1964, 1984) provided definitive reviews of the pathway enzymology [21]. Ornithine decarboxylase, the rate-limiting enzyme, was characterized by Pegg and Williams-Ashman in the 1960s, and its remarkably rapid proteasomal turnover (half-life of approximately 10 to 30 minutes, among the shortest of any mammalian enzyme) was established by subsequent investigations. S-adenosylmethionine decarboxylase was characterized by Pegg and colleagues. Spermidine synthase and spermine synthase were purified and characterized in the 1970s and 1980s [13, 14].

3.3 Discovery of eIF5A hypusination

The unique post-translational modification hypusination was discovered by Park and colleagues in 1981 [16]. Hypusine (N-epsilon-(4-amino-2-hydroxybutyl)lysine) is formed exclusively on a single conserved lysine residue (Lys50 in human eIF5A) through a two-step enzymatic process: deoxyhypusine synthase (DHPS) transfers the aminobutyl moiety from spermidine to the epsilon-amino group of Lys50, and deoxyhypusine hydroxylase (DOHH) subsequently hydroxylates the intermediate deoxyhypusine to produce hypusine. This modification is found only on eIF5A and

is essential for its function in translation elongation at polyproline stretches, in translation termination, and in mRNA turnover [16, 17]. The discovery established spermidine as an essential metabolite for translational regulation, distinct from its roles in chromatin compaction and membrane stabilization.

3.4 Autophagy and geroprotection

The connection between spermidine and autophagy was established by Eisenberg et al. (2009), who demonstrated that exogenous spermidine (administered in growth media at 0.2 to 4 millimolar) extended chronological lifespan in *S. cerevisiae* by approximately 4-fold, lifespan in *C. elegans* by approximately 15 percent, and lifespan in *D. melanogaster* by approximately 30 percent [1]. Crucially, lifespan extension was abolished in autophagy-deficient yeast (*atg7*-delta) and in *C. elegans* with RNAi knockdown of *bec-1* (the nematode beclin-1 homolog), establishing autophagy as the essential mediating mechanism. The same study demonstrated that spermidine treatment induced global hypoacetylation of histone H3, linking the polyamine to epigenetic regulation of autophagy gene expression.

The mechanistic refinement came with Pietrocola et al. (2015), who identified EP300 acetyltransferase as the principal molecular target [3]. Spermidine competitively inhibits the acetyltransferase activity of EP300, reducing acetylation of cytoplasmic autophagy proteins and of nuclear histones. The EP300 inhibition is structurally rationalized by the capacity of the polyamine to occupy the acetyl-CoA binding pocket, competing with the acetyl donor substrate.

3.5 Cardiovascular geroprotection and epidemiological validation

The Eisenberg et al. (2016) study in *Nature Medicine* marked the transition from invertebrate geroprotection to mammalian cardiovascular disease relevance [4]. This study demonstrated that lifelong oral spermidine supplementation in drinking water extended median lifespan in mice by approximately 10 percent, reduced age-related cardiac hypertrophy, preserved diastolic function, and enhanced cardiac autophagy and mitophagy in an ATG5-dependent manner. In Dahl salt-sensitive rats fed a high-salt diet, spermidine supplementation reduced blood pressure, attenuated cardiac hypertrophy, and delayed progression to heart failure. The epidemiological arm of the study, analyzing the Bruneck Study cohort, reported a significant inverse dose-response relationship between dietary spermidine intake (assessed by

validated food frequency questionnaire) and cardiovascular mortality (hazard ratio 0.62, 95 percent confidence interval 0.41 to 0.95 for the highest versus lowest intake tertile) over 20 years of follow-up.

3.6 Clinical translation

The transition to human interventional studies began with the Wirth et al. (2018) pilot trial of spermidine-rich wheat germ extract in older adults with subjective cognitive decline, followed by the SmartAge Phase IIb trial (Wirth et al. 2022) and by the ongoing POLYCAD cardiovascular trial (NCT05128331) [6, 7, 8]. The Madeo et al. (2018) review in *Science* consolidated the preclinical and epidemiological evidence and positioned spermidine as a leading caloric restriction mimetic candidate for clinical geroprotection [15].

4. Mechanism of Action

Spermidine exerts its biological effects through multiple molecular mechanisms. The principal characterized activities are autophagy induction via EP300 acetyltransferase inhibition, translational regulation through eIF5A hypusination, anti-inflammatory signaling, and direct interactions with nucleic acids and chromatin. These activities are not independent; they converge on cellular proteostasis, mitochondrial quality control, and inflammatory tone to produce the geroprotective phenotype observed across species.

4.1 Autophagy induction via EP300 acetyltransferase inhibition

The principal geroprotective mechanism of spermidine is the induction of macroautophagy through inhibition of the acetyltransferase EP300 (also designated p300 or KAT3B) [2, 3]. EP300 is a histone acetyltransferase that also acetylates cytoplasmic substrates including the core autophagy proteins ATG5, ATG7, ATG12, and LC3 (MAP1LC3B). Acetylation of these proteins by EP300 inhibits their participation in autophagosome formation and elongation. Spermidine, by competitively inhibiting EP300 acetyltransferase activity, reduces the acetylation of these substrates and thereby derepresses the autophagy machinery.

The mechanistic characterization of EP300 as the target was established by Pietrocola et al. (2015), who demonstrated that spermidine treatment phenocopied EP300 knockdown in multiple cell types, that EP300 overexpression blocked spermidine-induced autophagy, and that the spermidine-induced acetylproteome changes converged with those produced by EP300 depletion [3]. The autophagy induction was confirmed by multiple readouts: LC3-II accumulation, p62/SQSTM1 degradation, autophagosome formation (GFP-LC3 puncta), and autophagic flux measurements using lysosomal inhibitors.

The spermidine-EP300-autophagy axis is mechanistically distinct from the two other principal pharmacological routes to autophagy induction. Rapamycin (sirolimus) induces autophagy through direct inhibition of mTORC1 (mechanistic target of rapamycin complex 1), which phosphorylates and inhibits the ULK1 autophagy initiation complex. Metformin induces autophagy primarily through acti-

vation of AMP-activated protein kinase (AMPK), which activates ULK1 and inhibits mTORC1. Spermidine bypasses both the mTOR and AMPK signaling axes entirely, acting downstream at the level of autophagy protein acetylation. This mechanistic independence has implications for combination strategies and for the expected pharmacological profile in organisms or tissues with altered mTOR or AMPK signaling [2, 15].

4.2 eIF5A hypusination and translational regulation

Spermidine is the exclusive aminobutyl donor for the post-translational modification hypusination of eukaryotic translation initiation factor 5A (eIF5A) [16, 17]. Hypusination is a two-step process: deoxyhypusine synthase (DHPS) cleaves the aminobutyl moiety from spermidine and transfers it to the epsilon-amino group of a conserved lysine residue (Lys50 in human eIF5A), producing deoxyhypusine-eIF5A; deoxyhypusine hydroxylase (DOHH) then hydroxylates the deoxyhypusine intermediate to produce the mature hypusinated eIF5A. Hypusination is the only known biological function that is uniquely dependent on spermidine rather than on polyamines generally; putrescine cannot substitute, and spermine substitutes only inefficiently.

Hypusinated eIF5A functions in translation elongation at ribosomal stall sites, particularly at consecutive proline codons (polyproline stretches), and in translation termination [17]. The functional consequence of eIF5A hypusination is the efficient translation of a subset of mRNAs encoding proteins with polyproline motifs. Among these are mitochondrial proteins whose translation is necessary for mitochondrial biogenesis and respiratory chain assembly, and autophagy regulatory proteins. The Puleston et al. (2019) study demonstrated that spermidine-dependent eIF5A hypusination is required for efficient mitochondrial fatty acid oxidation and that hypusination deficiency contributes to nonalcoholic steatohepatitis progression [22]. The spermidine-eIF5A-hypusination axis has also been implicated in muscle stem cell activation, immune cell differentiation, and B-cell antibody production [23].

The eIF5A hypusination mechanism is relevant to the age-associated decline in spermidine: as tissue spermidine concentrations decrease with age, hypusination efficiency declines, translation of polyproline-rich mitochondrial and autophagy proteins is impaired, and mitochondrial dysfunction accumulates. Exogenous sper-

midine supplementation can restore hypusination in aged tissues in animal models, providing a second molecular rationale (beyond EP300 inhibition) for the geroprotective effects.

4.3 Anti-inflammatory signaling

Spermidine exerts anti-inflammatory effects through multiple pathways. Suppression of NF-kappaB activation has been demonstrated in macrophages and dendritic cells, resulting in reduced production of proinflammatory cytokines (tumor necrosis factor alpha, interleukin-1 beta, interleukin-6) [18]. The mechanism involves both autophagy-dependent clearance of inflammasome components and direct modulation of NF-kappaB signaling intermediates.

In the adaptive immune system, spermidine modulates T-cell differentiation. Puleston et al. (2021) demonstrated that spermidine promotes the differentiation of naive CD4+ T cells into regulatory T cells (Tregs) and inhibits the differentiation of proinflammatory Th17 cells, effects that depend on eIF5A hypusination and on mitochondrial function [24]. This immunomodulatory activity contributes to the anti-inflammatory phenotype observed in the cardiovascular and neurological disease models and may underlie the epidemiological association between dietary spermidine intake and reduced inflammatory disease burden.

4.4 Chromatin interactions and epigenetic modulation

As a polycation at physiological pH, spermidine interacts electrostatically with DNA, RNA, and chromatin. The interaction with DNA stabilizes the double helix and can condense chromatin structure, modulating gene expression. The inhibition of histone acetyltransferase activity (principally EP300) results in global histone hypoacetylation, which represents an epigenetic shift toward a more compact chromatin state with altered transcriptional output [1]. The extent to which the epigenetic modulation contributes independently to the geroprotective phenotype (as distinct from the cytoplasmic autophagy effects) is not fully resolved; both mechanisms are engaged simultaneously by spermidine treatment, and genetic dissection is complicated by the overlapping substrate profiles of EP300 in the nucleus and cytoplasm.

4.5 Mitochondrial quality control

Spermidine enhances mitochondrial quality through at least two convergent mechanisms: mitophagy (the selective autophagy of damaged mitochondria), stimulated through the EP300-autophagy pathway; and mitochondrial biogenesis, supported through eIF5A hypusination-dependent translation of mitochondrial proteins [4, 22]. The Eisenberg et al. (2016) cardiovascular study demonstrated that spermidine feeding enhanced mitochondrial respiration in aged cardiomyocytes and that this effect was abolished in cardiomyocyte-specific ATG5 knockout mice, confirming the dependence on autophagic mitochondrial turnover [4]. The composite of enhanced mitophagy and enhanced biogenesis produces a net rejuvenation of the mitochondrial pool in aged tissues, with functional consequences for cellular energetics, reactive oxygen species production, and apoptotic susceptibility.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Dietary spermidine is rapidly absorbed from the intestinal lumen. Animal studies using radiolabeled polyamines have demonstrated rapid uptake from the small intestine into the portal circulation, with absorption occurring through both active transport (polyamine transport systems on the apical membrane of enterocytes) and passive diffusion [15, 25]. The polyamine transport system is shared among putrescine, spermidine, and spermine and is subject to competitive inhibition among the polyamines.

The rate and extent of intestinal absorption are influenced by the food matrix. Spermidine from wheat germ extract, aged cheese, and fermented foods is absorbed with differing kinetics depending on the degree of matrix binding and the concurrent intake of other polyamines. The estimated bioavailability of dietary spermidine is not precisely characterized in humans owing to the complexity of polyamine interconversion in the intestinal wall and liver.

5.2 Distribution and metabolism

A critical pharmacokinetic feature of spermidine is its extensive presystemic metabolism. Studies in humans and mice have demonstrated that orally administered spermidine is substantially converted to spermine (through spermine synthase activity in the intestinal mucosa and liver) before reaching the systemic circulation [9, 10]. Schwarz et al. (2023) reported that high-dose spermidine supplementation (1.2 mg/day from wheat germ extract for 12 months, and purified spermidine at up to 40 mg/day for 28 days) did not significantly increase circulating spermidine or total polyamine concentrations in blood plasma or saliva [9, 10]. This finding indicates tight homeostatic regulation of circulating polyamine pools and suggests that the biological effects of oral spermidine are mediated through local tissue exposure (gastrointestinal tract, liver), through presystemic conversion to spermine, through modulation of gut microbiota polyamine production, or through redistribution into intracellular compartments that are not reflected in plasma measurements.

Intracellular spermidine concentrations are maintained through the balance of de novo biosynthesis (ornithine decarboxylase, spermidine synthase), interconversion (spermine to spermidine via SAT1 and PAOX), cellular uptake (polyamine transporters), and catabolism (diamine oxidase, polyamine oxidase). Tissue concentrations are highest in rapidly proliferating tissues (intestinal mucosa, liver, spleen, thymus) and decline with age in most organs [15].

5.3 Catabolism and elimination

Spermidine is catabolized through two principal routes. The first is N1-acetylation by spermidine/spermine N1-acetyltransferase (SAT1), producing N1-acetylspermidine, which is either excreted in urine or further oxidized by polyamine oxidase to putrescine. The second is oxidative deamination by diamine oxidase (DAO) or by serum amine oxidase, producing aminoaldehydes that are further metabolized. Urinary excretion of acetylated polyamines and of free putrescine constitutes the principal elimination route for polyamine metabolites [14].

5.4 Routes of administration

Oral administration is the only route used in human clinical studies. Spermidine is delivered either as a component of spermidine-rich wheat germ extract (containing approximately 0.8 to 1.2 mg spermidine per 750 mg extract) or as purified spermidine trihydrochloride. In preclinical studies, spermidine has been administered in drinking water (typically 3 to 6 millimolar, corresponding to approximately 5 to 30 mg/kg/day in mice), by intraperitoneal injection (typically 5 to 50 mg/kg), and by subcutaneous injection.

5.5 Dietary intake

Estimated daily dietary spermidine intake varies substantially across populations and dietary patterns. The Madeo et al. (2018) review estimated average intake at approximately 7 to 25 mg per day, with Mediterranean and Japanese diets providing higher intakes than Western diets [15]. The principal dietary sources are wheat germ (24 to 35 mg per 100 g), soybeans (8 to 15 mg per 100 g), natto (approximately 15 to 56 mg per 100 g, variable by fermentation conditions), aged hard cheeses (4 to 20 mg per 100 g), mushrooms (5 to 9 mg per 100 g), green peas (5 to 7 mg per 100 g), and whole grains (2 to 5 mg per 100 g) [15, 25].

6. Preclinical Pharmacology

6.1 Lifespan extension

Spermidine extends lifespan in multiple model organisms through autophagy-dependent mechanisms. In *S. cerevisiae*, exogenous spermidine extends chronological lifespan approximately 4-fold at optimal concentrations [1]. In *C. elegans*, spermidine extends lifespan by approximately 15 percent [1]. In *D. melanogaster*, spermidine extends lifespan by approximately 30 percent when administered in food throughout the adult life [1]. In mice, lifelong spermidine supplementation in drinking water extends median lifespan by approximately 10 percent; late-onset supplementation (beginning at 18 months of age) also extends remaining lifespan, though the magnitude is reduced [4]. In all model organisms tested, lifespan extension is abolished by genetic deletion or pharmacological inhibition of essential autophagy genes, confirming the requirement for functional autophagy [1, 4].

6.2 Cardiovascular protection

The Eisenberg et al. (2016) study demonstrated multiple cardiovascular endpoints in aged mice: reduced left ventricular wall thickness, preserved diastolic function (assessed by echocardiography and pressure-volume loop analysis), reduced cardiomyocyte hypertrophy, enhanced cardiac autophagy and mitophagy (assessed by LC3 flux and mitophagy reporters), enhanced mitochondrial respiration (assessed by high-resolution respirometry), and increased titin phosphorylation (which reduces cardiomyocyte passive stiffness and improves diastolic compliance) [4]. In Dahl salt-sensitive rats on a high-salt diet, spermidine supplementation reduced systolic blood pressure, attenuated cardiac hypertrophy, and delayed the progression to heart failure with preserved ejection fraction (HFpEF). The cardioprotective effects were abolished in cardiomyocyte-specific ATG5 knockout mice, confirming the autophagy dependence.

6.3 Neuroprotection

Spermidine has demonstrated neuroprotective effects in multiple preclinical models. In *D. melanogaster*, spermidine feeding protects against age-associated memory impairment through autophagy-dependent mechanisms [26]. In mouse models of neurodegeneration, spermidine reduces soluble amyloid-beta accumulation, improves microglial phagocytic activity, preserves mitochondrial function in neurons, and attenuates neuroinflammation [27]. The spermidine-eIF5A-hypusination axis has been implicated in maintaining synaptic plasticity and neuronal protein quality control during aging.

6.4 Anti-tumor immunosurveillance

Spermidine enhances autophagy-dependent release of ATP and other immunostimulatory signals from dying tumor cells, thereby potentiating the antitumor immune response. Pietrocola et al. (2016) demonstrated that spermidine treatment enhanced the efficacy of chemotherapy-induced immunogenic cell death in mouse tumor models and that this effect required functional autophagy in the tumor cells and an intact immune system in the host [28]. Epidemiological data correlating dietary spermidine intake with reduced cancer-related mortality are consistent with this mechanism, though direct human evidence for the immunosurveillance effect is absent.

6.5 Metabolic effects

Spermidine supplementation improves insulin sensitivity, reduces hepatic lipid accumulation, and improves glucose tolerance in aged mice and in high-fat-diet-fed mice [22]. The mechanism involves eIF5A hypusination-dependent translation of mitochondrial fatty acid oxidation enzymes and autophagy-dependent clearance of lipid droplets (lipophagy). These findings provide a metabolic rationale for the observed epidemiological association between dietary spermidine intake and reduced metabolic disease burden.

7. Clinical Evidence Base

7.1 Cognitive endpoints

The first interventional study of spermidine in humans was the Wirth et al. (2018) pilot trial, a randomized, double-blind, placebo-controlled, 3-month study of spermidine-rich wheat germ extract (providing an estimated 1.2 mg/day additional spermidine intake) in 30 older adults with subjective cognitive decline [6]. The study reported a trend toward improved mnemonic discrimination performance (a hippocampus-dependent memory task) in the spermidine group compared to placebo, though the primary outcome did not reach statistical significance in this small cohort.

The SmartAge Phase IIb trial (Wirth et al. 2022) was a monocentric, randomized, double-blind, placebo-controlled study of 12 months of spermidine-rich wheat germ extract supplementation (increasing daily spermidine intake by approximately 10 percent, corresponding to approximately 0.9 mg/day additional spermidine) in 100 cognitively normal older adults with subjective cognitive decline [7]. The primary outcome (change in mnemonic discrimination performance at 12 months) did not differ significantly between the spermidine and placebo groups. Secondary cognitive, neuroimaging, and blood biomarker outcomes were also not significantly modified. The authors noted the low absolute spermidine dose increase and the absence of measurable changes in circulating polyamine concentrations as potential explanations for the neutral result.

An earlier study by Wirth et al. (2019) examined the effect of spermidine-rich grain rolls (providing 3.3 mg/day of spermidine) consumed six times per week for 3 months in older adults with dementia [29]. This open-label study reported positive effects on cognition as assessed by the CERAD battery, supporting the possibility that higher doses may be required for clinical effect.

7.2 Cardiovascular endpoints

The POLYCAD trial (POLYamine treatment in elderly patients with Coronary Artery Disease; NCT05128331) is a Danish randomized, double-blind, placebo-controlled trial of high-dose spermidine (24 mg/day) versus placebo for 48 weeks in 187 pa-

tients aged 65 years or older with angiographically confirmed coronary artery disease [8]. The study was designed to evaluate effects on cardiac remodeling (assessed by cardiac MRI), exercise capacity (6-minute walk test), muscle mass (dual-energy X-ray absorptiometry), and systemic inflammation (C-reactive protein, interleukin-6). Enrollment was completed in August 2025 and trial completion is anticipated in August 2026. POLYCAD represents the first dedicated cardiovascular outcomes trial for spermidine at a pharmacological dose and will provide critical data on the translation of preclinical cardiovascular findings to human patients.

7.3 Metabolic and pharmacokinetic studies

The NCT05459961 trial (Metabolic Responses to Spermidine Supplementation) examined metabolic and pharmacokinetic parameters following spermidine supplementation. Schwarz et al. (2024) reported a double-blind, randomized, placebo-controlled study of purified spermidine at 40 mg/day for 28 days in healthy older men [10]. No significant changes in circulating polyamine concentrations, vital signs, clinical chemistry, or hematological parameters were observed, confirming the tight homeostatic regulation of systemic polyamine pools and the favorable safety profile at this dose.

7.4 Epidemiological evidence

Epidemiological studies provide the strongest current evidence for the health effects of dietary spermidine in humans. The Kiechl et al. (2018) analysis of the Bruneck Study cohort reported that individuals in the highest tertile of dietary spermidine intake had a 40 percent reduction in all-cause mortality (hazard ratio 0.60) compared to the lowest tertile, after adjustment for age, sex, caloric intake, and conventional cardiovascular risk factors [5]. The magnitude of the mortality difference between the highest and lowest spermidine intake tertiles was comparable to approximately 5.7 years of aging. Similar inverse associations have been reported in independent cohorts from Austria, Italy, and Japan, though residual confounding by overall dietary quality cannot be excluded from observational data.

8. Sourcing and Quality Verification

Spermidine is available commercially in two principal forms: as spermidine-rich wheat germ extract (the form used in most published clinical trials and in consumer supplements) and as purified spermidine trihydrochloride (the form used in pharmacokinetic studies and in laboratory research).

Wheat germ extract: Spermidine-rich wheat germ extract is produced by standardized extraction from *Triticum aestivum* germ and is typically standardized to contain 1 to 1.5 mg spermidine per gram of extract. The European Food Safety Authority authorized this preparation as a Novel Food in 2021 with a recommended upper intake of 6 mg/day of spermidine from this source [11]. Wheat germ extract contains additional polyamines (spermine, putrescine), wheat proteins (including gluten), and other bioactive wheat germ constituents; the extract is therefore not appropriate for individuals with celiac disease, wheat allergy, or non-celiac gluten sensitivity.

Purified spermidine trihydrochloride: Research-grade spermidine trihydrochloride is supplied by multiple chemical and pharmaceutical research suppliers (Sigma-Aldrich, Cayman Chemical, Tocris, MedChemExpress, Santa Cruz Biotechnology, Selleckchem) at greater than 98 percent purity by HPLC. Supply is in milligram to gram quantities. Pricing is moderate, in the range of \$30 to \$150 per gram at the time of monograph preparation.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity (greater than 98 percent)
- Mass spectrometry confirmation of molecular weight (146.26 for the protonated free base $[M+H]^+$)
- Nuclear magnetic resonance spectroscopy (1H -NMR) confirming the characteristic methylene resonances
- Karl Fischer analysis for residual water content (relevant for the hygroscopic trihydrochloride salt)

- Heavy metals analysis (particularly lead, arsenic, cadmium, mercury) for any preparation intended for in vivo research

For wheat germ extract preparations, additional quality parameters include total polyamine profiling (spermidine, spermine, putrescine by HPLC), gluten content determination, and microbiological testing.

9. Reconstitution and Handling

Spermidine trihydrochloride is freely soluble in water at room temperature. For research applications, the compound is supplied as a white crystalline powder and is reconstituted as needed.

Stock solutions: Aqueous stock solutions are typically prepared at 100 millimolar (approximately 25.5 mg/mL of the trihydrochloride salt) in sterile water or phosphate-buffered saline. The compound is also soluble in dimethyl sulfoxide (DMSO) for cell culture applications requiring high stock concentrations, though aqueous preparations are preferred to avoid DMSO-associated confounding effects on autophagy.

Storage: The solid compound should be stored desiccated at minus 20 degrees Celsius. Under these conditions, stability is maintained for at least 2 years from the date of manufacture. The free base form is a liquid at room temperature and is more susceptible to oxidative degradation than the trihydrochloride salt; the trihydrochloride is the preferred form for storage and for preparation of stock solutions.

Solution stability: Aqueous stock solutions at neutral pH are stable at 4 degrees Celsius for at least 2 weeks and at minus 20 degrees Celsius for at least 6 months. Repeated freeze-thaw cycles (up to 5) do not produce measurable degradation. Aliquoting is recommended for long-term storage to minimize freeze-thaw exposure. Solutions should be protected from prolonged exposure to strong oxidizing agents, though the compound is not particularly light-sensitive.

Cell culture use: For in vitro autophagy induction, spermidine is typically used at concentrations of 0.1 to 1 millimolar in cell culture media. Higher concentrations (greater than 5 millimolar) can be cytotoxic in some cell lines. The medium should be refreshed daily or every 48 hours when prolonged treatment is required, as spermidine can be oxidized by serum amine oxidases present in fetal bovine serum to produce cytotoxic aminoaldehydes. Aminoguanidine (1 millimolar) is sometimes added to culture media as a diamine oxidase inhibitor to prevent this oxidative conversion.

Drinking water preparations for animal studies: Spermidine is dissolved in the drinking water of mice at concentrations of 3 to 6 millimolar (approximately 0.44 to 0.88 mg/mL of the free base). The water is refreshed every 2 to 3 days. The free base form is used for drinking water preparations owing to its liquid state and its neutral taste profile at the studied concentrations.

10. Stack Interaction Considerations

Spermidine interacts with several compound classes through its effects on polyamine metabolism, autophagy induction, and translational regulation. Investigators conducting research with spermidine, particularly in combination with other geroprotective or autophagy-modulating compounds, should consider:

Other autophagy inducers: Concurrent administration with rapamycin (mTOR inhibitor), metformin (AMPK activator), or resveratrol (sirtuin activator) may produce additive or synergistic autophagy induction, as these compounds act through mechanistically independent pathways [2]. The Morselli et al. (2011) study demonstrated that spermidine and resveratrol converge on the acetylproteome through complementary mechanisms (EP300 inhibition and SIRT1 activation, respectively) and produce synergistic autophagy induction in cell culture [2]. Combination studies in animal models have shown additive lifespan effects. The clinical safety of autophagy inducer combinations has not been established.

Polyamine synthesis inhibitors: Difluoromethylornithine (DFMO, eflornithine), an irreversible inhibitor of ornithine decarboxylase, depletes intracellular putrescine and spermidine. Exogenous spermidine supplementation can bypass the DFMO-induced depletion at the spermidine level but does not restore putrescine. The combination has been used in cancer research to dissect the relative contributions of putrescine and spermidine to proliferative signaling [14].

SAT1 inducers: Compounds that induce spermidine/spermine N1-acetyltransferase (SAT1) expression, including certain NSAIDs and polyamine analogs, accelerate the catabolism of spermidine and spermine to putrescine. Concurrent use with exogenous spermidine would be expected to increase the rate of spermidine catabolism and may reduce the effective intracellular concentration.

Diamine oxidase inhibitors: Aminoguanidine and other diamine oxidase inhibitors prevent the oxidative degradation of spermidine in the gastrointestinal tract and in cell culture media. Concurrent use may enhance the effective dose of orally administered spermidine by reducing presystemic catabolism.

Caloric restriction and fasting: Hofer et al. (2024) demonstrated that spermidine is essential for the autophagy and longevity benefits of fasting in animal models, suggesting that fasting and spermidine supplementation engage overlapping molecular pathways [30]. Concurrent caloric restriction and spermidine supplementation may therefore produce less-than-additive effects on autophagy, as both interventions converge on the same EP300-dependent mechanism.

Acetyl-CoA precursors and ketogenic diets: Interventions that increase intracellular acetyl-CoA (including ketogenic diets, exogenous ketone supplementation, and citrate supplementation) may oppose the EP300 inhibition produced by spermidine by increasing the availability of acetyl donor substrate. The net effect on autophagy would depend on the relative magnitudes of EP300 inhibition (spermidine) and acetyl-CoA availability (ketogenic intervention).

Oncology considerations: Spermidine promotes cell proliferation in some contexts, and elevated polyamine levels are a hallmark of many tumor types. Concurrent use of spermidine supplementation with chemotherapy or with polyamine-targeting cancer therapies should be considered carefully. The immunosurveillance-enhancing effects of spermidine (through autophagy-dependent immunogenic cell death) may partially offset the proliferative effects, but the net impact on tumor growth is context-dependent and not resolved in the clinical literature [28].

11. Adverse Events and Safety

11.1 Human safety data

Spermidine supplementation has demonstrated a favorable safety profile in all completed human studies. The Wirth et al. (2018) 3-month pilot trial of spermidine-rich wheat germ extract (1.2 mg/day additional spermidine) in 30 older adults reported no adverse events attributable to the study intervention [6]. The SmartAge 12-month Phase IIb trial in 100 older adults reported no difference in adverse event rates between the spermidine and placebo groups [7]. The Schwarz et al. (2024) pharmacokinetic study of purified spermidine at 40 mg/day for 28 days in healthy older men reported no significant adverse events, with no differences in vital signs, body weight, clinical chemistry, or hematological parameters between the spermidine and placebo groups [10].

11.2 Reported adverse events

The adverse events reported in human spermidine studies have been mild and predominantly gastrointestinal:

- Mild gastrointestinal discomfort (bloating, nausea) in a minority of participants, generally transient and self-resolving
- No serious adverse events attributed to spermidine in any published trial
- No clinically significant changes in laboratory parameters, vital signs, or electrocardiographic findings

The gastrointestinal symptoms are attributed to the amine content of the preparations and to the wheat germ matrix in extract-based formulations rather than to spermidine-specific pharmacological toxicity.

11.3 Preclinical toxicology

A 90-day oral toxicity study in Sprague-Dawley rats using high-purity spermidine trihydrochloride reported no adverse effects at doses up to 728 mg/kg body weight per day for males and 829 mg/kg per day for females, establishing a high no-ob-

served-adverse-effect level (NOAEL) and confirming the compound is non-genotoxic in standard genotoxicity assays (Ames test, chromosomal aberration assay, in vivo micronucleus assay) [31].

11.4 Regulatory assessment

The European Food Safety Authority (EFSA) assessed spermidine-rich wheat germ extract as a Novel Food in 2021 and authorized its use at an intake providing up to 6 mg/day of spermidine [11]. The EFSA assessment considered the available toxicological data, the history of consumption of spermidine-containing foods, and the 90-day rat toxicity study. The 6 mg/day upper limit reflects the assessed safety margin relative to dietary intake and the available human data at the time of authorization, not a threshold for adverse effects.

11.5 Theoretical safety considerations

Polyamine-cancer interaction: Elevated polyamine concentrations promote cell proliferation and are observed in many tumor types. Ornithine decarboxylase is an oncogene product, and the polyamine pathway is a validated cancer drug target (DFMO is approved for African trypanosomiasis and is in clinical trials for cancer chemoprevention). The theoretical concern is that exogenous spermidine supplementation might promote growth of occult tumors. Against this concern, the epidemiological data consistently show an inverse association between dietary spermidine intake and cancer mortality [4, 5], and the Pietrocola et al. (2016) data demonstrate that spermidine enhances antitumor immunosurveillance through autophagy-dependent mechanisms [28]. The net effect in immunocompetent individuals appears protective rather than promoting, but the interaction in immunocompromised individuals or in individuals with active malignancy is not established.

Pregnancy and lactation: No human data are available on spermidine supplementation during pregnancy or lactation. Polyamines are essential for embryonic development and fetal growth, and physiological polyamine levels increase during pregnancy. Supplementation during pregnancy is not recommended in the absence of safety data.

Drug interactions: No clinically significant drug interactions with spermidine supplementation have been identified in published studies. Theoretical interactions with monoamine oxidase inhibitors (which also metabolize polyamines) and with immunosuppressive agents (through the immunomodulatory effects of spermidine) warrant caution but have not been observed in practice.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five geroprotective and autophagy-inducing compounds against spermidine, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five comparators were selected as the principal pharmacological candidates for geroprotection with substantive preclinical and clinical pharmacology programs, with priority on those that share spermidine's autophagy-inducing or caloric restriction mimetic positioning.

12.1 Rapamycin (Sirolimus)

Mechanism: Selective inhibitor of mechanistic target of rapamycin complex 1 (mTORC1). Rapamycin inhibits mTORC1 by binding to FKBP12, forming a complex that directly inhibits the mTOR kinase. mTORC1 inhibition activates the ULK1 autophagy initiation complex, induces autophagy, and suppresses mTORC1-dependent anabolic processes including protein synthesis, lipid synthesis, and cell growth [32].

Novelty: Low. Rapamycin is the canonical mTOR inhibitor and was the first pharmacological agent demonstrated to extend lifespan in mice (Harrison et al. 2009, NIA Interventions Testing Program) [33]. The mechanism is well characterized and has been the subject of extensive investigation for over 30 years.

Effect size: Substantial. Rapamycin extends median lifespan in genetically heterogeneous mice by approximately 10 to 15 percent when initiated at 20 months of age (Harrison et al. 2009) and by approximately 20 to 25 percent when initiated at 9 months [33]. This is the largest pharmacological lifespan extension demonstrated in mammals. Cardiovascular, cognitive, and immune aging endpoints are also improved in preclinical models.

Promising potential: Moderate. The PEARL trial (Participatory Evaluation of Aging with Rapamycin for Longevity) and the Dog Aging Project rapamycin arm represent the most advanced clinical geroprotection trials for any mTOR inhibitor

[34]. However, rapamycin is an immunosuppressant (approved for organ transplant rejection prophylaxis), and the therapeutic window between geroprotective and immunosuppressive doses is narrow and incompletely characterized.

Side-effect profile: Significant. Rapamycin produces dose-dependent immunosuppression, glucose intolerance (through mTORC2 inhibition at higher doses), hyperlipidemia, mucositis, and impaired wound healing. These effects are well characterized from transplant medicine and represent the principal barrier to geroprotective translation.

Overall validation: High. Rapamycin has the strongest preclinical evidence for lifespan extension of any compound, validated across multiple mouse strains, dosing regimens, and laboratories. Clinical translation is limited by the adverse-event profile.

Comparison to spermidine: Rapamycin and spermidine induce autophagy through independent mechanisms (mTORC1 inhibition versus EP300 inhibition) and are therefore mechanistically complementary. Rapamycin has stronger preclinical effect sizes on lifespan extension but a substantially worse side-effect profile. Spermidine has the advantages of endogenous presence, dietary availability, favorable safety data in human studies, and epidemiological validation. Rapamycin has the advantage of larger preclinical effect sizes and more advanced clinical geroprotection trials. The two compounds could in principle be combined for additive autophagy induction, though the safety of the combination has not been evaluated.

12.2 Resveratrol

Mechanism: Polyphenolic stilbene that activates sirtuin 1 (SIRT1), a NAD-dependent deacetylase. SIRT1 activation produces deacetylation of multiple substrates including histones, p53, FOXO transcription factors, and PGC-1alpha, resulting in enhanced autophagy, mitochondrial biogenesis, and metabolic adaptation [35]. Resveratrol also has antioxidant and anti-inflammatory activities independent of SIRT1.

Novelty: Low. Resveratrol is among the most extensively studied natural geroprotective compounds, with a literature spanning over 25 years and thousands of publications.

Effect size: Modest to negligible in lifespan extension. Resveratrol does not extend lifespan in normal-weight mice on a standard diet in the NIA Interventions Testing Program [36]. Lifespan extension has been demonstrated only in mice on a high-fat diet, where resveratrol protects against metabolic dysfunction. The compound has produced positive signals on metabolic, cardiovascular, and cognitive endpoints in some but not all preclinical and clinical studies, with small effect sizes and frequent failures of replication.

Promising potential: Low. Multiple clinical trials of resveratrol in cardiovascular disease, diabetes, cognitive decline, and cancer have produced inconsistent results, and no registration-quality efficacy has been demonstrated. Bioavailability is poor (less than 1 percent after oral administration), and the active metabolites are incompletely characterized.

Side-effect profile: Favorable. Resveratrol is well tolerated at doses up to 5 g/day in short-term human studies, with mild gastrointestinal effects as the principal adverse events.

Overall validation: Low to moderate. The extensive preclinical literature has not translated to consistent clinical benefit, and the compound is widely regarded as having failed to fulfill its initial geroprotective promise.

Comparison to spermidine: Spermidine and resveratrol act on complementary arms of the acetylproteome (EP300 inhibition and SIRT1 activation, respectively), and the combination produces synergistic autophagy induction in vitro [2]. However, spermidine has stronger epidemiological validation (dietary spermidine intake correlates with reduced mortality, whereas dietary resveratrol intake does not consistently do so), more robust preclinical lifespan extension data, and a more favorable pharmacokinetic profile (spermidine is absorbed and reaches target tissues, whereas resveratrol has very poor bioavailability).

12.3 Nicotinamide Mononucleotide (NMN)

Mechanism: NAD⁺ precursor. NMN is phosphorylated to NAD⁺ by nicotinamide mononucleotide adenylyltransferases (NMNATs). NAD⁺ is a cofactor for sirtuins (SIRT1 through SIRT7), poly(ADP-ribose) polymerases (PARPs), and CD38, and is essential for mitochondrial electron transport. NAD⁺ levels decline with age, and NMN supplementation aims to restore NAD⁺ to youthful levels, thereby supporting sirtuin-dependent metabolic and geroprotective functions [37].

Novelty: Moderate. NMN occupies a distinct mechanistic space (NAD⁺ repletion) from the autophagy inducers. The geroprotective thesis is indirect: NAD⁺ supports sirtuin activity, which in turn supports autophagy, mitochondrial function, and DNA repair.

Effect size: Moderate in preclinical models. NMN supplementation improves insulin sensitivity, exercise capacity, vascular function, and stem cell function in aged mice (Mills et al. 2016) [37]. Lifespan extension has not been consistently demonstrated in mice, limiting the comparability with spermidine and rapamycin.

Promising potential: Moderate to high. NMN has advanced to multiple human clinical trials evaluating metabolic, cardiovascular, and exercise endpoints. The Yi et al. (2023) randomized trial reported improved muscle insulin sensitivity in overweight older adults [38]. Commercial NMN supplementation is widespread, though regulatory status varies by jurisdiction.

Side-effect profile: Favorable. NMN is well tolerated in human studies at doses up to 1200 mg/day, with no serious adverse events reported.

Overall validation: Moderate. Preclinical evidence is substantial but lifespan extension is not established. Human trials have produced modest positive signals on metabolic endpoints. The compound is earlier in clinical validation than rapamycin but further advanced than spermidine.

Comparison to spermidine: NMN and spermidine act through independent mechanisms (NAD⁺ repletion versus EP300 inhibition). Spermidine has stronger preclinical lifespan extension data and stronger epidemiological validation for mortality reduction. NMN has more advanced clinical trial programs for metabolic endpoints. The two compounds are mechanistically complementary and could be combined; their interaction has not been systematically studied.

12.4 Urolithin A

Mechanism: Gut microbiota-derived metabolite of ellagitannins (found in pomegranates, walnuts, berries). Urolithin A induces mitophagy (selective autophagy of damaged mitochondria) through activation of the PINK1-Parkin mitophagy pathway, independent of general macroautophagy induction [39]. The compound does not act through mTOR, AMPK, or EP300.

Novelty: Moderate to high. The mitophagy-selective mechanism is distinct from the general autophagy induction produced by spermidine, rapamycin, and resveratrol. Urolithin A represents a newer entry in the geroprotective compound space.

Effect size: Moderate. Urolithin A extends lifespan in *C. elegans* by approximately 45 percent and improves muscle function and exercise capacity in aged mice [39]. In the Liu et al. (2022) randomized trial in older adults, urolithin A at 1000 mg/day for 4 months improved muscle endurance and plasma biomarkers of mitochondrial function [40].

Promising potential: Moderate to high. The Amazentis-sponsored clinical program has produced positive Phase 2 data in aging muscle function, and the compound has received GRAS (Generally Recognized as Safe) status from the FDA. The clinical trajectory is more advanced than spermidine in terms of registration-quality efficacy data for specific endpoints.

Side-effect profile: Favorable. Urolithin A is well tolerated in human studies at doses up to 2000 mg/day, with no serious adverse events reported.

Overall validation: Moderate. Clinical validation is emerging but limited to muscle function endpoints. The broader geroprotective thesis (cardiovascular, cognitive, metabolic) is supported by preclinical data but lacks clinical confirmation.

Comparison to spermidine: Urolithin A and spermidine have complementary autophagy mechanisms (mitophagy-selective versus general macroautophagy). Spermidine has broader preclinical evidence across cardiovascular, cognitive, and metabolic endpoints. Urolithin A has more advanced clinical efficacy data for muscle function specifically. The combination is theoretically attractive for comprehensive mitochondrial quality control (spermidine for general autophagy and mitophagy plus urolithin A for targeted mitophagy) but has not been studied.

12.5 Metformin

Mechanism: Biguanide antidiabetic that activates AMP-activated protein kinase (AMPK) through inhibition of mitochondrial complex I, resulting in increased AMP:ATP ratio. AMPK activation induces autophagy through ULK1 phosphorylation and mTORC1 inhibition. Additional mechanisms include inhibition of the mitochondrial glycerophosphate shuttle, modulation of gut microbiota composition, and anti-inflammatory effects through NF-kappaB suppression [41].

Novelty: Low. Metformin has been in clinical use for over 60 years as an antidiabetic and is the most widely prescribed oral hypoglycemic agent worldwide.

Effect size: Modest. Metformin does not extend lifespan in healthy mice in the NIA Interventions Testing Program at the tested doses, though it extends lifespan in some mouse strains at lower doses (Martin-Montalvo et al. 2013) [42]. The UKPDS (United Kingdom Prospective Diabetes Study) retrospective analysis reported that metformin-treated diabetic patients had lower all-cause mortality than non-diabetic controls, generating the hypothesis that metformin may slow aging [43].

Promising potential: High. The TAME (Targeting Aging with Metformin) trial, a large randomized controlled trial in older adults without diabetes, is the most prominent clinical geroprotection trial currently in progress [44]. Metformin has the advantage of an extensive clinical safety database from decades of use in millions of patients.

Side-effect profile: Well characterized. Gastrointestinal effects (nausea, diarrhea, abdominal pain) are common at initiation. Lactic acidosis is a rare but serious adverse event, principally in patients with renal impairment. Vitamin B12 deficiency occurs with chronic use. The side-effect profile is manageable and well understood from clinical experience.

Overall validation: Moderate to high. The clinical safety database is unmatched among geroprotective candidates. Preclinical lifespan extension data are inconsistent. The TAME trial will provide definitive human data.

Comparison to spermidine: Metformin and spermidine induce autophagy through independent mechanisms (AMPK activation versus EP300 inhibition). Metformin has the advantage of an enormous clinical safety database, established regulatory approval (for diabetes), and the TAME trial underway. Spermidine has the advantages of endogenous presence, dietary availability, stronger preclinical lifespan extension data, and epidemiological validation for mortality reduction that metformin lacks in non-diabetic populations. The two compounds are mechanistically complementary and could be combined.

12.6 Comparator summary

The five comparators occupy distinct positions in the geroprotective pharmacology space. **Rapamycin** has the strongest preclinical lifespan extension data but the most significant side-effect profile, limiting clinical translation. **Resveratrol** has the

most extensive research literature but the weakest clinical validation, with poor bioavailability and inconsistent efficacy. **Nicotinamide mononucleotide** has a distinct mechanism (NAD⁺ repletion) and positive early clinical signals but lacks lifespan extension data. **Urolithin A** has a novel mitophagy-selective mechanism and emerging clinical muscle function data. **Metformin** has the largest clinical safety database and the most prominent geroprotection trial (TAME) but inconsistent pre-clinical lifespan data.

Spermidine occupies a distinctive position among these candidates. It is the only compound that is both an endogenous metabolite and a dietary constituent with validated epidemiological associations with reduced mortality. It is the only compound in this panel for which dietary intake data are available in multiple large cohorts with long follow-up and with consistent inverse mortality associations. It is mechanistically independent from all five comparators (acting through EP300 inhibition rather than through mTOR, AMPK, sirtuins, PINK1-Parkin, or NAD⁺ pathways), making it compatible with combination strategies. Its safety profile is favorable, with no serious adverse events in any completed human study.

The principal limitation of spermidine relative to the comparators is the modest scale of completed clinical trials and the neutral result of the SmartAge Phase IIb cognitive trial. The SmartAge result may reflect inadequate dosing (the absolute spermidine dose increase was approximately 0.9 mg/day, well below the dose used in the POLYCAD cardiovascular trial at 24 mg/day and the pharmacokinetic study at 40 mg/day). The POLYCAD trial, if positive, would substantially advance the clinical validation of spermidine for cardiovascular geroprotection and would position the compound favorably relative to the comparators, none of which has produced a positive cardiovascular outcomes result in a dedicated randomized trial in an aging population.

The second limitation is the pharmacokinetic complexity: oral spermidine does not reliably increase circulating spermidine concentrations, raising questions about the mechanism of systemic biological effects and complicating dose-response characterization. This pharmacokinetic profile contrasts with rapamycin, NMN, and urolithin A, all of which produce measurable increases in circulating drug or metabolite concentrations at clinical doses.

Investigators considering spermidine for geroprotective research should expect epidemiologically supported but clinically unconfirmed benefit, should use doses in the range of 6 to 40 mg/day for human studies (the EFSA-authorized upper limit

and the highest dose tested without adverse effects, respectively), should consider the comparator landscape as context for clinical-translation expectations, and should evaluate the compound both as a standalone intervention and as a component of combination geroprotective strategies.

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