

Mechanisms of spermidine-induced autophagy and geroprotection

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Aging involves the systemic deterioration of all known cell types in most eukaryotes. Several recently discovered compounds that extend the healthspan and lifespan of model organisms decelerate pathways that govern the aging process. Among these geroprotectors, spermidine, a natural polyamine ubiquitously found in organisms from all kingdoms, prolongs the lifespan of fungi, nematodes, insects and rodents. In mice, it also postpones the manifestation of various age-associated disorders such as cardiovascular disease and neurodegeneration. The specific features of spermidine, including its presence in common food items, make it an interesting candidate for translational aging research. Here, we review novel insights into the geroprotective mode of action of spermidine at the molecular level, as we discuss strategies for elucidating its clinical potential.

Life expectancy has steadily increased during the past decades, while healthspan lags behind, leading to an increased risk of age-related disease. Fasting and reducing calorie intake without malnutrition (caloric restriction (CR)) are the best-studied nongenetic interventions to promote healthspan and lifespan across species borders and are comprehensively investigated in clinical settings^{1–5}. Like any harsh nutritional intervention, they are afflicted by several problems precluding their broad implementation. Few individuals succeed in complying with long-term CR or regular fasting. Also, CR is contraindicated in certain conditions such as infection or sarcopenia⁵. This spurred the search for pharmacological CR mimetics, which ignite (gero)protective pathways similarly to CR^{6–9}. CR mimetics, by definition, act as antiaging agents, thus prolonging healthspan or lifespan. At the forefront, the polyamine spermidine has been characterized as a natural autophagy inducer¹⁰.

In eukaryotes, the diamine putrescine and the polyamines spermidine and spermine are essential metabolites regulating important cellular functions, including growth and proliferation, RNA and DNA stability, RNA-to-protein translation, autophagy and immune responses¹¹. In mammalian tissues, spermidine and spermine are more abundant than putrescine¹². Still, their comprehensive molecular targets remain incompletely understood. We will review how the

polyamine pathway is affected by aging and how spermidine supplementation prolongs healthspan and lifespan in model organisms. In recent years, the notion has been confirmed that the antiaging effects of spermidine involve autophagy induction. However, its action may extend to other geroprotective mechanisms. Here, we will discuss its impact on each hallmark of aging¹³ and scrutinize the potential of spermidine for combating human diseases.

The polyamine pathway during aging

Polyamine metabolism is evolutionarily conserved among eukaryotes and deeply integrated into cellular bioenergetic pathways (Fig. 1). Experimental deletion or hereditary defects in the polyamine pathway cause severe cellular and organismal defects or compromise viability. Research into the antiaging properties of spermidine has been encouraged by reports on its age-dependent decline (Table 1). For example, spermidine concentrations decrease in chronologically aging *Saccharomyces cerevisiae*, a model for postmitotic aging¹⁴. Similarly, spermidine declines in the heads of middle-aged *Drosophila*¹⁵.

Due to strain-specific, sex-specific and tissue-specific differences and dietary preferences, the time-dependent trajectories of spermidine are more heterogeneous in mammals. In female C57BL/6 mice,

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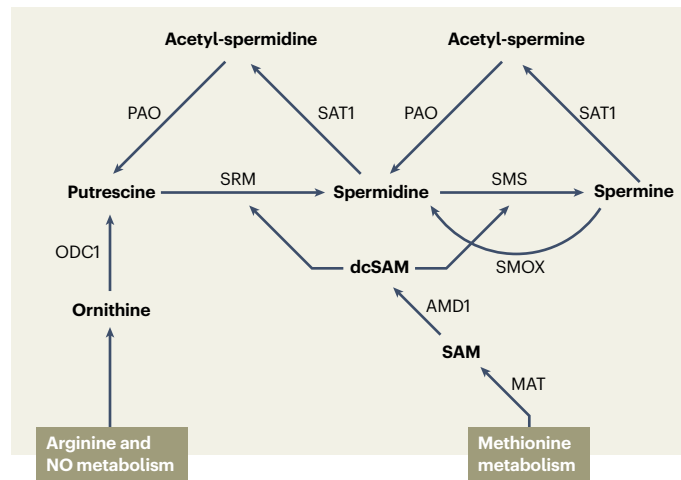


Fig. 1 | The intracellular polyamine pathway. MAT, methionine adenosyl transferase; PAO, polyamine oxidase; SMOX, spermine oxidase; SMS, spermine synthase; SRM, spermidine synthase.

Nishimura et al. measured a decrease in most tissues with few exceptions, including the brain, during the first 6 months of age¹⁶. Several studies investigated polyamines in aging rats: in 1964, a study reported reduced spermidine in several tissues during the first 9 months¹⁷, a tendency confirmed for selected tissues in longer-lasting studies^{18,19}. Interestingly, in male rats, spermidine sharply increased in kidneys at the age of 3 years, after an initial decline²⁰. This exemplifies the need for a systematic study of polyamines throughout the entire lifespan of mammals. One study found no effect or even a slight increase in several brain areas from Sprague-Dawley rats²¹, similarly to the aforementioned mouse study¹⁶. However, this contrasts with another report showing a constant decline of spermidine in cerebral cortex samples from aging rats¹⁹. Finally, circulating spermidine levels in serum from 4- and 13-month-old mice exhibited a 40% decrease when normalized to serum protein²². Given the animals' relatively young age in several of these studies, it is important to separate developmental from actual aging effects in future studies.

Two studies reported an age-dependent decline of spermidine in human blood^{23,24}. One of these found that healthy nonagenarians and centenarians retained levels comparable to those of middle-aged (45-year-old) participants²⁴, but this has not yet been confirmed. Spermidine declined with age in circulating peripheral blood mononuclear cells (PBMCs)^{25,26} and red blood cells²⁷. By contrast, a recent Japanese study reported an increase in whole-blood from aging humans²⁸. Post-mortem analyses of polyamines are rare, but two studies found stable levels throughout adulthood in human liver²⁹ and cortex³⁰ samples. Of note, polyamines were reported to rise in some aging-associated diseases, for example, renal failure³¹, cardiovascular disease³² and neurodegeneration^{33,34}, potentially as an adaptive response to cellular stress.

Spermidine (but not spermine) is utilized for the posttranslational hypusination of eukaryotic initiation factor 5A (eIF5A, a translation factor mainly involved in elongation and termination, despite its name³⁵), which stimulates autophagy^{25,36,37}. eIF5A hypusination declines with aging in vivo³⁸ and can be restored by dietary spermidine supplementation^{38,39}. Mechanistically, the depletion of spermidine and reduced eIF5A hypusination probably result from reduced activity of ornithine decarboxylase (ODC1), the rate-limiting step in polyamine biosynthesis. Indeed, ODC1 activity declines in several tissues of male rats¹⁹, which has been confirmed for liver⁴⁰ and heart⁴¹, while increased spermidine/spermine-*N*¹-acetyltransferase (SAT1) activity suggested activated catabolism upon aging⁴². Postmortem analysis of human liver samples

revealed decreased ODC1, but upregulated spermine oxidase, perhaps accounting for relatively stable spermidine levels²⁹.

In-depth studies of preclinical and human cohorts are missing to fully comprehend the impact of aging on polyamine metabolism. Since polyamines occur in soluble and bound forms (for example, complexed to nucleic acids or ribosomes), which are usually not distinguished by the current methods, it remains elusive whether aging affects these pools differently. Moreover, most studies do not consider the circadian aspect of sampling time points, or fail to report them. Polyamines and their metabolic enzymes vary with feeding status and circadian rhythms, with the amplitude of this nycthemeron-related fluctuation reduced in aged mice²². At the same time, spermidine may modulate cellular circadian phases, as shown in a mouse fibroblast cell line⁴³. Thus, when choosing heterogeneous sampling time points, age effects may be overlooked. Moreover, the dietary intake of polyamine and their precursors can be highly variable, especially from human diets, complicating conclusions about age-dependent changes in humans. Thus, the decline in polyamines observed in Westernized countries may be avoided in Asian regions owing to traditionally polyamine-rich diets⁴⁴. Therefore, variations in dietary habits and polyamine intake should be considered in clinical trials. Finally, many studies lack the statistical power to detect meaningful biological trends in a reproducible fashion. There is no consensus on the normalization of polyamines, especially for fluid biospecimens, for which it is not clear whether absolute or relative concentrations of analytes (for example, per volume or protein content) or ratios of analytes would be preferable (Table 1). Likewise, extraction and detection methods differ across studies, rendering their interpretation and cross comparison difficult.

Thus, future studies addressing the impact of aging on polyamines should consider the aforementioned points and perform systematic meta-analyses requiring methodological standardization and statistical normalization.

The molecular basis of geroprotection and autophagy induction by spermidine

Many cellular effects caused by spermidine have been mechanistically linked to autophagy¹⁴ (Fig. 2), a homeostatic, intracellular degradation and recycling mechanism. Autophagy enhancement harbors broad health-promoting potential and constitutes the molecular basis for several geroprotective strategies^{5,9,45}. Dysfunctional autophagy is linked to various age-related diseases^{46,47}, and the activation of autophagy by genetic (for example, overexpression of Atg5 in mice⁴⁸ or Atg1 in flies⁴⁹) or pharmacological (for example, via rapamycin^{50,51} or spermidine^{14,52}) means is sufficient for extending lifespan in model organisms, including flies and mice⁵³.

While evidence for autophagy's causal role in spermidine-mediated geroprotection emerged from several in vitro and in vivo studies, future work using in vitro cell culture models should carefully distinguish effects caused by mechanistic targets of spermidine and exogenously produced oxidative stress. Fetal bovine serum (a standard ingredient of many cell culture media) contains an amine oxidase capable of oxidizing polyamines and producing hydrogen peroxide, which itself promotes autophagy and might thus be partly responsible for some effects in vitro⁵⁴. A solution to this methodological problem is the use of the serum amine oxidase inhibitor aminoguanidine or the replacement of bovine serum by amine oxidase-free serum (for example, from human origin⁵⁵). Notwithstanding this caveat of in vitro experiments, the effects observed in vivo in animal models and clinical trials should remain valid. This also applies to the crucial autophagy enhancement in spermidine-mediated geroprotection.

Spermidine and acetylation

Spermidine modifies the epigenetic landscape by reducing histone acetylation and affecting the acetylation status of many cytoplasmic proteins^{14,56} (Box 1). In aging yeast cells, spermidine triggers

Table 1 | Evolution of spermidine levels during aging in various tissues and organisms

Organism (maximum lifespan)	Strain or origin	Method of normalization (length of experiment)	Cell or tissue type	Evolution of spermidine level	Ref.
<i>S. cerevisiae</i> (CLS 3–4 weeks)	BY4741	Cell number (until day 9)	Whole yeast cells	Constant decline	14
<i>D. melanogaster</i> (80–100 d)	w ¹¹¹⁸	Fly number (until day 30)	Heads	Constant decline	15
Mouse (3–3.5 years)	C57BL/6 (female)	Protein content (until week 26)	Pancreas	No change	16
			Thymus	Early decline	
			Spleen	Constant decline	
			Ovary	Constant decline	
			Uterus	No change	
			Intestine	Early decline	
			Liver	Early decline	
			Stomach	Early decline	
			Lung	Early decline	
			Kidney	Early decline	
			Brain	No change	
			Skin (ear)	Constant decline	
			Skin (abdomen)	Constant decline	
Heart	Constant decline				
Muscle	Early decline				
Rat (3.2–3.8 years)	Wistar rat (newborns both sexes, rest male)	Tissue wet weight (until month 9)	Liver	Constant decline	17
			Thymus	Constant decline	
			Spleen	Early decline	
			Kidney	Constant decline	
			Muscle	Constant decline	
			Brain	Constant decline	
	Wistar rat (male)	Tissue wet weight (until month 22)	Liver	Early decline	18
			Kidney	Early decline	
	Wistar rat (male)	Tissue wet weight (until week 85)	Cerebral cortex	Constant decline	19
	Wistar rat (male)	Tissue wet weight (until month 36)	Kidney	Early decline, increase at month 36	20
	Sprague-Dawley rat (male)	Tissue wet weight (until month 24)	Hippocampus CA1	Minor increase	21
			Hippocampus CA2/CA3	Minor increase	
			Dentate gyrus	No change	
			Prefrontal cortex	Minor increase	
			Entorhinal cortex	No change	
			Perirhinal cortex	No change	
			Postrhinal cortex	Early minor increase	
Temporal cortex	No change				
Human (~120 years)	Caucasian ancestry, based in central Italy (50% female)	Protein content (31–106 years)	Whole blood	Decline in old age but normal in 90 to 106 year olds	24
	Based in Japan (male)	Volume	Whole blood	Minor increase in 60 year olds	28
	Based in Austria (67.12% female)	Volume (until 80 years)	Serum	Constant decline	23
	NA	Protein content (until 80 years)	PBMCs	Constant decline	25
	NA	Protein content (until 80 years)	PBMCs	Constant decline	26
	Based in UK (47.8% female)	Cell number (until 79 years)	RBC	Early decrease in adulthood	27
	Based in Canada	Protein content	Cortex (postmortem)	No change in adulthood	30
	Based in Japan (35.9% female)	Protein content	Liver (postmortem)	No change	29

Many of the changes depicted in the table were indicated as statistically insignificant in the respective studies. CLS, chronological lifespan; NA, not available; RBC, red blood cell.

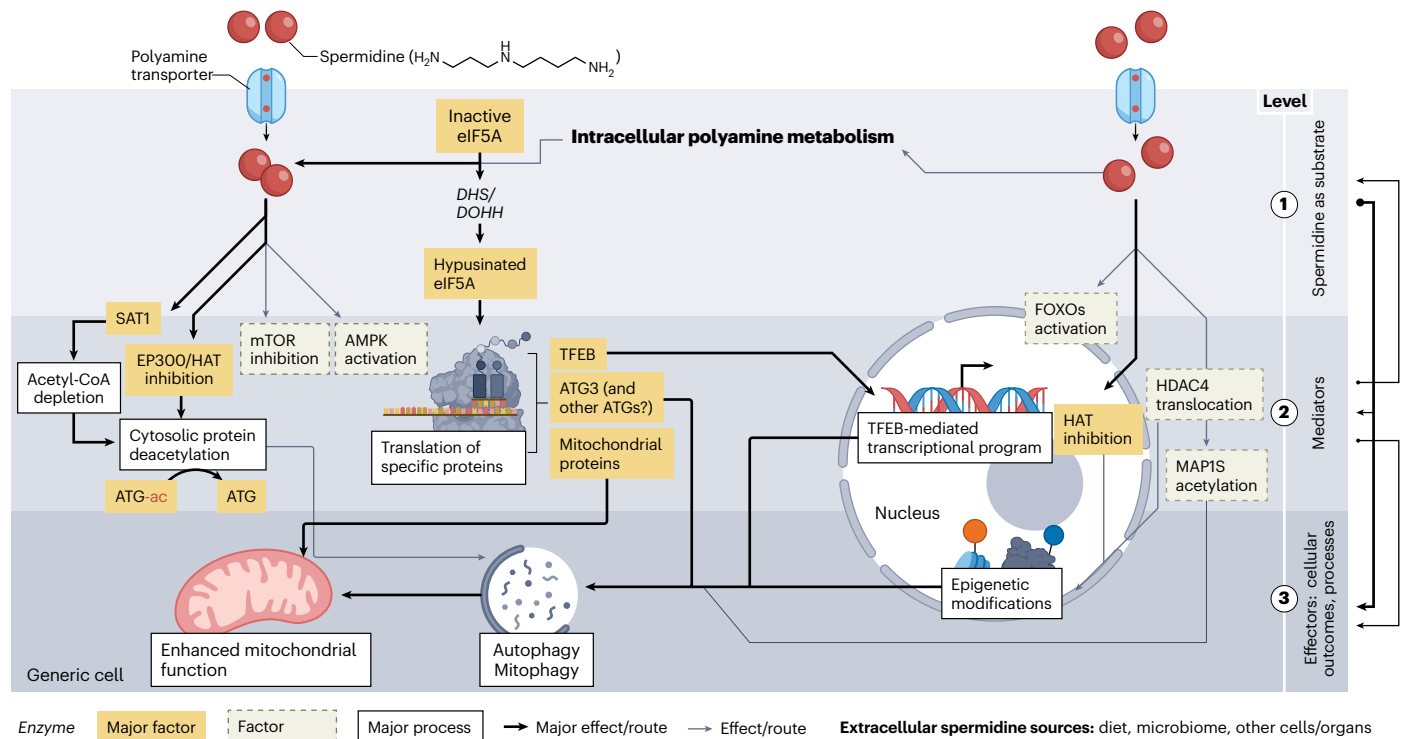


Fig. 2 | Major molecular and metabolic effects leading to spermidine-mediated lifespan extension. Based on our current knowledge of spermidine signaling, we attempted to classify the geroprotective mechanisms elicited by spermidine into three hierarchic layers. (1) Spermidine serves as a substrate for the hypusination reaction, activating eIF5A, hence facilitating the translation and synthesis of the pro-autophagic transcription factor TFEB and mitochondrial biogenesis. Many of the downstream effects of spermidine have been convincingly linked to its capacity to stimulate eIF5A hypusination. (2) Besides downstream mediators of hypusinated eIF5A (exemplified by ATG3, mitochondrial proteins, and TFEB), several factors and pathways have been described that are directly or indirectly affected. These include

acetyltransferases such as EP300, deacetylases (probably including HDACs and sirtuins), transcription factors (perhaps including FOXOs) and energy sensors (such as mTORC1 and AMPK), some of which regulate autophagy and cellular effects as described elsewhere in more detail¹⁰. (3) The cellular outcome is governed by increased autophagic flux, improved mitochondrial function and epigenetic reprogramming, accounting for the cellular, organismal and systemic antiaging effects. Note that the summarized effects have been identified in various cell types and organisms. Molecular effects that potentially contribute to autophagy induction and lifespan extension are depicted in dashed boxes with gray connectors. Ac, acetylated.

deacetylation of histone H3 by inhibiting histone acetyltransferases (HATs), thereby affecting autophagic gene transcription¹⁴. Autophagy induction by spermidine seemed independent of sirtuin 1 (SIRT1), a deacetylase, in one study⁵⁷ but dependent on the inhibition of the acetyltransferase E1A binding protein P300 (EP300)⁵⁶. However, two studies reported a spermidine-induced upregulation of SIRT1 and a functional SIRT1 dependency for its cellular effects^{42,58}. Nonetheless, autophagy was not characterized in those latter studies, and spermidine-mediated lifespan extension seemed independent of SIRT1 orthologs in yeast and worms⁵⁷.

EP300 acetylates and inactivates several autophagy-related genes (ATGs), including microtubule-associated proteins 1A/1B light chain 3B (known as LC3), adding further complexity to the autophagy-stimulating properties of spermidine⁵⁶. Spermidine also stimulates the translocation of histone deacetylase (HDAC) 4 to the nucleus, preventing the deacetylation of cytosolic microtubule-associated protein 1S (MAP1S)⁵² and promoting autophagic flux⁵⁹. Additional HDACs might be involved in the effects of spermidine^{60,61}. Activated polyamine metabolism can reduce protein acetylation by depleting acetyl coenzyme A (acetyl-CoA) pools⁶²⁻⁶⁴. For instance, overexpression of the acetyl-CoA-catabolizing enzyme SAT1 is sufficient to reduce acetyl-CoA pools in vivo⁶²⁻⁶⁴, favoring protein deacetylation and autophagy^{56,65}. Contrariwise, impaired polyamine synthesis and reduced hypusination alter the tricarboxylic acid cycle, hence increasing acetyl-CoA levels and HAT-mediated histone acetylation⁶⁶. Thus, polyamine metabolism acts

as an important nexus connecting cellular energy status and epigenetic modifications that govern cellular processes, including autophagy.

Interestingly, deacetylation of the transcription factor EB (TFEB), a target of eIF5A, favors its activity⁶⁷, suggesting a possible link between spermidine-induced protein deacetylation and TFEB activation. Similarly, eIF5A itself is regulated by acetylation events⁶⁸⁻⁷⁰, with deacetylation promoting its cytosolic location and activity⁷⁰. Whether EP300 is involved in this acetylation-based regulation is currently elusive. Future studies should address these unknowns to understand the cytoplasmic and epigenetic events linking high spermidine levels to the stimulation of autophagic flux. In a hypothetical scenario, spermidine rapidly stimulates autophagy through direct effects on cytoplasmic effectors but then mediates additional epigenetic, transcriptional and translational adaptations to sustain autophagic flux.

Spermidine, hypusination and autophagy: an intimate relationship

Many effects of spermidine can be traced to its use for posttranslational hypusination of eIF5A, a small, highly conserved and abundant protein containing 157 amino acids^{25,38,39,66,71} (Box 2). At least two isoforms of eIF5A (>80% sequence identity) have been identified in mice and humans. Both can be hypusinated and are partly tissue specific, with eIF5A2 mainly expressed in neuronal tissue. Hypusine (N⁶-(4-amino-2-hydroxybutyl)lysine) is a rare and unusual amino acid formed via a two-step enzymatic reaction: deoxyhypusine synthase (DHS) transfers

BOX 1**Spermidine and acetylation**

Acetylation of cellular and nuclear proteins is an important regulatory mechanism. Spermidine activates autophagy by inhibiting acetyltransferases and stimulating deacetylases, favoring autophagy-active states. Polyamine catabolism involves the consumption of acetyl-CoA and hence is coupled to protein deacetylation, while impaired spermidine synthesis and reduced eIF5A hypusination may increase acetyl-CoA generation by indirect mechanisms.

the 4-aminobutyl moiety from spermidine to the ϵ -amino group of a lysine residue in eIF5A (in humans Lys50) in an NAD⁺-dependent reaction. DHS is thought to be eIF5A exclusive, as no other protein is known to be hypusinated. The amino acid sequence surrounding the critical lysine residue is conserved across species. Then, deoxyhypusine hydroxylase (DOHH) transforms deoxyhypusine into hypusine by adding a hydroxyl group, which activates eIF5A. The high conservation of the DHS–DOHH–eIF5A pathway reflects its critical role in cellular growth and translational regulation. In yeast, the knockouts of *HYP2*, the eIF5A homolog, or *DYS1* (DHS) are lethal, and *Eif5a*-null or *Dhps*-null mice die during early embryogenesis. Postnatal conditional knockouts in mice compromise pancreas development⁷² and hematopoiesis²⁵ and cause major neurodevelopmental problems⁷³, underscoring the pathway's paramount ontological importance.

Hypusinated eIF5A regulates protein synthesis at translation initiation elongation, and termination stages. It helps to overcome ribosomal stalls, for instance, at poly-proline motifs and specific non-proline tripeptide sequences (a recent review details the molecular and physiological implications of eIF5A⁶⁹). Among others, it contributes to mitochondrial protein translation and, consequently, mitochondrial function^{66,74,75}. Accordingly, blockade of polyamine synthesis and consequent hypo-hypusination increases the tolerance to hypoxia by downregulating mitochondrial complexes and remodeling the mitochondrial network, which protects tissues from ischemia-induced damage⁷⁶. Blocking hypusination has also been tested in diabetes models, showing improvements in glucose tolerance⁶⁹, among other mechanisms, by causing a switch from glutaminolysis to glycolysis⁷⁷. This is corroborated by a recent study showing exacerbated diabetic pathophysiology in mice fed high doses of spermidine, although hypusination was not assessed in this study⁷⁸. Moreover, T lymphocyte differentiation critically relies on a functional polyamine–hypusine axis^{66,79}, which thus has an important role in shaping the immune system.

Importantly, hypusinated eIF5A facilitates the translation of certain ATGs^{36,38}, providing a direct link between increased hypusination and autophagy stimulation. eIF5A depletion in cell lines and *Caenorhabditis elegans* causes the rarefaction of autophagosomes (critical membranous structures formed to transport degradable cargo to lysosomes) and limits the lipidation of LC3, a key autophagosomal adaptor protein, and marker for autophagy^{36,37}. Similar effects, including the depletion of ATG3, can be achieved by pharmacologically blocking DHS by the spermidine-analog *N*¹-guanyl-1,7-diaminoheptane (GC7)³⁶. A recent study corroborated these findings in vivo by showing that spermidine increases ATG3 levels in *Drosophila* brains, depending on eIF5A hypusination³⁸.

Hypusinated eIF5A is also important for TFEB translation, the master inducer of lysosomal biogenesis and autophagy, in B and T lymphocytes. TFEB contains triproline motifs (one in mice and two in humans) and is decreased in immune cells from older adults²⁵. TFEB

BOX 2**Spermidine and hypusination**

Hypusination, the introduction of hypusine, is a rare posttranslational modification of a specific lysine residue in eIF5A, which functions as a translation elongation and termination factor. It strictly requires spermidine as a substrate. This rare and highly conserved modification activates eIF5A, which promotes autophagy by facilitating the translation of TFEB and ATGs. The spermidine–hypusination–TFEB–autophagy axis is responsible for at least parts of the geroprotective potential of spermidine.

drives the expression of genes with a CLEAR (coordinated lysosome expression and regulation) element and acts as a major transactivator of autophagy-relevant genes. TFEB is subjected to inhibitory phosphorylation by mechanistic target of rapamycin complex 1 (mTORC1), a major negative regulator of autophagy^{80–82}. Importantly, externally supplied spermidine elevates hypusination in mouse³⁹ and *Drosophila*³⁸ brains, in which aging is typically coupled to the decline of spermidine and hypusination^{15,38}. Cultured PBMCs from older (but not young) adult human donors also show increased hypusination upon in vitro spermidine treatment, alongside increased TFEB expression and autophagic flux²⁶. This suggests the functional incorporation of external spermidine into the hypusination–TFEB–autophagy axis both in vitro and in vivo.

The spermidine-mediated, eIF5A-driven increase of TFEB levels is probably one of the pathways explaining polyamine-mediated autophagy activation, at least in some tissues. The age-associated enfeeblement of this axis may compromise the function of immune cells, including B and T lymphocytes, some of which heavily depend on mitochondrial function^{25,26,66}. This probably explains the rate-limiting role of the polyamine pathway in neuronal tissues ('Human diseases and defects in the polyamine metabolism'), which is particularly energy consuming.

Interestingly, a self-amplifying loop in which spermidine enhances autophagic flux by EP300 inhibition, and autophagy favors polyamine synthesis, was recently described in kidney podocytes⁸³. The authors identified the transcription factor MAFB as an upstream regulator of polyamine metabolism in kidney cells⁸³. It remains to be studied whether this feedforward loop is active in other cell types. Beyond eIF5A, polyamines are known to directly modulate mRNA-to-protein translation, as exemplified by the autoregulation of polyamine enzymes that are translated less efficiently at high polyamine levels⁸⁴. As far as we know, this remarkable feature of translational control^{85,86} has not been investigated for aging-relevant and autophagy-relevant genes. Of note, aging per se leads to decreased translation efficiency and increased ribosomal pausing, thus favoring the collapse of proteostasis⁸⁷, while the age-associated decline of polyamine levels and hypusination is elusive in this context. At this point, it appears likely that polyamines affect multiple genes and proteins by intersecting transcriptional, translational or proteostatic pathways, requiring further mechanistic studies.

At the organelle level, mitochondria seem especially important for spermidine effects, as shown in cell cultures⁸⁸, worms⁸⁹, fruit flies^{38,39} and mice^{39,42,90–92}. Valuable lessons have been learned about the cellular consequences of impaired polyamine biosynthesis and reduced hypusination by using pharmacological and genetic strategies to target DHS or DOHH. For instance, genetic tools to reduce hypusination in *Drosophila* compromise mitochondrial respiration and ATP production alongside a general decrease in

mitochondria-related factors³⁸. Data from mammalian cell cultures corroborate the essential role of this axis in ensuring optimal mitochondrial function⁶⁶. Possibly, the upregulation of TFEB levels via eIF5A facilitates mitophagy, the autophagic removal of defective mitochondria, for enhanced mitochondrial quality control and optimization of cellular bioenergetics. As an alternative but nonexclusive possibility, spermidine may trigger mitochondrial biogenesis, potentially a necessary response to mitophagy. Indeed, oral spermidine increases the relative mitochondrial volume per myocyte⁹⁰ and the number of mitochondria in cardiac tissues from aged mice⁹³ and PGC-1 α in aged rat hearts⁴². Mechanistically, this may involve the SIRT1-dependent deacetylation and activation of PGC-1 α ⁴².

Both core autophagy genes and mitophagy-specific factors are required for the positive effects of spermidine on mitochondria and lifespan *in vitro*⁸⁸ and in flies^{38,39}, causally implicating mitophagy. TFEB and its homolog TFE3 are important stimulators of mitochondrial degradation processes⁹⁴ and metabolic flexibility⁹⁵, *inter alia* by increasing the expression of genes involved in mitochondrial biogenesis, oxidative phosphorylation and fatty acid oxidation. Thus, TFEB-like transcription factors also contribute to the activation and promotion of mitophagy, and loss of TFEB and TFE3 results in autophagic dysfunctions⁹⁶.

Other factors involved in geroprotection by spermidine

Several other mechanisms have been implicated in spermidine-induced autophagy, some of which may act independently of the abovementioned molecular cascade. Many autophagy triggers inhibit the negative regulator mTORC1 or activate the energy-sensing and autophagy-promoting AMP-activated kinase (AMPK). Spermidine caused a decrease in mTOR phosphorylation and increased AMPK activation in rat models of cardiac dysfunction *in vivo* and *in vitro*⁹⁷, but this does not apply to other systems^{25,56,57}. The importance of mTORC1 and other energy sensors might be time dependent, as suggested by a report on a transient, reversible decrease in mTORC1 activity upon spermidine supplementation *in vitro*⁷⁹. Of note, activation of mTORC1 has recently been shown to promote polyamine synthesis in cancer cells via *S*-adenosylmethionine decarboxylase 1 (AMD1), which generates the *N*-propylamine residue required for polyamine synthesis^{98,99}. mTORC1 also phosphorylates TFEB, inhibiting its translocation from the cytoplasm to the nucleus and preventing it from transactivating pro-autophagic genes^{100,101}. This provides another control layer of TFEB-mediated autophagy that may be influenced by spermidine. Hence, the interactions between spermidine, mTORC1 and TFEB remain to be elucidated in different contexts, and may depend on age, cell and tissue types, as well as on diets, as recently demonstrated for mTOR signaling after spermidine injections into mice¹⁰². Future studies should compare effects on primary rather than cancer cells, which often display rewired mTORC1 signaling.

Additionally, spermidine may suppress caspase 3-mediated Beclin1 cleavage, avoiding the destruction of an essential factor for autophagy initiation, which has been reported in a model of neuronal injury¹⁰³. Moreover, it elicits anti-inflammatory effects in dendritic cells by activating the transcription factor forkhead box O3 (FOXO3)¹⁰⁴ and recently spermidine treatment was shown to ameliorate neuroinflammation in an Alzheimer's disease mouse model¹⁰⁵. Whether FOXOs or other factors involved in inflammation regulation intersect with autophagy activation by spermidine remains unknown.

Interestingly, amine oxidase copper-containing 1 (AOC1) is downregulated in prostate cancer and its stimulation leads to increased spermidine oxidation, reactive oxygen species (ROS) production and anticancer effects¹⁰⁶. However, the implications of spermidine oxidation by amine oxidases *in vivo* remain largely unexplored.

Of note, autophagy-independent effects might additionally contribute to geroprotection by spermidine. Polyamines are potent scavengers of ROS, thus are able to prevent oxidative damage. This has been studied in various settings, including inflammatory conditions

*in vivo*¹⁰⁷. In fact, it remains elusive whether the anti-inflammatory action of spermidine observed in several studies is autophagy dependent^{10,108}. In a less explored line of research, spermidine might feed back on associated biosynthetic pathways. For example, methionine can be converted into *S*-adenosylmethionine (SAM) and decarboxylated SAM (dcSAM), an essential co-factor required for polyamine synthesis. SAM, the sole donor of methyl groups for the methylation of nucleic acids and proteins, may connect spermidine to autophagy-promoting, but also autophagy-independent, epigenetic changes. SAM inhibits methionine-starvation-induced autophagy via mTORC1 activation^{109,110}. Hence, increased spermidine synthesis could result in SAM consumption, thus alleviating SAM-mediated autophagy inhibition. However, whether externally supplied spermidine modulates SAM levels has not been formally established. Additionally, increased spermidine synthesis can reduce arginine bioavailability, which is used by arginase 1 (ARG1) for ornithine production. If external spermidine supply reduced the ARG1 activity, arginine would become available for its conversion into citrulline, generating nitric oxide (NO), which in turn has immunomodulatory and cardioprotective effects. Indeed, a spermidine-elicited NO augmentation has marked protective effects in models of arterial aging¹¹¹. NO has important functions, including signaling, vasodilation, metabolic regulation and stimulation of mitochondrial biogenesis¹¹², and its progressive decline might be involved in the pathogenesis of aging¹¹³. Finally, the aforementioned pathways have not been systematically studied in the context of spermidine-induced eIF5A hypusination, autophagy and geroprotection.

Since spermidine, hypusination of eIF5A, TFEB levels, autophagic capacity and mitochondrial function decline with aging, it appears plausible that their restoration via spermidine supplementation accounts for the extension of healthspan and longevity. Notwithstanding the heuristic value of the three-layer hierarchy of spermidine effects (Fig. 2), it will be important to perform careful epistatic analyses to understand whether combined interventions on specific elements of the hypothetical cascade will yield redundant effects (as this would occur if all elements act in a linear pathway) or rather may have additive antiaging effects (as this would happen if they were not arranged hierarchically). Hence, for theoretical and practical reasons, the model exposed in Fig. 2 should be subjected to further experimentation.

The effects of spermidine on the hallmarks of aging

Spermidine might be considered for both the prevention and treatment of (i) diseases caused by decreased autophagy or lysosomal function, (ii) metabolic disorders, (iii) diseases of energy-demanding tissues (for example, brain and heart) and (iv) inflammatory conditions and immune defects. Instead of discussing each disease individually, we refer interested readers to extensive reviews of this field by us¹⁰ and others¹¹⁴. Here, we outline the effects of spermidine on the hallmarks of aging¹³ (Fig. 3).

Genomic instability

During aging, DNA damage accumulates despite sophisticated repair mechanisms. It is largely unexplored whether polyamine supplementation impacts genomic stability, DNA repair or mutation frequency during aging. In a modified Ames test, measuring compounds' antimutagenic properties in bacteria, putrescine and spermidine were found to protect from DNA mutations¹¹⁵. Likewise, polyamines were crucial for homology-directed DNA repair, at least in hair follicles with induced DNA double-strand breaks¹¹⁶. ROS subserve important signaling functions, but excessive levels may cause DNA or membrane damage¹¹⁷. Polyamines can act as ROS scavengers^{118,119}, while their enzymatic degradation produces ROS^{120–122}, suggesting a dual role in oxidative homeostasis¹²³. Intriguingly, conditions tied to elevated polyamine levels (for example, some cancers, Snyder-Robinson syndrome (SRS)), as outlined below, experience oxidative damage. Along these premises and considering that most intracellular polyamines are bound to

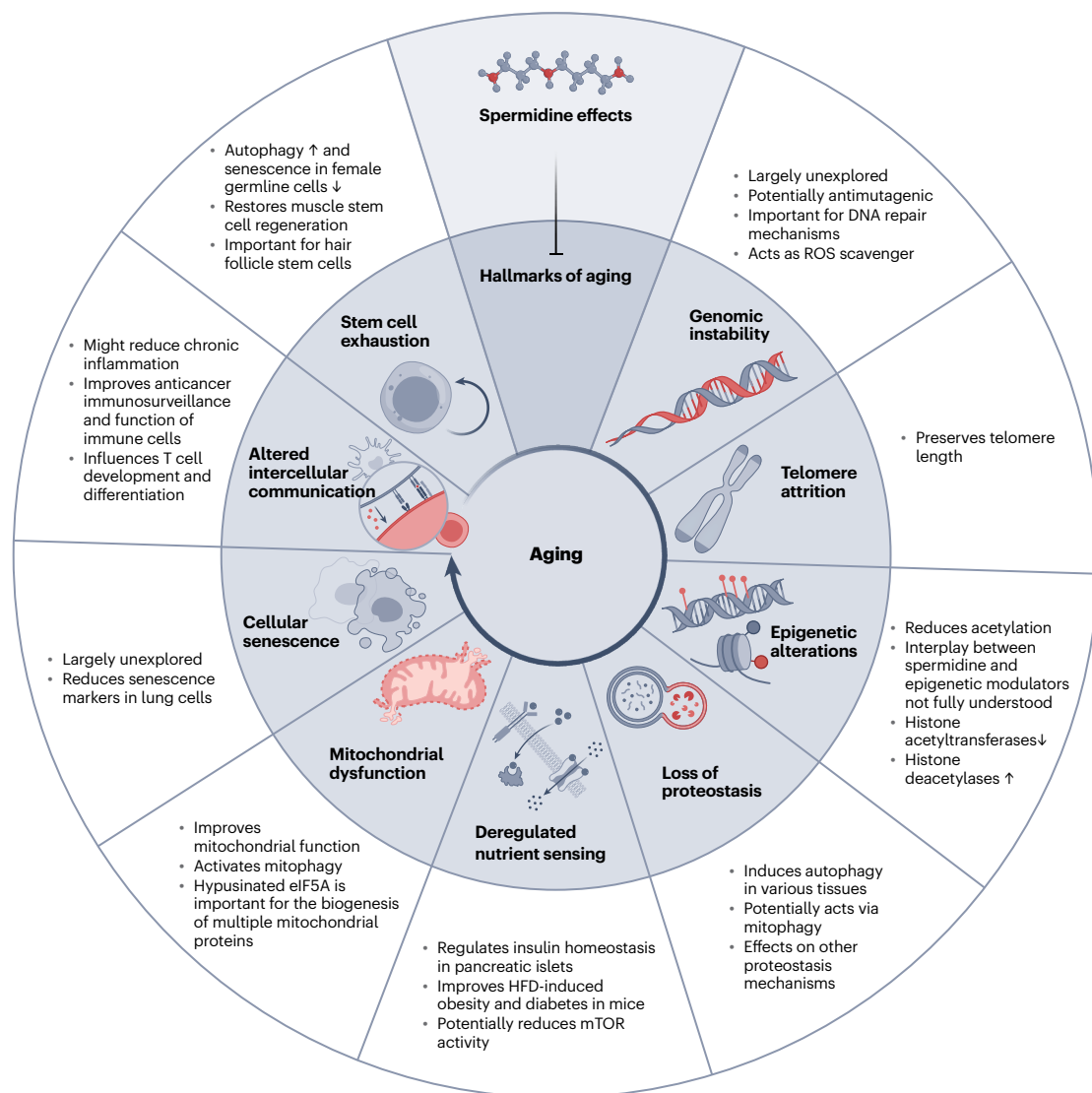


Fig. 3 | The effects of spermidine on the hallmarks of aging. For details and references see the main text. This figure was inspired by ref. 13. HFD, high-fat diet.

nucleic acids, influencing their stability and structure¹²⁴, this calls for a more detailed investigation of genomic instability and polyamines.

Telomere attrition

Telomeres are damage-susceptible, protective regions at chromosome ends and have been suspected of eroding with aging. Specialized DNA polymerases, telomerases, replicate these DNA ends but are absent or insufficiently expressed in many somatic cells, causing continuous telomere shortening. Protecting telomeres via genetic means is geroprotective in vivo but may come as a double-edged sword, potentially with carcinogenic effects. Spermidine supplementation preserves telomere length in aging mice¹²⁵. The exact mechanism is yet to be revealed and could be due to anti-ROS effects of polyamines and autophagy¹²⁶. It remains elusive whether spermidine directly modulates telomerases.

Epigenetic alterations

Aging is accompanied by epigenetic alterations (reversible DNA and histone modifications), probably reducing the flexibility of the transcriptome. Spermidine affects the epigenetic landscape in yeast and cell culture, which could be specific for certain promoter and gene regions, upregulating the transcription of autophagy-relevant genes¹⁴. In human dermal fibroblasts, spermidine inhibits HATs but

stimulates HDAC1 and SIRT1 (ref. 60). Similarly, in vascular smooth muscle cells, spermidine upregulates SIRT1, which mediates the inhibition of calcification⁵⁸. Spermidine also promotes the nuclear translocation of HDAC4 in cell lines and mice, preventing the deacetylation of cytosolic MAP1S, thereby promoting autophagy⁵². Conversely, in rat brains, spermidine was shown to promote phosphorylation and acetylation of non-histone chromosomal proteins¹²⁷, suggesting a more complex interplay of polyamines and acetylation than previously thought. Of note, (de)acetylation of polyamines and proteins might be executed by the same enzymes. For instance, HDAC10, an autophagy promoter¹²⁸, deacetylates *N*⁸-acetylspermidine for conversion to spermidine⁶¹. A concentration-dependent bimodal model has been suggested to explain how, in *Drosophila*, low spermidine concentrations promote, while higher concentrations reduce, HAT activity¹²⁹. Whether such a model may be applied to mammalian systems remains an open question.

Loss of proteostasis

Protein quality-control mechanisms include degradation and recycling pathways, for example, autophagy or the proteasome, and mechanisms to ensure proper folding and stabilization of proteins. Once proteostasis is out of balance, especially during aging, molecular protein

Table 2 | Spermidine-induced lifespan increases in model organisms

Organism	Strain	Sex	Starting time point	Dosing scheme	Lifespan extension	Maximum lifespan extension*	Median lifespan extension*	Comment	Ref.
<i>S. cerevisiae</i>	BY4741, DBY746	MATa, MATα	24 h after inoculation (stationary phase)	1 mM, 4 mM (via medium)	~4×	Yes	Yes	Chronological aging (4 mM) and replicative aging of pre-aged cells (1 mM); lifespan increase depends on functional autophagy (Atg7)	14 ^a
	BY4741	MATa, MATα	24 h after inoculation (stationary phase)	4 mM (via medium)	Up to 12×	Yes	Yes	Chronological aging; lifespan increase independent of SIR2	57
<i>C. elegans</i>	N2 (Bristol)	NA	Lifelong	0.2 mM (via food)	~15%	Yes	Yes	Lifespan increase depends on functional autophagy (<i>bec-1</i>)	14 ^a
	N2 (Bristol): control and vitamin B ₁₂ -deficient	NA	Lifelong	10 μM (via food)	~4% (control), 13% (B ₁₂ ⁻ deficient)	Yes	No (control); yes (B ₁₂ ⁻ deficient)		170
	N2 (Bristol)	NA	Lifelong	0.2 mM (via food)	18%	Yes	Yes	Lifespan increase independent of SIR2	57
<i>D. melanogaster</i>	w ¹¹¹⁸	Female	Hatched and aged on food containing spermidine	5 mM (via food)	~7–10%	No	Yes	Lifespan increase depends on functional hypusination pathway (CG8005/DHS)	38a
	w ¹¹¹⁸	Female	Lifelong	0.01–1 mM (dose-dependent increase; via food)	~15–30% (at 1 mM)	Yes	Yes	Lifespan increase depends on functional autophagy (ATG7); minor effects in males (only with 0.01 mM)	14 ^a
	w ^{Dah}	Female	48 h after eclosion	1 mM (via food)	~10%	No	Yes	Lifespan increase depends on glycine N-methyltransferase (GNMT)	135 ^a
Mouse	C57BL/6 (Charles River, Envigo, Janvier Labs or in-house breeding)	Female and male	Lifelong, starting at 4 or 18 months of age	0.3 mM, 3 mM (via drinking water)	~10%	No	Yes	Significant lifespan increase also with spermine, not with putrescine (3 mM)	90
	C57BL/6	Male	Lifelong	3 mM (via drinking water)	~24%	Yes	Yes	Lifespan increase depends on MAP1S-mediated autophagy	52 ^a
	Jc1:ICR	Male	Week 24	Via polyamine-enriched chow (putrescine, spermine and spermidine) ^b	NA	NA	Yes	High polyamine chow extended median lifespan; aging discontinued after 88 weeks	172
	Crj:CD-1 (Charles River Laboratories Japan Inc)	Female	Month 8	Via polyamine-increasing probiotic <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> (administered by gavage 3× per week)	NA	NA	Yes	Aging discontinued after 45 weeks	173
	<i>Zmpste24</i> ^{-/-} (progeroid model)	Male	Week 4	3 mM (via drinking water)	50%	Yes	Yes		171
	C57BL/6J (Charles River)	Female and male	Between months 19 and 21	Daily i.p. injection of 50 mg kg ⁻¹ spermidine (aqueous solution)	6% (female), 13% (male), (8.8% combined)	No	Yes	Small number of animals, lifespan increase not significant	102

Table 2 | Spermidine-induced lifespan increases in model organisms

Organism	Strain	Sex	Starting time point	Dosing scheme	Lifespan extension	Maximum lifespan extension*	Median lifespan extension*	Comment	Ref.
Rat	Sprague-Dawley	Male	Month 18	25 mg kg ⁻¹ d ⁻¹ (via drinking water)	None	No	No	Healthspan improvements (behavioral and neuroinflammatory parameters)	175
Human	Human immune cells (PBMCs) in vitro	NA	Blood donors <35 years old, spermidine added after 1 and 7 days in culture	0.2–20 nM (via medium)	3× on day 12 in culture (20 nM)	NA	Yes		14
	Dietary polyamine intake via FFQs in a North-Italian cohort	Women and men	NA	<62.2 μmol d ⁻¹ (lowest tertile), 62.2–79.8 μmol d ⁻¹ (middle tertile), >79.8 μmol d ⁻¹ (highest tertile) daily dietary intake	Estimated risk reduction equivalent to a 5.7-year difference between highest and lowest intake tertiles	NA	NA	Reduced mortality in highest polyamine intake tertile, controlled for confounding parameters	182

Atg7, autophagy-related 7; *bec-1*, *C. elegans* Beclin homolog; CG8005, *D. melanogaster* DHS homolog; FFQ, food frequency questionnaire; i.p., intraperitoneal; SIR2, silent information regulator 2; Zmpste24, zinc metalloproteinase STE24. *Studies that show a genetic dependency of lifespan extension on specific factors, thus suggesting causality of the associated pathway or process. ^bHigh polyamine chow: 1,540 nmol g⁻¹ spermidine, 374 nmol g⁻¹ spermine, 1,075 nmol g⁻¹ putrescine with average consumption of 7.7 g d⁻¹. This roughly equals 11.86 μmol spermidine, 2.88 μmol spermine and 8.28 μmol putrescine per day. Owing to the absence of water consumption data in most rodent studies, the daily doses of polyamine supplementation are difficult to compare.

errors, including accumulation of unfolded or misfolded peptides/proteins, inclusion bodies and dysfunctional protein complexes, lead to cellular and organismal perturbations. Improving proteostasis harbors great potential to ameliorate age-associated deteriorations and prolong lifespan and healthspan. Accordingly, autophagy is required for the lifespan-prolonging effects of spermidine^{14,15,52}. Likewise, in cardiac tissues from mice, properly functioning autophagy is needed for cardioprotection by spermidine⁹⁰. It can therefore be assumed that spermidine improves aging-associated proteostasis. However, this conjecture should be experimentally verified.

Deregulated nutrient sensing

Nutrient and energy states must be properly sensed for cellular and organismal functions. Aging leads to a disbalance in master regulators of nutrient sensing, including insulin-like growth factor 1 (IGF-1), mTORC1, AMPK and NAD⁺-dependent sirtuins. Inhibiting the trophic insulin/IGF-1 pathway and mTORC1 or stimulating low-energy-sensing AMPK and sirtuins elicits antiaging effects. Polyamines are abundantly present in pancreatic tissue of mice and rats¹³⁰, and putrescine and spermidine regulate insulin synthesis and release in pancreatic islets^{131,132}. Conversely, in mice fed a high-fat diet but not in controls, spermidine reversed hyperinsulinemia and insulin resistance, thus improving insulin signaling in the liver and white adipose tissue¹³³. In a mouse model of streptozotocin-induced type 1 diabetes, spermidine attenuated impaired testicular function¹³⁴. Conversely, high doses of spermidine (10 mM via drinking water) aggravated the diabetic phenotype of non-obese diabetic mice⁷⁸, highlighting the possibility that, in specific settings, spermidine may mediate toxic effects. The dose dependency of such effects has not yet been investigated. Interestingly, lifespan extension of a long-lived *Drosophila* strain with reduced insulin signaling was mediated by elevated spermidine levels¹³⁵, suggesting an interconnection between insulin signaling and polyamines during aging.

Moreover, polyamines regulate IGF–PI3K–Akt signaling in tumor-bearing mice¹³⁶, and spermidine suppresses Akt phosphorylation in skeletal muscle¹³⁷, suggesting another link between polyamines and

nutrient signaling relevant to aging. Conflicting effects of in vitro spermidine treatment on mTOR activity were found, but most evidence points to mTORC1 inhibition^{56,57,138,139}, especially in the short term⁹⁸. In mouse cardiomyocytes subjected to ischemia–reperfusion injury, spermidine reduced mTOR activity and activated AMPK⁹⁷. The latter was also shown in a mouse model of neurodegeneration¹⁴⁰. Because mTORC1 activation promotes polyamine synthesis in cancer cells via AMD1 (refs. 98,99), there may be bidirectional links between nutrient sensing and polyamine metabolism. However, the relationship between mTOR and the polyamine pathway is insufficiently understood in non-neoplastic cells.

Mitochondrial dysfunction

Mitochondrial integrity and function are compromised during aging. Mitochondrial dysfunction can accelerate aging, even independently from ROS levels¹⁴¹, by reducing cellular energy supply, engaging in inflammatory processes and igniting cell death pathways. Conversely, spermidine upregulates mitophagic subroutines^{39,89,90,142} and improves mitochondrial function in cell culture, flies and mice, partly depending on hypusination, autophagy and mitophagy^{38,39,42,88,90,142}. While these effects depend on mitophagic turnover and hence mitochondrial quality control (which eliminates dysfunctional organelles), the polyamine pathway also stimulates the biogenesis of mitochondrial proteins⁶⁶. Thus, in the fungus *Ganoderma lucidum*, the abundance of mitochondrial proteins is reduced upon genetic inhibition of spermidine synthesis or hypusination¹⁴³.

Cellular senescence

The state of cellular senescence is characterized by an arrest of the cell cycle, along with molecular alterations, including a specific secretome enriched in pro-inflammatory factors. One study showed that spermidine could suppress the induction of senescence markers by bleomycin in lung cells in vitro and in vivo¹³⁸. Accordingly, in female germline stem cells, spermidine reduced tumor protein 53 (TP53) levels and cyclin-dependent kinase inhibitor 2A (p16/CDKN2A)¹³⁹, markers of cellular

senescence¹⁴⁴. Yet another study showed a reduction of senescence markers in a cellular interleukin-1 β -induced senescence model and a mouse model of intervertebral disk degeneration¹⁴⁵. However, how spermidine treatment affects senescent cells in other tissues and, importantly, during physiological aging, remains elusive.

Stem cell exhaustion

During aging, the regenerative potential of tissues declines, including most stem cell compartments. Restoring stem cell function in aged organisms harbors the potential for reducing tissue damage and inflammation. In female germline stem cells, spermidine induced autophagy and reduced induced cellular senescence¹³⁹. Similarly, spermidine-induced autophagy restored the regenerative function of muscle stem cells¹⁴⁶ and increased myogenesis in vivo¹⁴⁷, suggesting its utility for reversing sarcopenia. Spermidine seems essential for hair growth and stem cell function of hair follicles in vitro and in vivo^{148,149}. Furthermore, high levels of AMD1 are required for embryonic stem cell pluripotency¹⁵⁰ and spermidine mediated the reprogramming of mouse embryonic fibroblast somatic cells to induced pluripotent stem cells¹⁵¹. Nonetheless, its potential to prolong stem cell function in different compartments in vivo in wild-type aging organisms has not been addressed.

Altered intercellular communication

Age-associated intercellular communication perturbations comprise disturbed nutrient sensing ('Deregulated nutrient sensing'), hormonal regulation alterations, chronic inflammatory reactions and reduced immunosurveillance. Little is known about how spermidine affects these hallmarks. Spermidine has anti-inflammatory properties in several mouse models^{10,104,152}. In ex vivo experiments, spermidine treatment improved the function of immune cells from aged human donors²⁶ and reduced viral propagation of severe acute respiratory syndrome coronavirus 2 in human lung cells¹⁵³. Spermidine, polyamine metabolism and eIF5A are critical factors for T cell development and differentiation^{26,79,154}. In mice, spermidine increased anticancer immunosurveillance through effects on both cancer and T cells^{155,156}. Conversely, polyamine-lowering approaches are being investigated in immune-invasive cancer types that rely on high polyamine levels¹⁵⁷. Combining polyamine transport and synthesis inhibitors reverses local immunosuppression in mouse tumors and increases T cell-mediated anticancer immune responses^{158,159}, a notion that is currently put forward in the first clinical trials (NCT03536728). In sum, spermidine may affect the cancer-immunity dialogue in a context-dependent fashion.

Interest in the microbiome as both a disease contributor and a therapeutic target has recently risen¹⁶⁰ and microbially produced polyamines are absorbed from the gut lumen, contributing to the systemic pool¹⁶¹. In mouse models of diet-induced obesity, spermidine feeding improved insulin resistance^{133,162}, and one study concluded that this could be due to gut microbiota changes¹³³. Furthermore, oligofructose treatment-associated changes in microbiota, leading to increased spermidine production, were found to be involved in reduced bacterial infection¹⁶³. In a mouse model of abdominal aortic aneurysm, spermidine feeding improved intestinal dysbiosis¹⁶⁴ and inflammation¹⁰⁸. Altogether this indicates an interdependent relationship between microbiota-produced polyamines, microbial composition and systemic health.

Spermidine-mediated lifespan extension

For potential geroprotective compounds or interventions, measurable increases in lifespan or healthspan are crucial benchmarks. Here, we summarize the effects of spermidine supplementation on lifespan in different model organisms (Table 2).

Unicellular and invertebrate models

The unicellular model organism yeast was at the forefront of basic biology achievements, including autophagy^{165–167}. In 2009, we found

that spermidine supplementation reversed its cellular decline, induced autophagy and prolonged survival in an ATG7-dependent manner¹⁴. This principle was conserved for *C. elegans*, *Drosophila melanogaster* and human PBMCs¹⁴. Consistent with the notion that autophagy induction by pharmacological or genetic means can extend lifespan¹⁶⁸, this places enhanced proteostasis at the mechanistic core of spermidine-mediated geroprotection. Several studies corroborated the lifespan extension in different fly strains, showing additional mechanistic dependencies on glycine *N*-methyltransferase¹³⁵ and DHS³⁸. Glycine *N*-methyltransferase is an important enzyme in the methionine cycle, providing the precursor SAM for polyamine biosynthesis. This suggests a possible link between methionine metabolism and increased lifespan by spermidine. As outlined before, spermidine-mediated longevity in flies depends on the hypusination pathway³⁸. In a fly model of Parkinson's disease, spermidine increased survival¹⁶⁹, suggesting potential effects against neurodegeneration. Initial reports of spermidine-mediated longevity in worms^{14,57} were recently corroborated by data showing that the shortened lifespan due to B₁₂ deficiency could be partially reversed by spermidine¹⁷⁰.

Lifespan extension in mammals

Lifelong spermidine supplementation via drinking water is sufficient to prolong the lifespan of adult female and male mice⁹⁰. Similar effects were observed for spermine but not for putrescine⁹⁰. Spermidine also sufficed to prolong the lifespan of pre-aged 18-month-old mice⁹⁰ and short-lived male mice with progeria (*Zmpste24*^{-/-} mice)¹⁷¹. Likewise, daily injections late in life caused a nonsignificant lifespan extension in mice¹⁰² and polyamine-enriched food prolonged the lifespan of aging mice¹⁷², suggesting that the mode of administration is of little importance for the longevity effects. Interestingly, the upregulation of intestinal polyamine production in 14-month-old mice by feeding the prebiotic arginine combined with the probiotic bifidobacteria LKM512 (equipped with the enzymatic machinery to convert arginine into spermidine) is sufficient to prolong lifespan and reduce the incidence of age-related diseases^{173,174}. While the cardioprotective effects of spermidine could mechanistically be linked to autophagy⁹⁰, only one mechanistic lifespan study has been conducted in mammalian models. In the study by Yue et al., lifespan extension and autophagy induction by spermidine feeding were abolished in autophagy-deficient male *Map1s*-knockout mice⁵². Fewer studies have been carried out on rats: in salt-sensitive *Dahl* rats, spermidine exerts systemic cardioprotective effects⁹⁰. One study found no impact on life expectancy but a significant healthspan-extending effect in middle-aged male Sprague-Dawley rats¹⁷⁵.

Several recent studies revealed protective effects of spermidine against high-fat or high-calorie diets, including reduced body weight gain or obesity, improved insulin/IGF-1 signaling and delayed metabolic syndrome^{102,133,152,162,176}. It will be interesting to test whether these benefits also translate to lifespan extension on such diets.

As a noteworthy control, in several mouse studies, orally or parenterally administered spermidine did not change food or water intake^{39,90,102,108}, excluding reduced caloric intake as a trivial reason for lifespan extension. Importantly, despite its tightly regulated metabolism, in mice, exogenous supplementation increases the levels of spermidine in plasma^{90,108} and cardiac tissue⁹⁰. Moreover, dietary spermidine passes the blood–brain barrier and integrates into the polyamine pools of neuronal tissues³⁹. A one-year-long clinical study detected increased spermine but constant spermidine plasma levels upon feeding polyamine-rich natto to healthy male volunteers²⁸. Data on the impact of spermidine supplementation on tissue levels and cellular polyamine metabolism are still scarce. A systematic investigation of these unknowns is warranted. Capturing aging-specific, sex-specific and organ-specific effects upon spermidine administration probably requires high spatiotemporal resolution and flux measurements, as steady-state quantifications might fail to detect changes in metabolic dynamics.

To date, there is a lack of systematic research to determine the optimal dose, required onset (whether there is a point of no return?) and timing (continuous or intermittent) of spermidine treatments. Moreover, there is insufficient information on possible gender-specific effects and mosaic aging. Does spermidine equally decelerate all hallmarks of aging in all organs, or does it preferentially affect specific features and tissues? And finally, would spermidine improve the quality of life and delay aging in nonhuman primates similar to rodents?

Spermidine in clinical trials and human diseases

Geroprotective compounds have been identified en masse in recent years¹⁷⁷, but the translation to human trials has been slow. Given its natural occurrence, spermidine seems an ideal candidate for clinical evaluation. The consideration that mammals probably have adapted to varying concentrations of dietary polyamine intake supports this idea. Of note, Janssens and Houtkooper compared the likelihood of side effects for putative geroprotectors by querying databases. They concluded that spermidine possessed the lowest chance of causing adverse effects¹⁷⁸, which is in line with our research regarding the safety of low-level spermidine supplementation¹⁷⁹ and suggestions by others¹⁸⁰. Accordingly, Partridge and colleagues ranked spermidine among the most promising interventions that may slow aging¹⁷⁷. Here, we discuss the current state of clinical and epidemiological research and disorders in polyamine metabolism (Box 3).

Spermidine in epidemiological studies

In humans, no interventional studies of polyamines on age-associated parameters or biomarkers (for example, epigenetic clocks or frailty-based aging estimates) have been reported. However, correlative studies suggest positive associations between spermidine intake and life expectancy in Asian countries¹⁸¹. Moreover, dietary spermidine is associated with reduced mortality in two non-interventional European cohorts¹⁸², and lower incidence of cardiovascular disease^{90,183}, cancer¹⁸² and cognitive impairment³⁹, withstanding potentially confounding factors. Spermidine intake is subjected to large variations and estimated as 5–15 mg per day^{6,161,184–189}. The highest spermidine intake had a lower estimated mortality risk corresponding to a 5.7-year younger age¹⁸². Interestingly, one study found spermidine and spermine in healthy nonagenarians and centenarians at comparable levels to the young-to middle-aged group (31–56 years), while the aged group (60–80 years) had significantly decreased levels²⁴. Hence, ‘successful aging’ might be linked to conserving high polyamine levels. This conjecture is supported by positive correlations between dietary spermidine intake and hippocampal volume and cortical thickness, which decline with old age¹⁹⁰.

However, the interpretation of epidemiological data requires care and caution, as underlying problems may blur these associations. For instance, food polyamine content varies notably between databases^{6,44,184,191} and the impact of seasons, food storage and preparation techniques on polyamine levels is insufficiently understood⁴⁴. Dietary assessments via questionnaires are afflicted by intrinsic problems, including subjectivity and compliance. Despite these limitations, it appears reassuring that the epidemiological correlations are endorsed by preclinical experimentation.

Randomized clinical trials using spermidine

The expanding preclinical literature on spermidine has spurred the design of clinical trials. In a 3-month pilot trial, older adults were supplemented with a wheat germ extract containing concentrated polyamines, leading to subtle amelioration of memory performance¹⁹². The same extract was tested for safety in aged persons and no safety issues were detected¹⁷⁹. Based on these results, a larger 12-month trial¹⁹³ was conducted on 100 participants aged 60–90 years with subjective cognitive decline. In this trial, the wheat germ extract failed to produce effects on memory function or other secondary outcomes, while

there was no detectable cognitive decline in the placebo group during the study duration. However, subgroup analyses focusing on participants with high compliance suggested beneficial effects on selected memory parameters and soluble intercellular adhesion molecule 1 levels¹⁹⁴, a marker for vascular inflammation and injury¹⁹⁵. Another small-scale study used wheat-germ-containing bread rolls to elevate daily spermidine intake by 3.3 mg in older adults in Austrian nursing homes. This study reported mild improvements in cognitive function in a subgroup affected by mild dementia¹⁹⁶. In line with accumulating preclinical data^{15,38,39,197,198}, this implies that spermidine might be useful for preventing or treating age-associated cognitive decline.

To the best of our knowledge, the longest interventional study was reported by Soda et al.²⁸: A one-year-long polyamine supplementation using natto, a polyamine-rich Japanese dish based on fermented soybeans (daily increases of 14.5 mg spermidine and 4.5 mg spermine) elicited decreased lymphocyte function-associated antigen 1 levels, suggesting anti-inflammatory effects. Another strategy for increasing polyamine availability consists in administering yogurt containing the precursor arginine and *Bifidobacterium animalis* subsp. *lactis*, which generates spermidine in the intestinal tract. This synbiotic combination of prebiotic and probiotic caused increased putrescine and spermidine levels in blood, decreased heart rates, improved endothelial function and improvements in the blood lipid profile^{199,200}. Moreover, spermidine supplementation reportedly prolongs the anagen phase of hair follicles in a randomized, placebo-controlled, double-blind trial²⁰¹, confirming similar results from preclinical studies^{148,202}.

Given the dependency of many cancers on polyamines, concerns have been raised that elevated dietary spermidine might favor oncogenesis^{203,204}. However, some epidemiological studies suggest a decreased cancer risk in people with naturally elevated spermidine intake^{182,205}. Preclinical experiments show that oral spermidine administration reduces the risk of inflammation-induced colon carcinogenesis and other cancers^{156,206}. Nevertheless, caution regarding increased polyamine intake should prevail in individuals with existing neoplastic diseases because spermidine reportedly has an ambiguous impact on cancer: under some circumstances, spermidine might reduce oncogenesis (possibly via the induction of oncosuppressive autophagy and the stimulation of cancer immunosurveillance). On the contrary, decreasing polyamine synthesis in neoplastic cells harbors the potential for halting tumorous growth and rendering cancer cells vulnerable to immune effectors^{157,207}. Hence, both polyamine-lowering therapies and spermidine supplementation could be considered for the prevention and treatment of cancer, calling for a more profound understanding before oncological clinical trials investigating spermidine supplementation are launched.

Several clinical trials (3–6 mg spermidine per day) are ongoing to improve hypertension in combination with first-line antihypertensive drugs (NCT04405388) or to explore effects on depression and sleep quality (NCT04823806). One study combines spermidine with exercise (along with shiitake powder, vitamins C, B₁ and zinc) to treat aged patients with heart failure with preserved ejection fraction (NCT05128331). Another small study plans to explore escalating doses of single postprandial spermidine supplementations (5, 10 and 15 mg), in combination with three different compounds, on inflammation (NCT05017428).

Human diseases and defects in the polyamine metabolism

Genetically determined polyamine pathway defects account for several human diseases. Faundes et al. noted that heterozygous *EIF5A* variants causing translational defects explain syndromes including developmental problems, cognitive disabilities, facial dysmorphism and congenital microcephaly²⁰⁸. In an *eIF5A*-knockdown zebrafish model recapitulating the human phenotypes, spermidine attenuated the severity of the pathology, suggesting a potential therapeutic avenue²⁰⁸. A genetic defect in spermine synthase leading to increased

BOX 3**Status of clinical research**

Given its natural occurrence in mammalian cells and foods, spermidine harbors a low risk of side effects in humans. Epidemiological data suggest systemic health benefits of elevated dietary intake. Translational clinical trials investigating spermidine are still rare but offer first hints at improvements in cognitive function and inflammation. Novel sequencing methods identified defects of the polyamine pathway in neurological and neurodevelopmental diseases, and altered polyamine levels have been identified in various medical conditions.

cellular spermidine and an enhanced spermidine/spermine ratio has been identified as the cause of the SRS^{209,210}. This X-linked, recessive mutation causes mental retardation and facial asymmetry, among other problems including osteoporosis and muscle weakness²¹¹. Surprisingly, SRS is characterized by lysosomal and mitochondrial damage, as well as by exacerbated oxidative stress and decreased autophagic flux²¹². The provision of stable spermine analogs rebalancing the polyamine system increases spermidine catabolism and ameliorates these disbalances, constituting a viable treatment option for SRS²¹³. The exact mechanistic relationship between excessive spermidine and SRS pathogenesis remains enigmatic. Neuronal cells are especially susceptible to defects in spermine synthase, perhaps due to the importance of spermidine-mediated hypusination for eIF5A function in mRNA-to-protein translation. Mutations in other translation factors (for example, EF2 and EF1a2) have also been linked to neurological, neurodegenerative and neurodevelopmental disorders²¹⁴.

Rare heterozygous gain-of-function mutations of ODC1 cause the Bachmann-Bupp syndrome, leading to accumulating putrescine levels, developmental delay and macrocephaly (in contrast to loss-of-function mutations that cause microcephaly)^{215–218}. Repurposing the ODC1 inhibitor difluoromethylornithine (DFMO) has been suggested as a potential therapy²¹⁹, which was originally investigated as a putative treatment for cancers characterized by high polyamines, although with limited effects.

Disbalances in polyamine levels might be involved in a series of human diseases and have been suggested as potential biomarkers^{220,221}. Amin et al. summarized various conditions linked to dysregulated polyamine levels²²². Elevated polyamine levels and increased biosynthetic capacities have been identified in human cancers and were investigated as potential drug targets and biomarkers^{98,222,223}. Thus far, despite numerous preclinical studies, polyamine-targeted agents have not been successful as anticancer therapies. Furthermore, deregulated polyamines have been intensively investigated in neurodegenerative diseases, such as Alzheimer's²²⁴ and Parkinson's²²⁵. A recent study suggested that higher serum spermidine in patients with mild cognitive impairment could be predictive of developing Alzheimer's disease²²⁶. This, however, stands at odds with another study showing the opposite association²²⁷. A comprehensive review of altered polyamine metabolism in Alzheimer's disease has recently been published²²⁸.

Interestingly, despite accumulating data on the cardioprotective effects of spermidine in preclinical models and the positive association of dietary intake with reduced cardiovascular risk factors, a recent Chinese study reported higher serum levels to be associated with increased stroke risk²²⁹. Conversely, serum spermidine levels were positively related to good prognosis of patients with myocardial infarction²³⁰. This interpretation is supported by a preclinical study showing that exogenous spermidine protects rats against acute myocardial

infarctions²³¹. In sum, the effects of blood spermidine levels on the human cardiovascular system are still controversial.

Despite many studies investigating polyamines as biomarkers, technical limitations, small-scale trials and nonsystematic evaluation of the pathway have hindered a comprehensive understanding of polyamines in diseases. By the nature of such biomarker studies, an increase or disbalance of polyamine levels in a disease state does not necessarily mean a causal involvement in that condition and instead could reflect a homeostatic stress response. Moreover, to understand pathologic alterations in the polyamine pathway, flux studies should be performed.

Conclusion

The intricate relationship between polyamines and human disorders implies a delicate balance of the polyamine system to maintain homeostasis. As can be inferred from the pathogenic effect of gain-of-function *ODC1* mutations, the accumulation of polyamines in SRS and the reliance of many cancers on elevated polyamines may mediate toxic or pro-tumorigenic effects, calling for caution in dose-escalation studies. The same degree of caution needs to be applied for long-term spermidine supplementation. Its clinical evaluation must go hand in hand with rigorous safety evaluations. The variety of diseases linked to disturbed polyamine levels warrants further research considering state-of-the-art techniques²³² to quantify polyamines, their precursors and degradation products reliably. A systematic assessment of polyamines as biomarkers in the aforementioned medical conditions, in combination with metabolic flux analysis of the pathway, may help to distinguish physiological and pathophysiological changes in aging and diseases in future studies and could provide a better understanding of pathogenesis and disease progression. In this context, disbalances in the polyamine pathway that contribute to normal or progeroid aging should be investigated.

Outlook

Spermidine emerges as a bona fide geroprotector that naturally occurs in food and is vital for cellular and organismal function through a highly conserved pathway. Seven lines of evidence support its antiaging properties: (i) spermidine levels decrease in most, but not all, investigated tissues and species during aging. (ii) Spermidine supplementation elongates the healthspan and lifespan of multiple species. (iii) Epidemiological data support the notion that elevated dietary intake alleviates, delays or halts age-associated deteriorations, including cancer, cardiovascular disease and cognitive dysfunction. (iv) Metabolic processes within and adjacent to the polyamine pathway are well understood, pharmacologically targetable and highly conserved across species, allowing the informed use of models and the development of targeted interventions. (v) Spermidine affects most of the hallmarks of aging. (vi) Spermidine seems safe for humans. (vii) The bioavailability of spermidine seems reasonably good in mammals.

Due to its intricate interactions with cellular metabolism, spermidine probably exhibits multiple modes of action and molecular targets that may vary in a cell-type-specific, organ-specific and context-specific manner. This may be advantageous for challenging aging as a multifactorial process, but the lack of one unique mode of action may constitute an obstacle to the targeted pharmacological use of the compound. Alternatively, the synthetic design of polyamine-related compounds could overcome some limitations that are intrinsically present for these bioactive body-endogenous substances. Recently, this concept was successfully tested: the polyamine-related compound 1,8-diaminooctane mimicked or even exceeded spermidine-mediated geroprotection in worms. This compound also activated mitophagy and conferred cytoprotection to human cell lines²³³. Important questions remain to be answered for the future clinical application of spermidine. These concern its uptake by human tissues, the monitoring of autophagy or specific signaling pathways as pharmacodynamic markers in humans,

optimal dosing and scheduling, and the effects of externally supplied spermidine on intestinal, cellular and systemic polyamine metabolism. We hope that further research into these questions will improve the design of future registration trials that evaluate the antiaging properties of spermidine.

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Author contributions

S.J.H. wrote the first draft. S.J.H., G.K. and F.M. conceptualized the review. M.B. and S.J.H. collected information for the tables. All authors proofread, edited and contributed substantially to the final version.

Competing interests

F.M. and G.K. are scientific cofounders of Samsara Therapeutics, a company that develops new pharmacological autophagy inducers. F.M. and T.E. have equity interests in and are advisors of The Longevity Labs (TLL). A.K.S. is consulting for TLL and Oxford Healthspan. G.K. is a scientific cofounder of everImmune, Osasuna Therapeutics and Therafast Bio. G.K. is holding research contracts with Daiichi Sankyo, Eleor, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Tolllys and Vascage. S.J.H. and M.B. declare no competing interests.

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