REVIEW SUMMARY

AGING

Spermidine in health and disease

Frank Madeo,*† Tobias Eisenberg,† Federico Pietrocola, Guido Kroemer*

BACKGROUND: As the world population ages, chronic diseases such as diabetes, cardiovascular disease, cancer, and neurodegeneration become ever more prevalent. Interventions that favor healthy aging would constitute powerful strategies with which to limit human diseases that have a broad socioeconomic impact. Fasting regimens such as intermittent fasting or dietary adaptations such as caloric restriction are among the few regimens that extend life and beneficially affect health in all tested model organisms, including rodents and nonhuman primates. However, few people seem capable of changing their dietary routines for extended periods. Thus, supplementation with caloric restriction mimetics (CRMs), which would pharmacologically mimic the beneficial effects of caloric restriction or fasting, has gained attention as an attractive and potentially feasible strategy. The naturally occurring polyamine spermidine, the abundance of which declines during the process of aging, has emerged as a well-tolerable CRM targeting various molecular and physiological age-associated adversities.

ADVANCES: Conceptually, healthy aging requires the retardation of multiple molecular and cellular alterations that drive the aging process and induce age-associated pathologies. These include genomic instability, epigenetic alterations, loss of protein degradation capacity (leading to neurodegeneration), deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, and chronic inflammation. Spermidine displays pleiotropic effects that include anti-inflammatory properties, antioxidant functions, enhancement of mitochondrial metabolic function and respiration, as well as improved proteostasis and chaperone activity. Many anti-aging effects of spermidine are causally connected to the capacity of this polyamine to induce cytoprotective autophagy. Autophagy ensures general cell homeostasis and proteostasis and is directly involved in the degradation of damaged, potentially toxic organelles and harmful protein aggregates, thus removing and recycling cytoplasmic material that otherwise would accumulate during aging. Consistently, extra supply of spermidine prolongs the life span across species in an autophagy-dependent manner and counteracts age-associated pathologies such as cardiovascular disease, neurodegeneration, and cancer. For instance, dietary spermidine supplementation ameliorates age-induced memory impairment in flies and protects from autoimmune-directed demyelination of neurons in a mouse model for multiple sclerosis. Spermidine also reduces the growth of transplantable tumors, stimulates anticancer immune surveillance in combination with chemotherapy, and suppresses tumorigenesis induced by chemical insults in mice. Moreover, elevated dietary polyamine uptake correlates with reduced cardiovascular and cancer-related mortality in human epidemiological studies. Because spermidine is already present in daily human nutrition, clinical trials aiming at increasing the uptake of this polyamine appear feasible.

OUTLOOK: Although spermidine induces autophagy and autophagy inhibition curtails many of the health-promoting effects of spermidine, additional mechanisms have been proposed to explain the beneficial effects of spermidine on aging. These potentially autophagy-independent mechanisms include direct antioxidant and metabolic effects on arginine bioavailability and nitric oxide (NO) production. However, it has not been formally determined whether these routes act in a completely autophagy-independent manner or are interrelated with autophagy (in an additive or synergistic way) (see the figure), and it will be important to define actionable molecular targets that explain the beneficial effects of spermidine in diverse pathophysiological settings. In this sense, it will also be of interest to explore synergisms of spermidine with other CRMs that initially act through different mechanisms.

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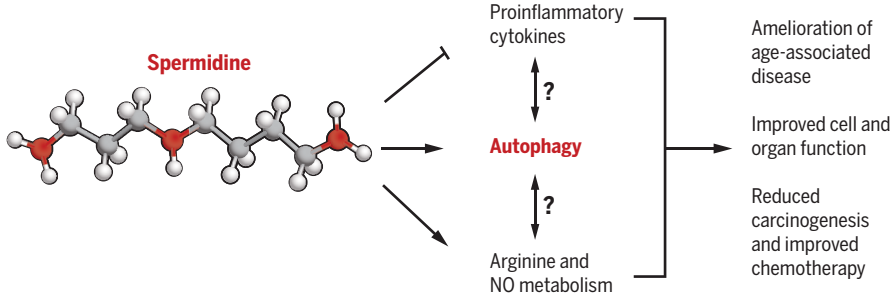
Another unresolved enigma resides in the tissue specificity of spermidine-induced health effects. For instance, the mechanisms through which oral spermidine intake can mediate systemic effects on blood metabolites and proteins remain to be elucidated. Similarly, it remains elusive whether spermidine acts exclusively on leukocytes to suppress chronic low-grade inflammation, and to what degree other organs may explain the increased bioavailability of arginine upon spermidine supplementation.

One strong argument in favor of the exploration of spermidine as a CRM in clinical trials is its low toxicity yet strong efficacy. Spermidine is an abundant natural polyamine contained in all organisms from bacteria to men and is naturally present in reasonable but varying amounts in human diets. Nevertheless, based on preclinical results, possible contraindications of spermidine administration such as advanced cancer and renal failure have to be defined. Last, it may be interesting to explore the development of artificial spermidine analogs with increased potency or ameliorated pharmacokinetic characteristics. ■



Schematic outline of the mechanisms of spermidine-mediated health effects. The natural polyamine spermidine has prominent cardioprotective and neuroprotective effects, ameliorates aging-associated metabolic decline, and stimulates anticancer immunosurveillance in animal models. Autophagy is required for several of these health-promoting effects of spermidine. Spermidine also suppresses proinflammatory cytokines and improves the bioavailability of arginine required for NO biosynthesis. It remains an open question whether all these effects depend on the autophagy-stimulatory properties of spermidine.

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Interventions that delay aging and protect from age-associated disease are slowly approaching clinical implementation. Such interventions include caloric restriction mimetics, which are defined as agents that mimic the beneficial effects of dietary restriction while limiting its detrimental effects. One such agent, the natural polyamine spermidine, has prominent cardioprotective and neuroprotective effects and stimulates anticancer immunosurveillance in rodent models. Moreover, dietary polyamine uptake correlates with reduced cardiovascular and cancer-related mortality in human epidemiological studies. Spermidine preserves mitochondrial function, exhibits anti-inflammatory properties, and prevents stem cell senescence. Mechanistically, it shares the molecular pathways engaged by other caloric restriction mimetics: It induces protein deacetylation and depends on functional autophagy. Because spermidine is already present in daily human nutrition, clinical trials aiming at increasing the uptake of this polyamine appear feasible.

As the world population ages, chronic maladies such as diabetes, cardiovascular disease, cancer, and neurodegeneration become ever more prevalent because life expectancy is increasing at a quicker pace than does health span (*1*). Interventions that favor healthy aging could constitute powerful strategies to limit human diseases that have a broad socioeconomic impact. Caloric restriction (CR), which is defined as the chronic reduction of calorie intake without malnutrition, is among the few regimens that extend life and beneficially affect health in all tested model organisms, including rodents and nonhuman primates (*2*, *3*). However, it is difficult to set the optimal intensity of CR to avoid undernourishment and other unwanted effects, for which reasons CR is contraindicated in elderly and diseased persons (*1*, *4*, *5*). Moreover, only few people seem capable of changing their dietary routines for extended periods (*6*). Thus, the supplementation of caloric restriction mimetics (CRMs), which would pharmacologically mimic the beneficial effects of caloric or dietary restriction, has gained attention as an attractive and potentially feasible strategy (*7*).

Conceptually, healthy aging requires the attenuation or retardation of several molecular and cellular alterations that drive the aging process and induce age-associated pathologies. These include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, chronic inflammation, and altered intercellular communication (*2*, *8*–*10*). Accordingly, regimens that extend life span of model organisms often display pleiotropic effects that include but are not limited to anti-inflammatory properties, enhancement of mitochondrial metabolic function and respiration, as well as improved proteostasis. This holds true also for the application of CRMs, most of which enhance cytoprotective autophagy (examples of CRMs are summarized in table S1), although additional effects such as on energy metabolism cannot be excluded (*11*). Autophagy ensures general cell homeostasis and proteostasis and is directly involved in the degradation of damaged, potentially toxic organelles or long-lived protein aggregates (*12*). Through controlled sequestration of cytoplasmic material into double-membraned autophagosomes, autophagy directs macromolecules (including proteins, lipids, and nucleic acids) or whole organelles to lysosomal degradation. This process detoxifies and recycles potentially harmful material that accumulates during aging. Consistent with its protective function against aging and disease (*13*–*15*), autophagy is also required for the life-span–extending effects of CR and of several CRMs (*2*, *12*). These CRMs mostly induce autophagy via a common mechanism that involves the deacetylation of cytosolic as well as nuclear proteins (*7*, *16*–*19*). However, the molecular targets accounting for these effects vary among CRMs. CRMs can act as inhibitors of the synthesis of acetyl coenzyme A (CoA), which is essential for enzymatically catalyzed acetylation reactions, for direct inhibitors of acetyltransferases, or as activators of deacetylases (in particular sirtuins) (table S1).

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The diamine putrescine and the polyamines spermidine and spermine are ubiquitously occurring polycations that are associated with several important cellular functions and help maintain general cell homeostasis. Polyamines are essential for cell growth and proliferation and tissue regeneration. They bind and stabilize DNA and RNA, have antioxidative activities, modulate enzyme functions, and are required for the regulation of translation. These characteristics have been reviewed elsewhere (*20*–*22*). Polyamines have also been characterized in the control of apoptosis (*20*, *22*) and in the context of learning and memory (*23*). Spermidine acts as a natural autophagy-inducer (*24*) and anti-aging compound that shares many beneficial traits with CR and thus can be considered as a CRM. We review the potential health-promoting effects of polyamines on aging and its comorbidities. Such effects include cardiovascular, neuroprotective, and anti-tumorigenic effects and are induced by dietary or otherwise externally applied spermidine, which will be the primary focus of this article.

Spermidine concentrations decline with age: Bioavailability and metabolism

Polyamine concentrations in mammals are determined by their nutritional supply, synthesis by the intestinal microbiota, uptake, cellular biosynthesis, catabolism, and urinary excretion. Tissue spermidine concentrations decline with age in model organisms as well as in humans (*24*–*27*). This may, at least in part, result from a decline in the biosynthetic activities of polyamine-producing enzymes [reviewed in (*28*)].

Polyamine metabolism and transport

The intracellular spermidine content is the final result of polyamine uptake from the extracellular space, endogenous biosynthesis, catabolism, and excretion (outlined in Fig. 1). Biosynthesis is achieved from the precursor ornithine forming putrescine, spermidine, and spermine (Fig. 1) in tightly regulated step-wise reactions (*20*, *22*, *29*). Catabolism of polyamines involves, on the one hand, the oxidative degradation of spermine to spermidine. On the other hand, the degradation and secretion of both spermidine and spermine requires their acetyl-CoA–dependent acetylation by spermine-/spermidine-N1-acetyltransferase 1 (SSAT1) and subsequent oxidation (Fig. 1) (*30*, *31*). In a recent study, histone deacetylase 10 (HDAC10), a key regulator of autophagy and cell survival (*32*), has been proposed as yet another mediator of polyamines metabolism owing to its ability to deacetylate spermidine (*33*).

Polyamines also interconnect with disease-relevant amino acid metabolism. Synthesis of spermidine and spermine requires the formation of decarboxylated S-adenosyl methionine (dcSAM) from SAM. SAM serves as an important cofactor for methylation of proteins, including histones, as well as of DNA, both of which are necessary for the epigenetic control of gene regulation (Fig. 1). Through the putrescine precursor ornithine, polyamine biosynthesis affects

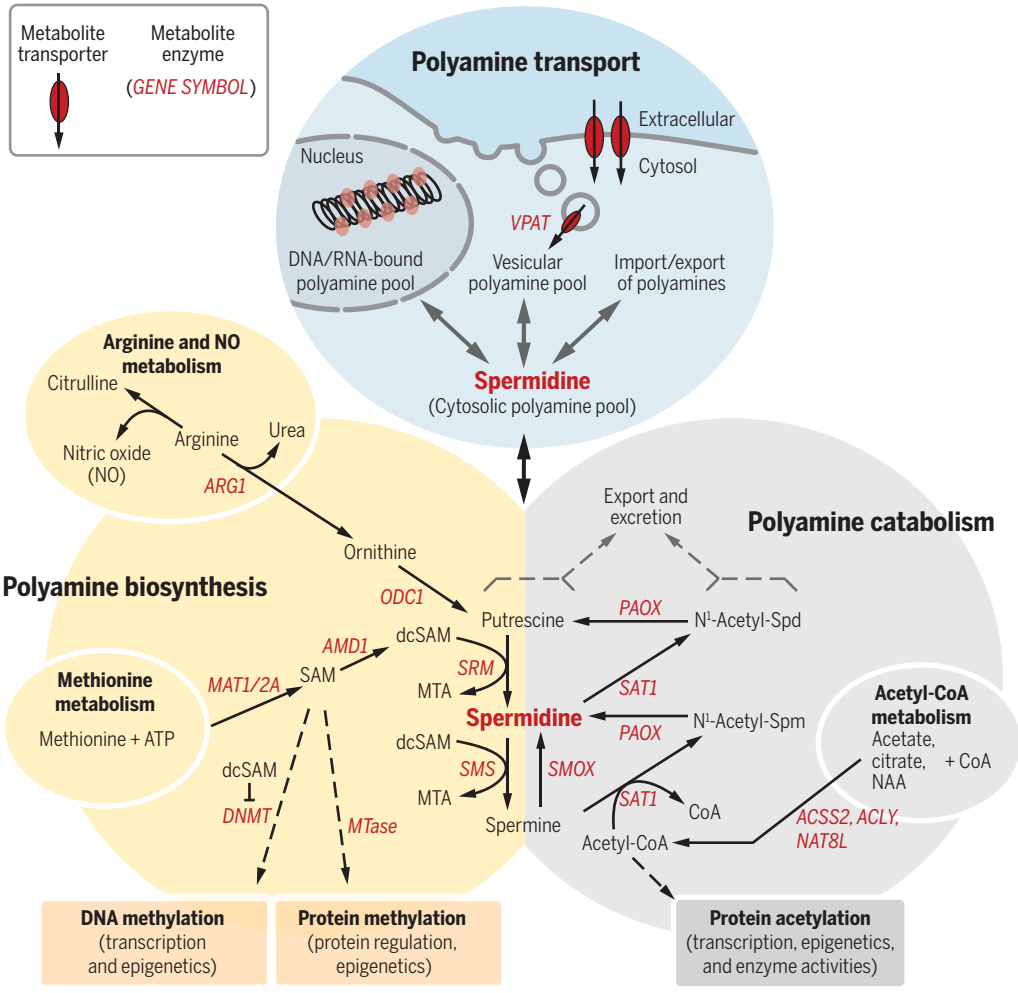

Fig. 1. Regulation of the intracellular spermidine pool. Major routes and enzymes of mammalian polyamine metabolism. The cytosolic spermidine pool results from uptake, biosynthesis, catabolism, and transport. Spermidine is formed from its precursor putrescine or by degradation from spermine. Polyamine biosynthesis connects to arginine and NO metabolism via ornithine (as part of the urea cycle). Spermidine catabolism is mainly mediated through acetylation and subsequent oxidation reactions. Through the cofactors dcSAM (polyamine biosynthesis) and acetyl-CoA (polyamine catabolism), polyamine metabolism interrelates to protein/DNA methylation and protein acetylation, respectively, and thus indirectly influences epigenetic regulation of gene expression. ACLY, ATP-citrate synthase; ACSS2, acetyl-coenzyme A synthetase, cytoplasmic; AMD1, *S*-adenosylmethionine decarboxylase proenzyme; ARG1, arginase-1; ATP, adenosine triphosphate; CoA, coenzyme A; dcSAM, decarboxylated *S*-adenosylmethionine; MAT1/2A, *S*-adenosylmethionine synthase isoform type 1/2; NAA, *N*-acetylaspartate; NAT8L, *N*-acetylaspartate synthetase; NO, nitric oxide; ODC1, ornithine decarboxylase; PAOX, peroxisomal *N*(1)-acetyl-spermine/spermidine oxidase; SAM, *S*-adenosylmethionine; SMO, spermine oxidase; SAT1, spermidine/spermine *N*(1)-acetyltransferase 1; SMS, spermine synthase; Spd, spermidine; Spm, spermine SRM, spermidine synthase; VPAT, vesicular polyamine transporter.

the bioavailability of arginine, which is important for the production of nitric oxide (NO), an important signaling molecule that mediates vasodilation, protects from maladaptive cardiac remodeling, affects mitochondrial biogenesis and function, and has vast immunomodulatory effects (*34*).

The transport of polyamines in mammals is less well understood but may involve plasma membrane transporters as present in yeast and bacteria (*35*). Cellular polyamine uptake and secretion may also be mediated via endocytosis and exocytosis, respectively (Fig. 1). In support of this notion, a vesicular polyamine transporter (VPAT) was identified in astrocytes (*36*) and mast cells (*37*) that may have important neuro- and immune-modulatory implications (*37*, *38*).

Polyamine uptake and excretion

In addition to cellular biosynthesis, two other sources are equally important for the systemic availability of spermidine. These sources are (i) external (oral) uptake with the food and (ii) production by intestinal microorganisms (Fig. 2).

Selected unprocessed plant-derived food items are naturally enriched in polyamines, as exemplified by the Durian fruit. Moreover, fermentation processes involving bacteria and fungi used in the food industry cause microbial generation of polyamines, which may contribute to the sometimes subjectively malodorous properties of milk and soy products such as mature cheese and natto, respectively. Ingested spermine and spermidine are quickly adsorbed from the intestinal gut and distributed without degradation (*39*). Thus, diet influences the blood polyamine concentration, which is highly diverse in humans (*40*). The average daily nutritional intake of spermidine varies from ~7 to 25 mg and more, with the highest amounts in the Mediterranean diet, which is often described to improve health in humans (*41*, *42*). These values still lie below the amount of spermidine, when calculated based on the optimal food composition proposed by the Swedish Nutrition Recommendations Objectified (*43*).

In mice, oral supplementation of spermidine increases amounts in whole blood (*44*), serum (*45*), and tissues (*44*). Supplementation of the polyamine precursor arginine (alone or in combination with probiotics) may also suffice to increase spermidine concentrations in the colon and blood (*46*, *47*). Indeed, the intestinal luminal concentration of spermidine critically depends on colonic microbiota in mice (*48*), and this may hold true for humans as well (*49*). Together, these studies outline the importance of dietary polyamines and polyamine-producing bacteria for the bioavailability of spermidine (Fig. 2). Given the high content of polyamines in certain types of food, it appears plausible that a polyamine-rich diet could overcome the age-associated decline of polyamines. Indeed, daily intake of 50 to 100 g of natto over a 2-month period significantly increased the whole-blood spermine content of healthy human volunteers (*40*). A cross-sectional observation in central Italy revealed that whole-blood spermidine and spermine content were decreased in elderly humans but remained at the levels of younger (middle-aged) individuals in healthy nonagenarians and centenarians (*25*). Further studies are warranted to address whether dietary or genetic factors explain the high

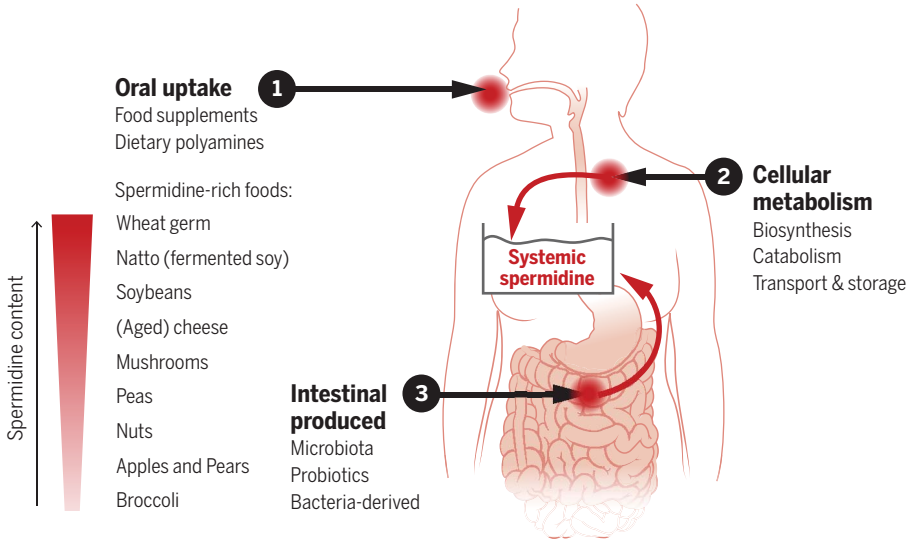

Fig. 2. Sources of systemic spermidine. Scheme depicting the sources crucial for spermidine bioavailability in the whole organism. In addition to cellular metabolism (outlined in Fig. 1), spermidine is taken up orally from dietary sources or produced by commensal gut bacteria. Subsequently, spermidine can be resorbed by intestinal epithelial cells and is distributed through systemic circulation. Examples of spermidine-rich foods are enumerated. Food supplements, including the polyamine precursor arginine and probiotics (polyamine-producing bacteria), increase the intestinal production of polyamines.

spermidine content in healthy centenarians and whether they represent a cause or a consequence of healthy aging.

Systemic concentrations of polyamines are also controlled through renal secretion. Thus, putrescine and acrolein—a toxic product and by-product, respectively, of spermidine and spermine catabolism by serum amine oxidase—increase in the plasma of patients with chronic renal failure (*50*). Therefore, dietary supplementation of spermidine in such patients should be carefully reviewed for potential adverse effects.

Health effects of dietary spermidine

Beyond their capacity to improve cellular fitness—as documented in yeast, plants, and in cultured mammalian cells (table S2)—polyamines (in particular, spermidine) increase life span of multicellular organisms, including nematodes, flies, and mice. Cardiovascular disease and cancer are the two major causes of death in humans and mice. Similarly to CR regimens, which partially protect against these pathologies, spermidine can delay their manifestation and reduce their severity in rodent models.

Life-span extension by spermidine

Health- and life-span–promoting effects are documented for dietary or otherwise externally supplied spermidine (Fig. 3A). Spermidine supplementation confers life-span extension both to invertebrate model organisms (*19*, *24*) and to mice (*44*, *51*). Moreover, a diet rich in polyamines reduces mortality of aged mice (*52*). As true for many other CRMs, spermidine extends life span in a sex-independent manner (table S1). Supplementation of polyamine-producing probiotic bifidobacteria—alone or, moreso, in combination with the polyamine precursor arginine—increases blood concentrations of spermidine and spermine and decreases mortality and the prevalence of cutaneous pathologies in old mice (*46*, *47*). Furthermore, oral spermidine administration counteracts the age-associated disruption of circadian rhythm in mice (*45*). Spermidine may also ameliorate menopausal decline. Indeed, bone loss induced by ovariectomy in mice, a model of postmenopausal osteoporosis, is prevented with oral spermidine supplementation via inhibition of the formation of (bone-resorbing) osteoclasts (*53*). The total amount of food polyamines significantly correlates with human life expectancies across distinct Asian countries (*54*), although this epidemiological study did not adjust for confounding factors typically associated with longevity. Future well-controlled epidemiological studies are warranted to explore the effects of dietary spermidine on human health span and life span.

Polyamine effects on tumorigenesis

Polyamines are essential for cell proliferation and growth, and dysregulation of polyamine metabolism is a defining signature of many tumor types; increased polyamine concentrations caused by enhanced biosynthesis can be found in skin, breast, colon, lung, and prostate cancers (*35*). Given the proliferation-enhancing and cytoprotective effects of polyamines on cultured human cancer cells or xenografted human tumors evolving in immunodeficient mice [reviewed in (*55*)], polyamines might have procarcinogenic properties. Efforts have been made to suppress polyamine biosynthesis by inhibiting ornithine decarboxylase (ODC) with difluoromethylornithine (DFMO) for the treatment of established cancers in mice, but clinical trials using DFMO have been abandoned because of its toxicity (*35*). Potentially less toxic, a competitive inhibitor of AdoMetDC (SAM486A), leading to low spermidine concentrations, has been tested in clinical trials for various tumor types, with moderate success in non-Hodgkin's lymphoma (*35*, *56*).

In contrast to potential procarcinogenic properties of polyamines, spermidine supplementation can reduce tumorigenesis in mice. Oral supplementation of probiotic polyamine-producing bacteria (*Bifidobacterium animalis* subsp. *Lactis* LKM512) lowers the incidence of visible skin tumors in aging Crj:CD-1 female mice (*47*). Dietary spermidine reduces the severity of liver fibrosis and the incidence of hepatocellular carcinomas induced by chemical insults in mice (*51*). Spermidine administration also slows the growth of CT26 colorectal tumors transplanted into immunocompetent mice (*57*). Increased intake of dietary polyamines (using chow enriched in spermidine, spermine, and putrescine) causes a delay in chemically induced tumorigenesis in young BALB/c male mice, although the maximum size of tumors increased (*58*). Thus, in mice polyamines may inhibit colon carcinogenesis yet favor tumor growth, once a cancer has developed. In humans, one study suggested that dietary intake of polyamines above the median could be associated with an elevated risk of developing colorectal adenoma, particularly in women (*59*). Nevertheless, a subsequent prospective study performed by the same authors failed to confirm this positive association and rather revealed an inverse correlation of high polyamine intake with the risk of colorectal cancers, at least in overweight women with a body mass index (BMI) ≤ 25 (*60*). Interventional studies are needed to explore the possible effect of dietary spermidine on cancer risk.

Spermidine supplementation reduces the growth of transplantable tumors in mice treated with chemotherapies. This spermidine effect is shared by other CRMs (*61*) as well as by fasting or hypocaloric diets (*62*, *63*) and is mediated by the stimulation of immunosurveillance. Thus, spermidine and other CRMs enhance the anticancer immune response as they deplete immunosuppressive cells such as regulatory T lymphocytes (T_{reg} cells) from the tumor bed (*61*). Tumors growing in mice that lack cytotoxic T lymphocytes fail to reduce their growth in response to spermidine or other CRMs (*61*). Beyond its effects on adaptive immunity, spermidine synthase (SRM)–mediated spermidine production appears to be a determinant for antitumor actions of tumor-associated macrophages (TAMs) in the context of colorectal cancer progression (*57*). These findings may explain why external supply of spermidine has chemopreventive effects in vivo (that are likely mediated by immunostimulatory effects), although it enhances the proliferation of cancer cells in vitro.

Future studies should address associations of dietary spermidine with other types of cancer, especially in light of the potential requirement of polyamines for cancer cell growth. Whether a

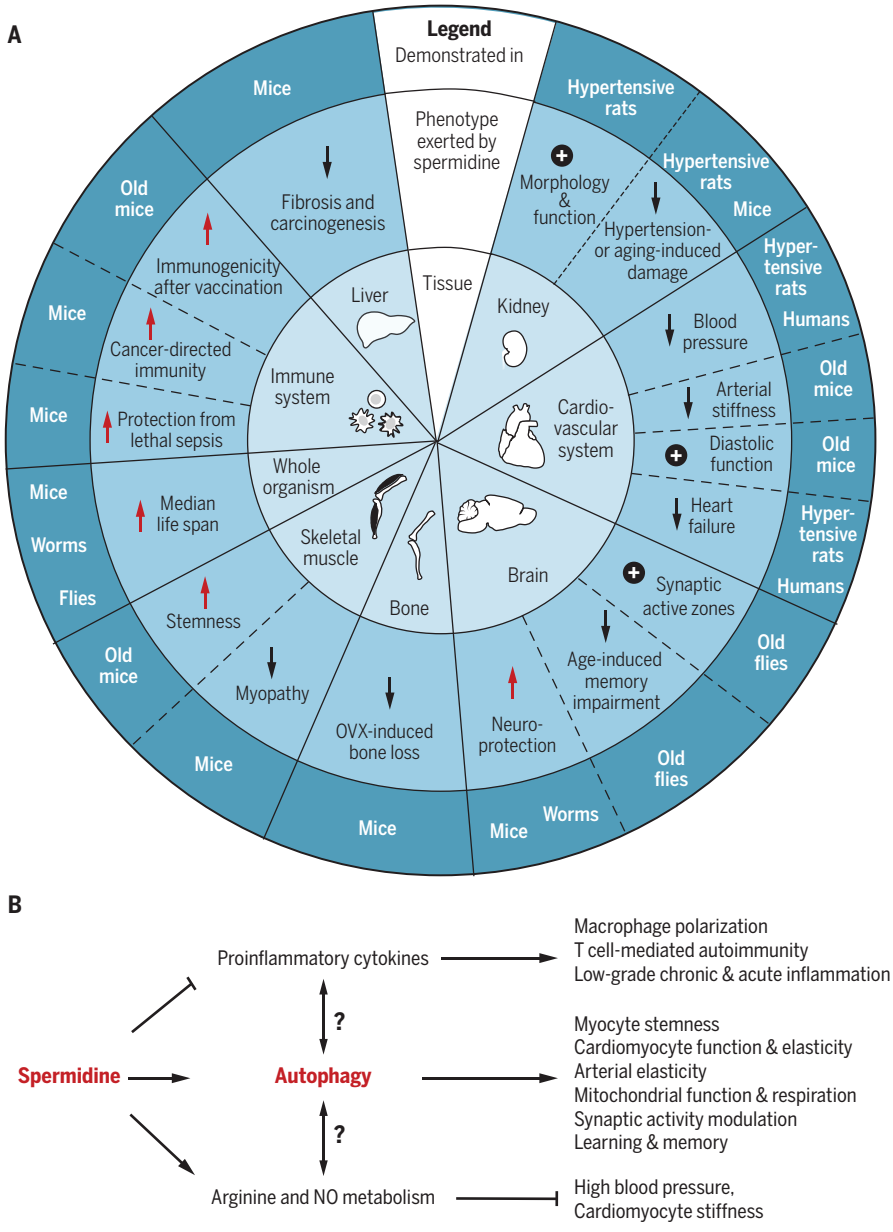

Fig. 3. Spermidine-mediated health effects. (**A**) Summary of effects elicited by dietary or otherwise supplemented spermidine in different organ systems. Plus symbols indicate "improved." (**B**) Spermidine-enhanced autophagy is required for several of the health-promoting effects presented in (A). Spermidine also suppresses proinflammatory cytokines and improves the bioavailability of arginine required for NO biosynthesis, mediating immunomodulatory and antihypertensive effects. It remains an open question to which extent autophagy contributes to spermidine-mediated changes in cytokine production and arginine or NO metabolism.

diet low in polyamines slows growth of specific cancers and whether a polyamine- or specifically spermidine-rich diet would accelerate tumor growth in human patients remain open questions, requiring in-depth investigation before such diets can be broadly recommended, at least for this specific risk group.

Spermidine in cardiovascular and muscle-related disease

Dietary spermidine protects from cardiac aging. It improves diastolic function, left ventricular elasticity, and mitochondrial function in old mice (*44*). First results from epidemiological studies corroborate these findings in humans: Intake of dietary spermidine (or that of spermidine and spermine combined) inversely correlates with the incidence of cardiovascular disease (CVD) and death in the Bruneck cohort (*44*). A cross-sectional regression meta-analysis of nutritional polyamine content with CVD-caused mortality rates by using publicly available data from 48 Western countries identified negative associations of spermidine and spermine with CVD (*64*).

Spermidine reversed age-induced arterial stiffness with a reduction in oxidative damage of endothelial cells in old mice (*65*) and alleviated the formation of atherosclerotic plaques in apolipoprotein E–deficient ($ApoE^{-/-}$) mice fed a high-fat diet (HFD) for 20 weeks (*66*). In Dahl salt-sensitive rats fed a high-salt diet (a model of hypertensive heart failure), oral supplementation of spermidine reduced high blood pressure and delayed the transition to heart failure (*44*), further documenting the antihypertensive (*67*) and vascular health–promoting (*65*, *66*) functions of dietary spermidine. Humans belonging to the higher tertile of dietary spermidine uptake in the Bruneck cohort had lower diastolic and systolic blood pressure as compared with those of the lower tertile subjects (*44*).

One of the hallmarks of aging is the decline in stem cell function, resulting in impaired tissue regeneration and immunosenescence (*8*). As a first indication that polyamines might facilitate the manifestation of stem cell–like features, spermidine favored the reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) of mouse embryonic fibroblasts in vitro (*68*) and promoted hair growth and epithelial stem cell function in human hair follicle organ cultures (Fig. 4F) (*69*). Short-term administration of spermidine to old mice reversed the age-associated defect of autophagy and mitophagy in muscle stem cells (satellite cells), preventing their senescence and improving muscle regeneration (*70*). Similarly, spermidine reactivated autophagy and ameliorated the myopathic defects of collagen-VI–deficient mice, which are normally characterized by invalidating mitochondrial defects (*71*). Skeletal muscle atrophy in D-galactose–treated rats was inhibited by spermidine application (or spermidine combined with exercise), concomitant with induction of autophagy and mitochondrial improvements (*72*). The spermidine-mediated ultrastructural and functional improvement of mitochondria from aged cardiac muscles (*44*) and from skeletal muscle (stem) cells (*70*–*72*) further support the potential utility of spermidine in the treatment of muscle-related disorders.

Polyamines in metabolic syndromes

Whether polyamines might be useful in treatment of obesity and type 2 diabetes is an important topic for future research. Daily administration of spermine to mice eating a HFD prevented adiposity and improved glucose tolerance (*73*). The white adipose tissue (WAT) or liver-specific depletion of nicotinamide *N*-methyltransferase (Nnmt) [an enzyme that methylates the nicotinamide adenine dinucleotide (oxidized form) (NAD^+) precursor nicotinamide by using SAM as methyl-group donor and therefore links polyamines metabolism to NAD^+ metabolism and Sirtuin signaling (Fig. 1)] rendered mice resistant against HFD-induced obesity (*74*); the down-regulation of Nnmt resulted in an augmented polyamine flux (as corroborated by the simultaneous activation of ODC-mediated spermidine biosynthesis and SSAT1-driven spermidine elimination) and led to an increased energy expenditure

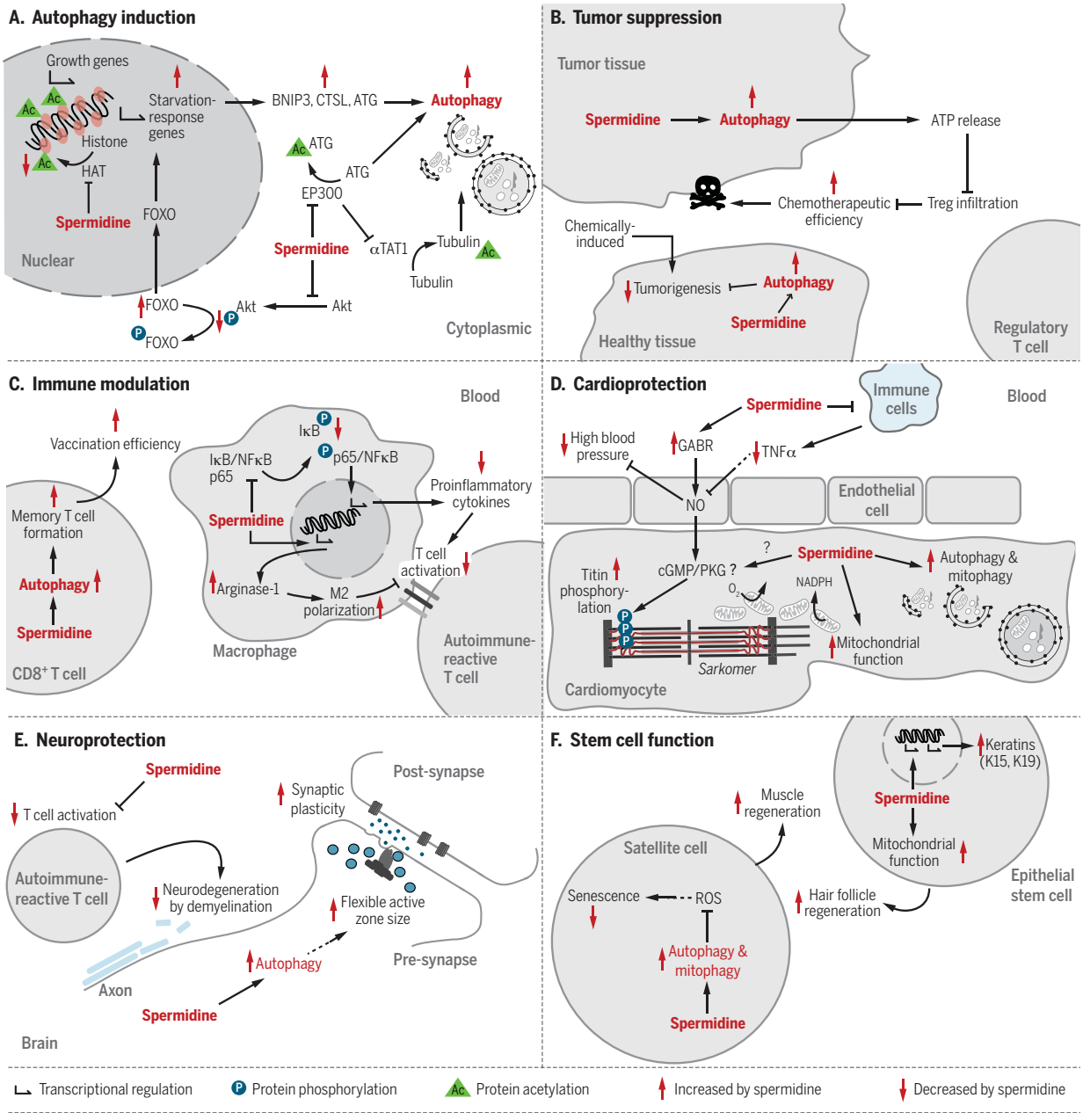

Fig. 4. Cellular and molecular mechanisms of spermidine-mediated health protection. Mechanistic models of the cellular and molecular effects elicited by spermidine through transcriptional, posttranslational (affecting protein acetylation and phosphorylation), as well as metabolic effects. (**A**) Rapid autophagy induction by spermidine administration through inhibition of the acetyl transferase EP300, primarily resulting in autophagy-relevant cytosolic protein deacetylation. Sustained autophagic control by spermidine is mediated through induction of autophagy-relevant gene transcription. This involves the regulation of FOXO transcription factor as well as inhibition of histone acetyl transferases, resulting in epigenetic transcriptional reprogramming. (**B**) Spermidine may suppress tumorigenesis through induction of autophagy in healthy cells. In autophagy-competent tumor cells, spermidine favors the autophagy-dependent release of ATP, which in turn favors immunosurveillance. (**C**) Anti-inflammatory effects of spermidine are explained through its effects on macrophages, promoting M2 polarization and the suppression of NFκB-dependent proinflammatory cytokines. These inhibitory macrophages then suppress autoimmune-reactive T cells. At the same time, spermidine favors formation of $CD8^+$ memory T cells via induction of autophagy. (**D**) The suppression of circulatory cytokines, such as TNFα, also contributes to cardiovascular protection, possibly via a concerted action with arginine-derived nitric oxide, which leads to vasodilation and promotes the cGMP/cGMP-dependent protein kinase (PKG)–dependent phosphorylation status of titin. Spermidine-enhanced autophagy and mitophagy also contribute to cardiomyocyte elasticity and mitochondrial functionality. (**E**) The inhibitory effect of spermidine on autoimmune-reactive T cells (C) further translates into prevention of neurodegeneration by demyelination. Neuroprotection is also mediated via autophagy-dependent proteostasis of presynaptic active zones, assuring the maintenance of synaptic plasticity. (**F**) Suppression of stem cell senescence by spermidine depends on autophagy (in satellite cells), whereas spermidine promotes mitochondrial function and production of keratins in epithelial stem cells. These stemness-enhancing effects ensure muscle and hair follicle regeneration, respectively. Red arrows (up or down) indicate changes (increased or decreased, respectively) that are observed after spermidine supplementation.

(74). Similarly, whole-body depletion of the RNA polymerase III repressor MAF1 (which leads to a reduced expression of Nnmt) increases life span and confers resistance to HFD-induced obesity associated with enhanced polyamine flux and activation of autophagy (75). Of note, autophagy is required for full weight loss upon acute starvation and counteracts weight gain– and obesity-related pathologies in mice fed hypercaloric diets. Spermidine attenuated weight gain and the comorbidities of obesity induced by hypercaloric regimens, correlating with autophagy induction in WAT (76).

Neuroprotection by spermidine

Autophagy (and particularly mitophagy) have a major role in maintaining normal brain function and protecting from neurodegeneration (13, 77), and spermidine supplementation exerts neuroprotective effects in vivo (Fig. 3A). In flies, spermidine feeding protects from age-induced memory impairment (26) and loss of locomotor activity (78) in an autophagy-dependent manner. This has been explained by the autophagy-dependent rejuvenation of synaptic active zone composition (Fig. 4E), which is important for synaptic flexibility and plasticity (79). In a mouse model of experimental autoimmune encephalomyelitis, a model for multiple sclerosis, oral supplementation of spermidine attenuates disease progression and improves visual functions through the reduced demyelination of optic nerve and spinal cord and decreased loss of retinal ganglion cells (80, 81). Similarly, spermidine promotes optic nerve regeneration and retinal ganglion cell survival after optic nerve injury in vivo in mice (82) and blunts retinal degeneration in a mouse model of normal tension glaucoma (83). Spermidine biosynthesis induced through up-regulation of arginase-1 is required for spontaneous axonal regeneration after peripheral lesion of dorsal root ganglion neurons in mice (84). Spermidine further prevents α-synuclein neurotoxicity in invertebrate models (85), reduces frontotemporal lobar dementia (FTLD-U) in mice expressing transgenic transactive response DNA–binding protein 43 kDa (TDP-43) (86), and attenuates laurate-induced cerebral small vessel disease (87). Striatal injection of spermine improved the recognition memory deficit in a rodent model of Huntington's disease (88), and up-regulation of colonic polyamines through orally administered arginine in combination with the probiotic bifidobacteria LKM512 improved the spatial learning and memory capabilities of old mice (46).

Altogether, these studies suggest a wide range of neuroprotective effects of exogenously applied spermidine with relevance to several neurodegenerative motor disorders and dementias. The underlying mechanisms are discussed below in detail.

Cellular and molecular mode of action of spermidine

Spermidine supplementation induces autophagy and exerts additional and potentially autophagy-independent metabolic and transcriptional effects in vivo. These changes may explain the disease-modulatory and anti-aging mode of action of polyamines.

Autophagy and mitophagy induction through spermidine administration

Spermidine administration stimulates autophagy in aging yeast, flies, and worms (24) and in cultured mammalian cells (18, 19, 70). In mice, acute application of spermidine (for example, by means of intraperitoneal injection) induces autophagy in vivo in multiple tissues such as heart, liver, and muscle within a few hours (4 to 24 hours) (19, 61). Concomitantly, spermidine induces major changes in the metabolome of the plasma, heart, skeletal muscle, and liver that resemble those triggered by other CRMs or fasting (61), which is in line with a common mechanism of action of these pharmacological and nutritional interventions. Oral supplementation of spermidine in drinking water triggers autophagy after 2 to 4 weeks in cardiac tissue (44) and reverses the age-dependent decline of autophagy in the aorta (other tissues remain to be tested) (65). At least in cardiomyocytes in vivo and in various cultured cell lines, spermidine also increases the autophagy-dependent selective degradation of mitochondria (that is, mitophagy) and thus contributes to mitochondrial health and functionality (44, 70, 72, 89).

Given the predominantly cytoprotective role of autophagy and mitophagy, the induction of autophagy may explain the health-promoting effects of spermidine (Fig. 3B), like those of other CRMs (7, 90). In support of this, genetic inhibition of autophagy abolishes spermidine-induced extension of life span in flies, worms (24), and mice (51) and prevents restoration of memory performance and synaptic flexibility in aged flies (26, 79). Inhibition of autophagy partially reduces the spermidine-mediated resistance to paraquat-induced loss of locomotor activity in *Drosophila* (91). Moreover, spermidine fails to promote cardioprotective effects in cardiomyocyte-specific $Atg5^{-/-}$ mice (44) and does not restore stem cell–proliferative function in autophagy-deficient ($Atg7^{-/-}$) Pax7-expressing cells (Pax7 represents a marker and regulator of muscle progenitors and satellite cells) (70). Last, the spermidine-mediated reduction of atherosclerotic lipid accumulation and necrotic core formation that is normally observed in $ApoE^{-/-}$ mice is absent in $ApoE^{-/-}Atg7^{-/-}$–deficient (autophagy-incompetent) animals (66).

Several CRMs induce autophagy by decreasing acetylation of multiple cellular proteins (7). Spermidine diminishes cytosolic protein acetylation by inhibiting the activity of several acetyltransferases, including that of E1A-associated protein p300 (EP300) (19, 24), and EP300 inhibition is sufficient to acutely induce autophagy (18). EP300 directly inhibits acetylation of several autophagy-essential autophagy-related (ATG) proteins (92, 93) and indirectly stimulates deacetylation of tubulin by inhibiting α-tubulin acetyltransferase 1 (αTAT1) (94). Therefore, the inhibition of EP300 by spermidine causes deacetylation of ATG proteins and increases acetylation of tubulin, thus stimulating autophagic flux (Fig. 4A). In addition, autophagy induction and extension of life span in aging mice depends on spermidine-mediated stabilization of the pro-autophagic microtubule-interacting protein microtubule-associated protein 1S (MAP1S) and may involve the depletion of cytosolic HDAC4 (51). Long-term, chronic effects of spermidine on the rate of basal autophagic flux may be additionally explained by transcriptional effects on autophagy-related genes (Fig. 4A). For instance, the transcription factor FoxO3, which is inhibited by protein kinase B (PKB, best known as Akt), is necessary and sufficient for the induction of autophagy in mouse skeletal muscle in vivo (95). The spermidine-mediated reactivation of muscular autophagy in collagen VI–deficient mice coincides with the dephosphorylation (and hence inactivation) of Akt and in turn with increased transcription of FoxO3-dependent target genes (71). Support for direct inhibition of acetyltransferases by spermidine comes from in vitro activity assays, in which nuclear extracts were incubated with spermidine (24) and enzymatic assays with recombinant EP300 protein that reveal spermidine as a competitive inhibitor that is particularly active when acetyl-CoA concentrations are low (18). Spermidine may also reduce the activity of acetyltransferases by reducing the availability of acetyl-CoA because the catabolism of spermidine requires acetylation and hence consumes acetyl-CoA (Fig. 1). Depletion of the cytosolic-nuclear pool of acetyl-CoA induces autophagy in cultured mammalian cells, and in mice, aging yeast, and flies (16, 17, 96), and thus may represent an important mode of action of CRMs that induce protein deacetylation (table S1) (7). Given the pleiotropic action of spermidine on autophagy-related gene transcription, protein stability, and posttranslational regulation, it will be important to molecularly define the key events that are essential for autophagy induction by spermidine.

The protein deacetylase Sirtuin 1 inhibits (97) whereas cytosolic acetyl-CoA and EP300 promote the activity of the autophagy-inhibitory mechanistic target of rapamycin complex 1 (mTORC1) (17). Indeed, spermidine and several other CRMs cause mTORC1 inhibition (7, 17, 18) and activate phosphorylation of 5′ adenosine monophosphate–activated protein kinase (AMPK) (17), which antagonizes mTORC1 at the functional level and may further facilitate autophagic response to these CRMs. Induction of mitophagy by spermidine has also been linked to ataxia-telangiectasia mutated protein kinase (ATM)–dependent activation of the phosphatase and tensin homolog (PTEN)–induced putative kinase 1 (PINK1)/Parkin-signaling pathway (89), which has an important role in mitochondrial quality control (98). Spermidine induces mitochondrial depolarization and mitophagy in cultured human fibroblasts but does not do so in cells lacking ATM or treated with the ATM inhibitor KU55933. However, the precise mechanism by which spermidine favors the activation (and phosphorylation) of AMPK and ATM remain elusive. Spermidine-treated cells exhibit

changes in phosphorylation of multiple proteins (*99*), pointing to a sophisticated cross-talk of the protein acetylation and deacetylation machinery and protein kinase cascades that requires further in-depth investigation.

Immunostimulatory and anti-inflammatory effects of spermidine

The success of current anticancer treatments, including chemotherapies, essentially relies on the immune cell–dependent clearance of tumor cells. This is particularly important for the long-term efficacy of cytotoxic agents that induce immunogenic cell death (*100*–*102*). Autophagy is required for cancer cells to adapt to cell-intrinsic and -extrinsic stress. However, premortem autophagy also promotes immune recognition of cancer cells because it facilitates the release of adenosine 5′-triphosphate (ATP), which serves as a chemotactic factor for myeloid cells, including dendritic cell precursors (*103*, *104*). By reducing the stimulation of immunosuppressive adenosinergic receptors, autophagy also prevents the recruitment of T_{reg} cells into the tumor bed (*105*). Autophagy induction by spermidine or by the CRM hydroxycitrate favors the release of ATP from neoplastic cells in mice and therefore ameliorates immunosurveillance, resulting in a delay in tumorigenesis of autophagy-competent (but not autophagy-deficient) mutant KRAS-induced lung cancers in vivo (Fig. 4B) (*61*). Thus, the administration of spermidine or hydroxycitrate, similar to that of acute nutrient deprivation (*106*), can favor chemotherapeutic efficiency against autophagy-competent cancers. Spermidine also directly improves the generation and function of memory lymphocytes, similar to what is known for other autophagy inducers such as rapamycin (*107*) or metformin (*108*). T cell function declines with age, and this decline is associated with impaired autophagy, which can be observed in cells isolated from elderly human donors (*109*). In old mice, $CD8^+$ T cell responses to influenza vaccination are restored by oral spermidine treatment in an autophagy-dependent manner (*110*), suggesting important roles for autophagy and spermidine in memory cell formation (*111*). Of note, in isolated T cells spermidine apparently induces autophagy independently from mTORC1 because it fails to affect the phosphorylation of the mTORC1 substrate ribosomal S6 kinase (*110*).

Exogenously supplied spermidine and spermine suppress the secretion of proinflammatory cytokines in numerous pathophysiological settings (table S2). These effects are in part secondary to autophagy induction because autophagy has broad anti-inflammatory effects that rely in part on the inhibition of inflammasome activation (*112*). Dietary spermidine suppresses the age-associated chronic low-grade increase in plasma concentrations of tumor necrosis factor–α (TNF-α) and other cytokines in mice (*44*, *46*) and inhibits lethal sepsis (*113*).

Lymphocyte function–associated antigen–1 (LFA-1) is crucial for regulating immune cell adhesion and migration, and its expression increases with age in human blood cells (*114*). Spermidine and spermine both suppress LFA-1 expression in human lymphocytes in vitro and, when orally supplied, in aging Jc1:ICR male mice in vivo (*58*, *115*). Blood spermine concentrations inversely correlate with abundance of LFA-1 in blood obtained from healthy human volunteers (*114*). The underlying mechanism may involve age-associated alterations in DNA methylation (*58*, *115*) or inhibitory effects of polyamines on proinflammatory transcription factors (Fig. 4C), including nuclear factor–κB (NFκB) in macrophages (*81*).

Neuromodulatory and neuroprotective mechanisms of spermidine

The neuromodulatory actions of spermidine and spermine result from their interaction with ionotropic receptors—most importantly, *N*-methyl-D-aspartate (NMDA) receptors [reviewed in (*23*)]. Neuroprotection conferred by spermidine may also result from increased autophagy in neuronal or glial cells, as well as from suppression of maladaptive inflammation. Old flies deficient in Atg7 fail to recover their memory function and synaptic flexibility when fed with spermidine (*26*, *79*). This is consistent with a general age- and neuroprotective function of autophagy in this model organism (*12*, *13*). At first sight counterintuitively, spermidine causes a decrease in presynaptic active zone scaffold proteins (Bruchpilot and Rim-binding protein) and a concomitant reduction in synaptic vesicle release (Fig. 4E) (*79*). Through this effect, spermidine restores synaptic dynamics that are otherwise pushed to their dynamic limits at old age, causing memory impairments. Thus, spermidine appears to ensure proteostasis of synaptic active zones in an autophagy-dependent fashion. In addition, spermidine prevents the toxicity of transgenic α-synuclein expression in flies and nematodes (*85*), which is consistent with the idea that (spermidine-enhanced) autophagy clears polyubiquitin-associated and other neurotoxic protein aggregates.

In spite of the potent neuroprotective capacity of spermidine in invertebrates, it remains elusive whether spermidine is able to cross the mammalian blood-brain barrier or even to regulate its integrity (*116*, *117*). At least after forebrain ischemia, spermidine is transported into several areas of the rat brain (*117*). Alternatively, spermidine may act on nervous tissue via systemic routes that involve regulation of inflammatory cytokines and immune cells capable of invading cerebral tissue. In line with this idea, the preventive action of spermidine against axon demyelination (*80*) is mediated through inhibition of autoimmune-reactive (demyelinating) T cells (*81*). Spermidine prevents activation of autoimmune-reactive T cells indirectly by favoring the polarization of circulatory macrophages to T cell inhibitory M2 cells. This process is mediated by NFκB-dependent suppression of proinflammatory cytokines and by transcriptional activation of arginase-1 (Fig. 4, C and E), an important modulator of both adaptive and innate immune responses (*118*).

In mouse models of Alzheimer's disease, increased monoamine oxidase type B (MAOB) activity has been measured in reactive astrocytes. This MAOB activity favors the conversion of putrescine to the gliotransmitter γ-aminobutyric acid (GABA), which drives Alzheimer's-associated pathology in APP/PS1 mice that carry mutations in the amyloid precursor protein (APP) and the γ-secretase presenilin-1 (PS1) (*119*). MAOB protein levels are elevated in the brain of Alzheimer's patients, and a higher activity is observed in plaque-associated astrocytes (*120*). Therefore, potential adverse effects of dietary polyamines in such patients should be carefully considered and weighed against their neuroprotective potential.

Mode of action of cardiovascular protection by spermidine

Beyond the induction of autophagy and mitophagy that result in improved mitochondrial structure and function in cardiomyocytes (*44*), spermidine protects from cardiovascular pathologies via complementary pathways (Fig. 4D). In Dahl rats fed a salt-rich diet, spermidine increases the bioavailability of arginine (likely because arginine is no more needed for endogenous polyamine biosynthesis), which may translate into enhanced production of the vasodilator NO, reducing arterial hypertension (*44*). At the same time, spermidine ensures cardiomyocyte elasticity through phosphorylation of the myosin filament–associated protein titin (*44*). This molecular event is favored by suppression of proinflammatory cytokines and the availability of nitric oxide (NO) with subsequent guanosine 3′,5′-monophosphate (cGMP)–dependent protein kinase–dependent signaling. The capacity of spermidine to reverse arterial aging has also been linked to enhanced NO bioavailability as well as to the suppression of oxidative damage in endothelial cells (*65*). Thus, metabolic effects of spermidine may include the feedback inhibition of endogenous polyamine biosynthesis and limiting consumption of arginine for ornithine biosynthesis, thus facilitating arginine-dependent NO biosynthesis (Fig. 1). The antioxidant action of spermidine on in vitro–cultured mouse arteries appeared to be autophagy-dependent because it was blocked by the autophagy inhibitor chloroquine (*65*). Thus, it remains to be investigated whether the effects of exogenous spermidine on arginine and NO metabolism and autophagy occur in an independent manner or are somehow interlinked.

Outlook: Diagnostic and therapeutic potential of spermidine in humans

Several pathologies have been associated with increased polyamine concentrations, raising the possibility of considering polyamines as disease biomarkers. This applies in particular to cancer, neurodegenerative disease, stroke, and renal failure [reviewed in (*121*)], as well as heart failure and cardiac infarction (*122*, *123*). Furthermore, disturbed polyamine and arginine metabolism is observed in mouse models of Alzheimer's disease (*124*) and in human patients with mild cognitive impairments (MCIs) (*125*). Several metabolites

including spermidine were differentially affected in stable MCI patients and patients that later developed Alzheimer's disease (*125*). In subjects developing Alzheimer's disease, putrescine may be preferentially channeling into production of spermidine and spermine, whereas the stable MCI condition favors the conversion of putrescine to *N*-acetylputrescine and 4-aminobutanal (*125*). In light of these findings, quantitative high-throughput methods to analyze polyamines and related metabolites (*122*, *126*) could be used on vast sample collections to further evaluate the relationship of such compounds to disease evolution.

Increased concentrations of whole-blood spermidine and spermine are associated with longevity in healthy nonagenarians and centenarians (*25*). Therefore, the sole measurement of the polyamines spermidine and spermine in blood or tissue specimens will likely not suffice as biomarkers for general health or specific diseases. Moreover, the association of increased spermidine with various human pathologies does not necessarily argue in favor of its causal involvement. On the one hand, increased biosynthesis of spermidine could be a homeostatic response to stress that is activated to prevent excessive damage (such as by inducing autophagy). This has been proposed, for instance, for the compensatory cytoprotective response of spermidine observed in uncoupling protein 1 (UCP1) transgenic mice, which is a model for skeletal muscle–specific mitochondrial dysfunction (*127*). On the other hand, increases in polyamine biosynthesis could result from disease-associated alteration of other interlinked metabolic pathways. The complexity of age-associated effects on polyamine metabolism is further emphasized by diverse and region-specific changes in polyamine levels of memory-associated brain structures (*128*).

Future studies should directly address the potential causality of alterations in polyamine metabolism as well as associated pathways (such as arginine metabolism) for disease development and progression. It will be fundamental to test whether exogenously supplemented spermidine would counteract pathological polyamine biosynthesis activities (as indicated by increases in spermidine levels) through a direct or indirect metabolic feedback regulation. Mechanistically, such feedback effects may thus overcome maladaptive changes in the associated pathways (arginine and methionine or SAM metabolism) and define a protective "metabolic" action of dietary spermidine.

Conclusions and open questions

Dietary supplementation of spermidine prolongs life span and health span by protecting from a range of age-associated pathologies in several animal models. Although spermidine induces autophagy and autophagy inhibition curtails most of the spermidine effects, alternative mechanisms have also been implicated in the health-improving action of this polyamine. These potentially autophagy-independent mechanisms include direct antioxidant and metabolic effects on polyamine and associated metabolic pathways—in particular, increased arginine bioavailability and NO production (*44*, *65*). However, it has not been formally determined whether these routes act in a completely autophagy-independent manner or are interrelated with autophagy (in an additive or synergistic way), and it will be important to define actionable molecular targets that explain the beneficial effects of spermidine in diverse pathophysiological settings. In this sense, it will also be of interest to explore synergisms of CRMs that initially act through different mechanisms. For instance, the combination of spermidine and resveratrol shows synergistic effects on autophagy induction (*19*). Combinations with other CRMs, as well as with agents that act independently from protein acetylation such as rapamycin (table S1), remain to be tested. Mechanistic relations of spermidine to the autophagy inducer coffee may also exist (*129*). Coffee consumption inversely associates with total and cause-specific mortality in humans (*130*), and coffee induces autophagy and cytoplasmic protein deacetylation in mice (*129*). Although it appears that both effects are independent from caffeine content, the active principle(s) of coffee must be defined in molecular terms. It also remains to be determined whether combinations of distinct autophagy inducers can be used to obtain additive or synergistic health benefits or whether they rather would trigger unphysiological, potentially toxic levels of autophagy. Another important question is the tissue specificity of spermidine-induced health effects. Which are the prime targets of spermidine in aging and disease? Although cardiac and vascular tissues appear to be directly targeted, the kidney may similarly benefit from increased spermidine bioavailability because spermidine-rich diets ameliorate the functional decline of kidneys in animal models of aging and hypertension (*44*, *52*). The origin of spermidine-induced systemic effects on blood metabolites and proteins is another demanding problem. It remains unknown whether spermidine acts solely on leukocytes to suppress chronic low-grade inflammation and which organ is responsible for the increased bioavailability of arginine upon spermidine supplementation.

One of the strongest arguments for spermidine as a favorable CRM for future clinical trials is its low toxicity yet strong efficacy, even at moderate concentrations. Spermidine is an abundant natural polyamine contained in all organisms from bacteria to men and is naturally present in reasonable but varying amounts in human diets. It is therefore not surprising that life-long supplementation of spermidine does not seem to have any negative side effects in mice (*44*). Enhancing spermidine uptake could be achieved with a variety of strategies in clinical studies, namely by (i) supplementation of synthetic spermidine, (ii) changes in diet composition in favor of polyamine-rich food items, (iii) use of natural plant extracts rich in polyamines, or (iv) administration of prebiotics and probiotics that favor microbial polyamine synthesis in the gut. A polyamine-rich diet including daily servings of natto was well tolerated for a period of 2 months in human volunteers (*40*) and led to an effective increase in circulating spermine levels. A clinical trial on supplementation of spermidine-rich plant extracts to elderly is currently ongoing (https://clinicaltrials.gov/ct2/show/NCT02755246) (*131*). Nevertheless, possible contraindications of spermidine administration have to be carefully defined in clinical trials and may include patients with advanced cancer and renal failure. A low-polyamine diet may also be beneficial in some specific cases, as has been suggested in rats infected with *Trypanosoma brucei brucei* (*132*). Last, it may be interesting to develop spermidine analogs that act at lower doses or have a more stably pharmacokinetic profile for the prevention or treatment of specific diseases.

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SUPPLEMENTARY MATERIALS

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Spermidine in health and disease

Frank Madeo, Tobias Eisenberg, Federico Pietrocola, and Guido Kroemer

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Having your longevity and eating too

Although caloric restriction has clear benefits for maximizing health span and life span, it is sufficiently unpleasant that few humans stick to it. Madeo *et al.* review evidence that increased intake of the polyamine spermidine appears to reproduce many of the healthful effects of caloric restriction, and they explain its cellular actions, which include enhancement of autophagy and protein deacetylation. Spermidine is found in foods such as wheat germ, soybeans, nuts, and some fruits and vegetables and produced by the microbiota. Increased uptake of spermidine has protective effects against cancer, metabolic disease, heart disease, and neurodegeneration.

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