ANNUAL REVIEWS

Annual Review of Nutrition

Nutritional Aspects of Spermidine

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Annu. Rev. Nutr. 2020. 40:135–59

First published as a Review in Advance on July 7, 2020

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

https://doi.org/10.1146/annurev-nutr-120419-015419

Keywords

healthy diet, polyamines, autophagy, aging, longevity, health span

Abstract

Natural polyamines (spermidine and spermine) are small, positively charged molecules that are ubiquitously found within organisms and cells. They exert numerous (intra)cellular functions and have been implicated to protect against several age-related diseases. Although polyamine levels decline in a complex age-dependent, tissue-, and cell type–specific manner, they are maintained in healthy nonagenarians and centenarians. Increased polyamine levels, including through enhanced dietary intake, have been consistently linked to improved health and reduced overall mortality. In preclinical models, dietary supplementation with spermidine prolongs life span and health span. In this review, we highlight salient aspects of nutritional polyamine intake and summarize the current knowledge of organismal

and cellular uptake and distribution of dietary (and gastrointestinal) polyamines and their impact on human health. We further summarize clinical and epidemiological studies of dietary polyamines.

Contents

1. INTRODUCTION

The diamine putrescine and the natural polyamines (PAs) spermine and spermidine are aliphatic bioactive amines that carry two or more amino groups [see **Figure 1** and the sidebar titled Definition of (Natural) Polyamines]. Due to their polycationic nature (i.e., the amino groups are fully protonated at physiological pH), PAs stabilize negative charges of macromolecules, including DNA, RNA, and proteins (150). PAs are essential for cell growth and proliferation (8) but fulfill many other functions, including promoting antioxidative activities, affecting ion channels, and controlling protein translation, and influence complex processes, such as apoptosis (138), autophagy (34), and immune regulation (55, 122, 136). Although seemingly every type of cell is

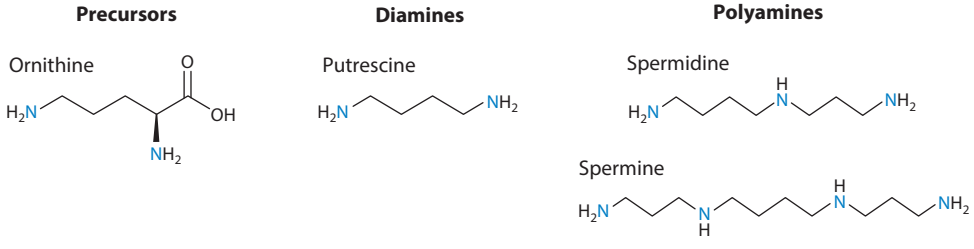

Figure 1

Structural formulas of the polyamine precursor ornithine, the diamine putrescine, and the polyamines spermidine and spermine.

DEFINITION OF (NATURAL) POLYAMINES

Polyamines (PAs) are a subclass of bioactive amines that perform a variety of functions in living cells (132). Many of the biogenic (i.e., biologically formed) amines are generated from different amino acids or related metabolites by decarboxylase reactions (142). For example, the diamine putrescine is produced from its precursor ornithine (catalyzed by ODC1) by the ornithine decarboxylase (ODC) reaction. Subsequently, the PAs spermidine and spermine (the only naturally occurring PAs that carry more than two amino groups) originate from their precursor putrescine by distinct metabolic reactions (115, 138). The functions of spermidine and spermine differ from those of diamines and other biogenic amines at the cellular and organismal levels in several respects, particularly regarding their health-promoting effects (32, 61–63, 83).

Therefore, we do not consider putrescine, spermidine, and spermine (which are often collectively referred to as natural PAs) to be equivalent. We use distinct terms within this review to differentiate them: Natural polyamine refers to spermidine and spermine, and diamine refers to putrescine. We thus stress the importance of studying separately the functions of the two natural PAs and the diamine putrescine.

capable of producing PAs through de novo biosynthesis, a substantial part of PAs comes from external sources, outlining the importance of diet for maintaining PA pools in the body (7, 100, 182). Importantly, external administration of spermidine has general antiaging effects (32, 34, 82, 83, 145, 178), as it confers a variety of positive effects on different organs, including the brain (50–52, 140, 169, 180), the cardiovascular system (1, 23, 32, 76, 174, 179), the skeletal muscles (26, 39, 47), the immune system (122), the liver (178), and the kidney (32, 73, 145), during aging. In line with this, PA levels, including those of spermidine, decline in human serum and whole blood with age (116, 121), which corroborates findings from rodent models showing that age inversely correlates with PA levels in most but not all tissue types (102). Healthy nonagenarians and centenarians display levels of spermine and spermidine comparable to those of 30- to 50-year-old adults (121) and higher than those of 60- to 80-year-olds.

Whereas its cellular metabolism and basic cellular functions have been studied for a long time, the health-promoting effects of dietary spermidine represent a relatively young field that has just begun to approach clinical translation. This review focuses on the importance of dietary PAs, particularly spermidine, while summarizing the current knowledge of spermidine content in human nutrition, the molecular basis of its uptake and distribution within the body, and its importance for human health.

2. SPERMIDINE CONTENT IN FOOD AND HUMAN NUTRITION

The natural PAs spermidine and spermine are ubiquitously present in living organisms of different phyla, including bacteria, plants, fungi, and animals, and are heat resistant (i.e., they are not destroyed by cooking). As a consequence, PAs are abundant in the daily nutrition of humans (100, 113). However, the PA content of different foods greatly varies (**Figure 2**). One of the most comprehensive compilations was reported in 2011 by Atiya et al. (7), who summarized the PA content of various types of foods reported in prior reports and databases (9–11, 15, 27, 35, 65–68, 75, 77–79, 97, 101, 102, 105, 106, 108, 130, 143, 164, 181). A more recent summary of the PA content of food categories is also available (100). Moreover, several studies have focused on specialized food types, such as various kinds of cheeses (38, 149).

One reason for this disparity in PA content is that organismal PA levels vary among different species, cell types, and tissues, and consequently among foods of animal, fungi, or plant

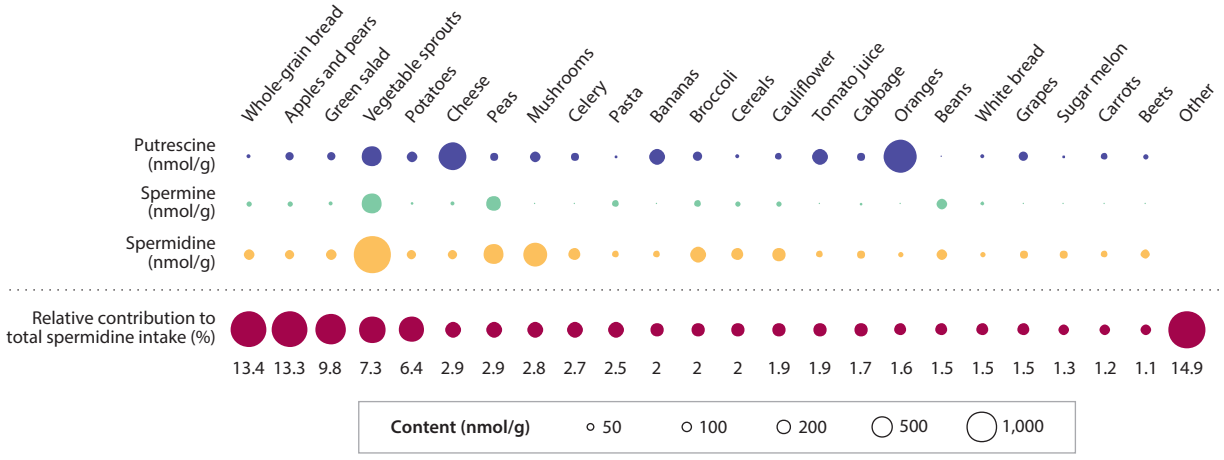

Figure 2

An example of polyamine content and the contribution of food to daily spermidine intake taken from a distinct regional population in South Tyrol, Italy. Data were adapted from polyamine levels and nutritional information previously published in the epidemiological Bruneck Study (71 and references therein) and from personal communication with S. Kiechl. The relative spermidine intake (% of total intake) is depicted (the size of the *circles* linearly corresponds to the relative intake values) and compared with the absolute mean concentrations of the diamine putrescine and the polyamines spermidine and spermine for indicated food categories. Note that the relative contribution of a given food category to total spermidine intake is calculated from both its spermidine content and the actual amount ingested (not shown). Therefore, both large intake of certain foods (despite moderate spermidine content) and high spermidine content (despite low intake) may result in a high relative contribution to total spermidine intake.

origin. Plant- and fungi-derived products represent the most relevant sources for spermidine. Whole-grain products, vegetables, and legumes are among the highest spermidine-containing food categories in common human nutrition. Examples of spermidine-rich foods are wheat germ (0.35 mg/g); soybeans (0.070–0.180 mg/g); select mushrooms (0.060–0.160 mg/g), such as eringi (a.k.a. king trumpet mushroom); and various nuts and seeds, such as pine nuts (0.060 mg/g) (102).

Animal-derived foods generally contain less spermidine than the average plant- or fungi-derived foods. However, they display comparable levels of spermine and therefore have a higher spermine-to-spermidine ratio (7, 100). Among animal tissues, innards such as liver, spleen, and kidney contain the highest amount of spermidine, with average concentrations as high as 0.161 mg/g in cow liver (65), whereas classical meat and meat products range from 0.001 to 0.020 mg/g (7, 66). Note that the published literature reports high variations in spermidine levels for select food items, as exemplified for bovine liver, for which the spermidine level ranges from 0.0068 to 0.390 mg/g (65). Such variation might mirror regional, species-, or food production–related differences, or may even indicate methodological problems with respect to the robustness of PA measurements, and thus clearly requires further investigation. Another relevant source for spermidine from animal-derived products is aged cheese, which contains levels of spermidine as high as 0.073 mg/g (and exceptionally high levels of up to 0.200 mg/g in aged cheddar) (11), although again the levels reported for different kinds of cheeses vary considerably (38, 149). Of note, cheese may contain high concentrations of other biogenic amines, such as putrescine and histamine, among others (38, 149). The high concentration of biogenic amines in aged cheese compared with that in raw milk likely results from microbial activities during the fermentation process (108).

Importantly, common portion sizes or actual intake amounts should be considered when discussing the relevance of certain foods regarding their contribution to overall spermidine intake. Diverse food items differ in magnitude in their contribution to the dietary intake of spermidine,

depending on two combinable variables: (*a*) the amount of food ingested and (*b*) the spermidine content of the ingested food (**Figure 2**). Therefore, food items with moderate levels of spermidine may still contribute to the daily spermidine intake at a similar or even higher degree than spermidine-rich foods if comparably ingested at higher amounts. For instance, whole-grain products, green salad, apples and pears, and potatoes, which are all rather moderately rich in spermidine, were among the top five spermidine-contributing foods (**Figure 2**) in a food frequency questionnaire (FFQ)-based epidemiological study (32). Another study, also based on FFQ-based data, reported green peas as the main source of spermidine (182).

The remarkable differences in the spermidine content of various foods, as well as the regional and cultural variances in human nutrition, have resulted in country-specific estimates of spermidine intake. A positive association between gross domestic product and the total PA per energy content of food for 33 Asian countries was reported (15). Gross domestic product is a representative indicator of socioeconomic status and correlates with health span and life span (15 and references therein), arguing for a potential positive association of PAs in food with healthy diet. However, the cause–effect relationship of this correlation remains elusive and needs to be determined in future intervention trials.

As noted above, the average estimated intake of spermidine differs by country. The daily intake is calculated to be approximately 13 mg for Asian countries (15), 8 mg for the United States, and as low as 5 mg for Turkey (19). For Europe the available data suggest an average intake similar to that of Asian countries, of approximately 13 mg per day (182). However, noticeable variations are present among European countries (summarized in 100): Spermidine intake values range from 10 mg (Sweden) to 12 mg (Italy) to almost 15 mg (Spain) per day. Regional differences are likely present within countries. For instance, the typical Mediterranean diet contains significantly higher levels of spermidine than the average southern European diet, with an estimated intake of up to 26 mg of spermidine per day (7, 9). On the basis of calculations using the recommended composition of a standard healthy diet according to the Swedish Nutrition Recommendations Objectified (SNO), the recommended daily amount of spermidine has been estimated to be approximately 30 mg for males and 25 mg for females (6). As a word of caution, all these estimates rely on calculations from generalized food consumption estimates, which may not always reflect real-life and individual situations.

An epidemiological study reported similar mean values of spermidine intake based on individual assessments of dietary habits using high-standard food questionnaires assisted by dieticians. A cohort of 829 study participants in Bruneck, a small town in northern Italy, consumed on average approximately 10 mg of spermidine per day (71). However, realistic table values of spermidine have not been determined directly, and present intake estimates are based solely on previously determined PA contents from similar foods. Due to the strong variations in spermidine content, even within specific food items, direct assessments of actual spermidine levels in food samples would be desirable and would allow more rigorous studies of the impact of spermidine intake on human health.

3. STABILITY OF SPERMIDINE IN FOODS AND DURING FOOD PROCESSING

Many reports have focused on the actual PA content of select foods, but very little is known about how agricultural and food-processing techniques may influence these levels. Large variations, particularly in animal-derived products such as innards or cheese, suggest that the specific origin and processing of the raw material significantly impact spermidine content. Storage temperature, the stage of ripening, and contact with oxygen may all affect the stability of PAs in stored foods and thus determine the final content of spermidine before consumption (3, 119).

PA stability during storage appears quite diverse. For instance, refrigerated meat displayed stable levels of spermidine over 8 days, however, with a gradual decrease in spermine content and, surprisingly, an increase in putrescine levels (54). In eggplant, spermidine content remained stable over 12 days independent of the storage temperature (3°C versus 20°C), whereas putrescine content increased at low temperature (29). In contrast, spermidine content decreased during a storage period of 12 days when eggplants were subjected to heat (35°C) for 1 h prior to storage, which also prevented an increase in putrescine (29). Such a decrease in spermidine levels, accompanied by an increase in putrescine, in response to chilling injury occurs in other types of fruits, including lemons (89), unripe peaches stored at 1 and 5°C (163), and zucchini squash (139), among others (see 29). In general, these effects may be explained by a metabolic stress response in fruits following storage at low temperatures, leading to the activation of PA biosynthesis, an effect that is avoided by a short-term application of moderate heat. This stress response likely accounts for increased putrescine levels and may even result in the recovery of spermidine, which at first glance seems to be degraded when food is stored. Metabolic adaptations to stress should thus be considered when PA stability in foods is investigated.

Fermentation processes are generally believed to favor the production of several biological amines (142). For instance, fermented fish contains elevated levels of spermidine (177). Yet spermidine appears less affected by fermentation than do other biogenic amines (including putrescine and histamine), implying that not all fermented foods contain increased levels of spermidine. Ripening may potentially impact spermidine levels as well. However, although some variations occurred (a decrease of up to 10–20% depending on the genotype), spermidine content was relatively stable during banana ripening and was barely affected by cooking or frying (17).

In general, spermidine appears to be relatively stable when boiling foods (7, 66). Nevertheless, spermidine and spermine may be significantly lost from foods and released into the cooking broth or water (66, 181). For instance, cowpeas and other common beans cooked in abundant water had 50% less spermidine than their raw, uncooked form (31). However, higher temperatures achieved by baking, grilling, or deep-frying may destroy spermidine (75).

In summary, the effects of food processing, such as various cooking techniques, storage conditions, and local differences in the production process, have been only partially studied. Additional work is needed to understand their impact on the abundance of spermidine in food items.

4. SPERMIDINE UPTAKE IN CELLS

4.1. Cellular Spermidine Import in Unicellular Organisms and Plants

Intracellular PA levels are stringently controlled by a triad of synthesis, uptake, and degradation/secretion. The uptake mechanisms of PAs into unicellular organisms have been studied for decades and are well understood, contrasting with the relatively scarce knowledge of PA transport into mammalian cells (59).

In *Escherichia coli*, two operon-organized adenosine 5′-triphosphate (ATP)-binding cassette (ABC) transport systems are relevant to PA and diamine metabolism: PotABCD for spermidine and PotFGHI for putrescine. Both systems consist of ATPases, channel-forming units, and substrate-binding proteins (e.g., PotD binding spermidine via four acidic residues), in which all partners are essential for metabolite uptake (59).

In yeast cells, multiple factors mediate cellular uptake of spermidine and PAs. *AGP2*, which was initially misidentified as a PA importer, harbors mainly PA-sensing properties that mediate the expression of other uptake-relevant factors (4, 5). The main membrane-bound PA importers are encoded by *DUR3* and *SAM3* (159) and *GAP1* (158). Another pH-sensitive PA transporter

in yeast cells is encoded by *TPO1*, which catalyzes PA uptake under alkaline conditions and controls export at acidic pH (161). In the unicellular parasites *Leishmania major* and *Trypanosoma cruzi* and the bacterium *Neisseria gonorrhoeae*, transporters bound to the plasma membrane (LmPOT1, TcPOT1.2/TcPAT12, and PotFGHI, respectively) have been identified, and such transporters present potential targets for antibiotics (20, 48, 53).

In plant cells, PA transport systems involve multiple divergent proteins that likely exhibit parallel, redundant, and less-specialized functions compared with proteins in bacterial systems (44). In *Arabidopsis thaliana*, members of the L-type amino acid transporter (AtLAT) family (especially plasma-membrane-bound RMV1/AtLAT1, a presumed amino acid permease) have been reported to be involved in PA uptake (43, 44). In rice (*Oryza sativa*), the gene *PA uptake transporter 1* (*OsPUT1*) was identified as a preferred transporter of spermidine (99).

4.2. Spermidine Import Mechanisms in Mammals

Intracellular depletion of PAs [e.g., by treatment with difluoromethylornithine (DFMO), an ODC inhibitor] (44), stimulation with growth factors (16), and proliferation (155, 175) all lead to increased uptake of PAs. Enhanced proliferation is indeed linked to elevated uptake of PAs into lung, liver, and cancer cells (44, 137). Several PA analogs, including D-lysine spermine (MQT-1426), N^1-spermyl-L-lysinamide (OR1202), and D-Lys(C_{16}acyl)-spermine (AMXT1501), reduce cellular PA uptake. Preclinical studies have shown that a combination of PA-uptake inhibitors (e.g., AMXT1501) and DFMO drastically reduces intracellular PA levels, thereby exerting a synergistic antiproliferative effect on cancer cells, in vitro and in vivo, in preclinical mouse models (45, 46). However, so far, specific uptake systems in mammalian cells are poorly understood, and single genes/proteins involved in the uptake process have not yet been holistically characterized. The complex picture of the mammalian PA transport system and its regulation has been reviewed elsewhere (2, 120) but likely involves tissue- and cell type–specific factors, suggested by the multiple PA transporter candidates described.

Various transport systems have been suggested to partly mediate PA transport into mammalian cells (**Figure 3**). These include solute carrier family 7 member 1 (SLC7A1), cation-chloride cotransporter 9 (CCC9), and organic cation transporter 6 (OCT-6), which function as permeases for lysine, arginine, and ornithine; inorganic ions; and cations, anions, and zwitterions, respectively (92, 120). This idea is supported by findings of PA transporters in synaptic vesicles, neurons, and glial cells (85). *SLC18B1*-encoded vesicular PA transporters may be responsible for storage of vesicular spermidine/spermine in (hippocampal) neurons (56) and are also expressed in mast cells that release PAs (152). More recently, SLC3A2, which forms heterodimers with various partners transporting different molecules, has been identified as a major PA transporter in neuroblastoma. Knockdown of SLC3A2 decreased spermidine uptake, and DFMO-mediated inhibition of synthesis increased SLC3A2 levels (46). SLC3A2 is strongly overexpressed in some cancer cell lines (46). Nonetheless, SLC3A2 has previously been implicated in PA export processes, implying a bidirectional role of intracellular PA regulation (160, 162) and suggesting level-, tissue-, and cell type–specific functions.

In addition to these putative permease-based transport systems, it is believed that the uptake of spermidine and other PAs in mammalian cells may be mediated by endocytosis (12) (**Figure 3**). In particular, glypican-mediated and caveolin-mediated endocytosis have been suggested to enable PA internalization into mammalian cells (12, 13, 120, 160). The ATPase cation transporting 13A2 (ATP13A2) (also called PARK9) was recently suggested to be a lysosomal PA transporter (favoring spermine and spermidine), facilitating endocytosis-mediated PA uptake (via subsequent export to the cytosol from endolysosomes) (165). Knocking out ATP13A2 results in lowered intracellular

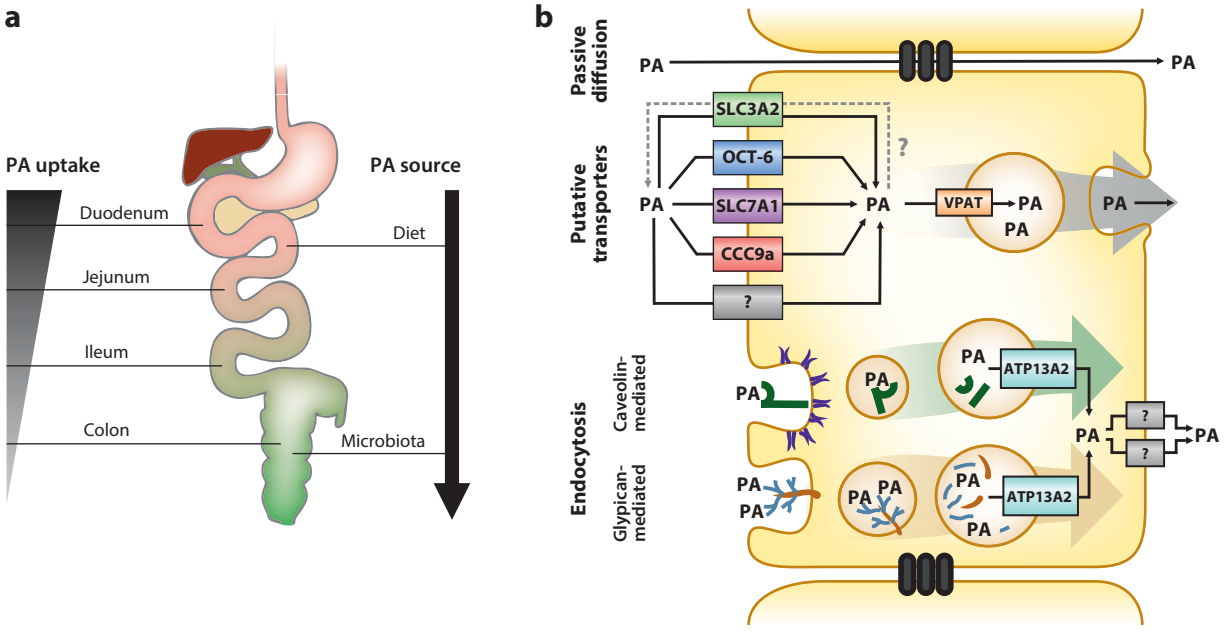

Figure 3

Intestinal uptake and putative cellular transport mechanisms of PAs in mammals. (*a*) PAs absorbed in the gastrointestinal tract are derived mainly from food and the gut microbiota, with most PAs being resorbed in the upper part of the small intestine from dietary sources. PAs taken up in the lower part of the gastrointestinal tract originate mainly from microbiota. (*b*) Putative cellular PA import systems in mammals include two distinct modes of endocytosis, several proposed transporters, and paracellular passive diffusion. Depending on the tissue, putative routes of PA export include the release of vesicular PA stores (as suggested for neurons and mast cells) and transport via yet uncharacterized transporters. Abbreviations: ATP13A2, ATPase cation transporting 13A2; CCC9a, cation-chloride cotransporter 9a; OCT-6, organic cation transporter 6; PA, polyamines (spermine, spermidine) and putrescine; SLC3A2, solute carrier family 3 member 2; SLC7A1, solute carrier family 7 member 1; VPAT, vesicular polyamine transporter.

PA levels and dysfunctional ATP13A2 impairs lysosomal PA export to the cytosol, resulting in cell death and possibly neurodegeneration (which corroborates its genetic implication in parkinsonisms) (165). ATP13A2 thus emerges as a new and potentially central player in cellular PA homeostasis, regulating both cytosolic and lysosomal PA levels.

Thus, the exact interplay of different uptake mechanisms of spermidine and spermine in mammalian cells and their individual contribution to intracellular PA levels remain to be determined, and the emerging picture will likely involve tissue- and cell type–specific factors.

5. UPTAKE, BIOAVAILABILITY, AND TRANSPORT OF DIETARY SPERMIDINE FROM THE GASTROINTESTINAL TRACT

PAs in the gastrointestinal tract originate mainly from food and the intestinal microbiota but also from dying cells and from secretions by the pancreas and gall bladder (100) (**Figure 3**). Although the exact contribution of the different sources of PAs to the available, gastrointestinal PA pool remains unclear, it has been debated that in humans most PAs in the small intestine are of dietary origin and PA levels in the colon depend more prominently on intestinal microbiota (125). Consequently, PA levels are normally in the low millimolar range (0.5–1 mM) in the intestinal lumen of healthy humans (86) and vary shortly after meals (high PA levels) and upon fasting (low PA levels)

(93). The small intestine (particularly the duodenum and proximal jejunum) is the major site of PA absorption (125).

Intestinal epithelial cells are the first barrier between (nutritional) luminal PAs and circulation (**Figure 3**). Epithelial cells of the intestinal mucosa are also highly proliferative and thus require fair amounts of PAs, which partly result from luminal PA uptake (125). Nevertheless, it is well established that dietary spermidine and other PAs reach the bloodstream and peripheral tissue, with kinetics suggesting rapid uptake and/or elimination from luminal compartments (93, 125, 135). For instance, in rats, 61–76% of labeled PAs administered to the jejunum (performed ex vivo using isolated organ blocks consisting of the small intestine with adjacent blood vessels) were found in the veins after 10 min, suggesting a rapid transition from the intestinal lumen to the bloodstream (157). No significant differences between the uptake of spermidine and that of other PAs were observed (157).

There are two main routes of passage from the intestinal lumen into the bloodstream: (*a*) uptake in epithelial cells via transporters (as discussed above) in the apical membrane followed by intracellular metabolism or cellular release across the basolateral membrane; and (*b*) paracellular transport via solute gradients and passive diffusion through the intercellular space between cells, which is likely responsible for most dietary PAs that find their way into the bloodstream (93) (**Figure 3**). Whereas putrescine seems to be readily metabolized in intestinal epithelial cells, spermidine and spermine appear to be spared from conversion at this site (125).

In blood, spermidine levels tend to be higher than spermine and putrescine levels (137) and are typically in the micromolar range (125). Plasma contains lower PA levels than does whole blood (28). Indeed, most circulating PAs are readily taken up by red blood cells and white blood cells (which exhibit the highest concentrations of PAs). In blood, PAs may be bound by various plasma proteins, mostly by electrostatic interactions (28, 94, 98). However, even in healthy adults, PA levels in blood show a high degree of variability and may be influenced by individual parameters, diseases, and age (22, 144). Thus, several lines of transport distribute dietary or microbially produced PAs to the systemic circulation and then supply peripheral tissues (**Figure 3**).

6. DISTRIBUTION OF POLYAMINES TO ORGANS AND ITS EFFECTS

Preclinical studies of mice (32, 145) and other model organisms, including *S. cerevisiae*, worms, and flies (34, 151), show that supplementation with spermidine significantly promotes life span. In mice, this is true for both lifelong feeding (starting at 4 months of age) and late-in-life feeding (starting at 18 months of age) (32).

Upon uptake in the gastrointestinal tract, dietary spermidine becomes available to different organs (11). Accordingly, a PA-enriched diet increases the PA levels in plasma in mice and humans (148). The highest concentrations of PAs in mammalian tissues are found in pancreas (58; F. Madeo, T. Eisenberg, S. Hofer, T. Pendl, M. Poglitsch & C. Magnes, unpublished observations), spleen (F. Madeo, T. Eisenberg, S. Hofer, T. Pendl, M. Poglitsch & C. Magnes, unpublished observations), intestine, thymus, and liver (10, 153), with levels of ~0.6 mg spermidine/g wet weight tissue in murine pancreas. This value corresponds to a concentration that is three- to sixfold higher than that found in spleen, thymus, liver, and intestine (F. Madeo, T. Eisenberg, S. Hofer, T. Pendl, M. Poglitsch & C. Magnes, unpublished observations). Still, in humans, the impact of dietary and exogenously applied spermidine on specific organs remains largely unexplored, although several reports point toward a systemic and multi-organ effect.

For instance, increased dietary intake of spermidine has been directly associated with improved cardiovascular health in a prospective, population-based cohort (71). According to preclinical data, cardiovascular improvement by spermidine in rodents might rely on its capability to promote

macroautophagy. In mouse cardiomyocytes, spermidine induces mitophagy, a specific form of autophagy that clears dysfunctional mitochondria (32). If damaged, mitochondria can overproduce detrimental reactive oxygen species and ignite cell death pathways, in which case their elimination improves cellular function (33). This is especially important for cells that display a high mitochondrial content due to their relatively high energy demand (like cardiomyocytes). In line with this consideration, intraperitoneally delivered spermidine (and spermine) reversed age-associated alterations in myocardial morphology and inhibited apoptosis in cardiomyocytes (179). This finding was further corroborated by a study showing that spermidine could counteract induced myocardial infarction in a rat model by enhancing autophagic flux (174). Further, administration of spermidine during pregnancy could prevent detrimental effects of hypoxia, such as structural defects in mitochondria, reduced mitochondrial biogenesis, and respiratory dysfunction, on neonatal rats (23), highlighting a potentially protective basis similar to that previously demonstrated for cardiomyocytes (32).

Besides its capacity to induce autophagy within cardiomyocytes, spermidine may improve cardiac function by systemic actions, including dampening chronic inflammation [by diminishing circulating levels of tumor necrosis factor α (TNF-α)] and reducing blood pressure. The latter effect may be explained by enhanced bioavailability of arginine, which increases the levels of the vasodilator nitric oxide (32). Moreover, spermidine may reduce blood pressure through autophagy-mediated improvement of renal function. Indeed, animals supplemented with spermidine exhibited increased renal spermidine content and elevated autophagy levels (179). In *Dahl* rats, which have impaired renal salt metabolism, treatment with spermidine delayed the appearance of several signs of hypertensive injury to the kidney (32).

The proautophagic capacity of spermidine suggests it can affect normal brain function and protect against neurodegeneration, which are tightly connected to autophagy (42). Indeed, the first pilot trial of a cohort of individuals with subjective cognitive decline (SCD), which is characterized by self-experienced deterioration in cognitive performance that cannot (yet) be detected objectively by formal neuropsychological examination, suggests that administration of spermidine-rich wheat extract can enhance memory performance (172). This potential for neuroprotection is supported by several preclinical studies (83). For example, in a mouse model of multiple sclerosis, orally administered spermidine abated disease progression (50, 176). Spermidine also reduces frontotemporal lobar dementia in mice expressing transgenic transactive response DNA–binding protein 43 kDa (TDP-43) (169). A recent report shows neuroprotective effects of spermidine against rotenone-induced Parkinson disease (PD) in rats by avoiding the PD-associated loss of dopaminergic neurons in substantia nigra pars compacta and nerve terminals (140). In line with these observations, spermidine feeding protects worms and flies against neurotoxicity exerted by α-synuclein, which is believed to be a crucial toxic trigger of PD (18). Further studies of fly models have shown that administration of spermidine protects against age-induced loss of memory (52) and locomotor activity (95). In these models, induction of autophagy may play a fundamental role, possibly by promoting synaptic flexibility and plasticity (51).

Given the neuroprotective effects of spermidine, the extent to which nutritional spermidine is able to cross the blood-brain barrier requires further investigation. In rats, 5 s after an acute intraarterial injection of labeled spermidine, brain uptake index (BUI) values of approximately 5–6% could be determined (note that nonpassable substances could give BUI values of less than 2%, which suggests a low capability of spermidine to pass the blood-brain barrier) (49, 141). Another study using arterial and intravenous injection of tracer spermidine suggests that a specific transport mechanism may enable spermidine to access the brain (30). Still, more work is required to clarify the neuroavailability of dietary spermidine.

Importantly, several preclinical studies of mice support the anticancer activity of spermidine as suggested by the Bruneck and the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) studies (71, 72). Besides its role in increased immunosurveillance (118), spermidine production by spermidine synthase is crucial for the antineoplastic activity of tumor-associated macrophages in colorectal cancer (90). Moreover, dietary administration of spermidine to mice attenuates chemically induced liver fibrosis and reduces the incidence of hepatocellular carcinomas (178) and colon tumorigenesis in young animals (147). In the latter study, however, the maximum size of established tumors increased (147). Thus, spermidine might be able to block tumorigenesis but stimulate the growth of existing tumors, which rely on autophagy for further growth and stress resistance. Future studies will need to explore in detail the possible impact of dietary spermidine on the growth of existing tumors.

Systemic administration of spermidine has also been linked to improved immune recognition of neoplastic cells in mice (118). Spermidine-induced autophagy in cancer cells promotes the release of ATP, which acts as a chemotactic factor for myeloid cells, thus initiating an anticancer immune response (80, 91). Accordingly, mice treated with spermidine presented an autophagy-dependent and T lymphocyte–dependent inhibition of tumor growth in the context of chemotherapy (118). Other preclinical studies have further evinced the multifaceted effects of spermidine on the immune system. For instance, memory lymphocyte function, which has been shown to decline in humans with ongoing age (117), can be enhanced in mice by spermidine treatment (122). Moreover, spermidine shows strong anti-inflammatory properties in rodents (25, 111), a finding that may be relevant to several pathophysiological scenarios. This is connected to the suppression of several proinflammatory modulators and cytokines, including TNF-α (32, 70), which is slightly but chronically increased in plasma during aging.

Other diseases, such as metabolic syndrome–related pathologies like obesity and type 2 diabetes, may be influenced by PAs. Although this needs further investigation, several preclinical studies suggest application of exogenous spermidine and/or spermine has protective effects. For instance, mice fed a high-fat diet supplemented with spermine showed reduced adiposity and improved glucose tolerance (131). Moreover, weight gain and obesity-related pathologies were reduced in mice fed a hypercaloric diet administered with spermidine (41). Of note, this reduction was correlated with induced autophagy in white adipose tissue (41).

Collectively, these preclinical studies reflect the multipronged activity of spermidine, which in turn explains its protective potential against multiple diseases. In fact, the preclinical evidence has initiated several clinical trials and a prospective epidemiological cohort study (72, 116, 173) that address the possible impact of dietary intake of spermidine on longevity, cardiovascular disease, and age-dependent cognitive decline.

7. CROSS TALK BETWEEN POLYAMINES AND THE GUT MICROBIOTA

Nutrition and the gut microbiota, which may consist of myriad species (123), are closely intertwined and affect each other in multiple ways. In fact, a series of microorganisms in the intestine are equipped with pathways to fully or partially produce PAs (156). Accordingly, the administration of probiotics (live bacteria) may be another way to increase luminal PA levels (156). In a small-scale study, consumption of yogurt containing the probiotic bifidobacteria strain LKM512 and free arginine (Arg) led to increased levels of putrescine and spermidine in serum (87), suggesting the feasibility of this approach. In rodents, the same procedure extended life span and improved several markers of physiological and cellular senescence, and Arg alone led to increased putrescine levels in the gut and increased spermidine and spermine levels in blood (70).

Overall, although data are still scarce, it appears plausible that a PA-rich diet might influence the gut microbiota. Likewise, intestinal microorganisms may produce PAs from different dietary substrates, modulate nutritional PA content, or both. Thus, future dietary studies of PA intake in humans should closely explore the interplay between these factors, as they may influence each other in ways that are not yet fully understood.

8. EXCRETORY SYSTEMS

The effective intracellular concentrations of spermidine are dictated both by its (partly reversible) conversion to other PAs and by its secretion from cells. In either case, spermidine acetylation, a process controlled mainly by cytoplasmic spermidine/spermine N^1-acetyltransferase (SSAT), plays a major role (**Figure 4**).

SSAT is instrumental in the conversion of spermidine to its upstream precursor, the diamine putrescine, and in the regeneration of spermidine from its downstream product, spermine. On the one hand, SSAT produces N^1-acetylspermidine, which can be converted back to putrescine by acetyl polyamine oxidase (APAO). On the other hand, SSAT acetylates spermine to generate N^1-acetylspermine, which can be a substrate of APAO, thereby regenerating spermidine (reviewed in 100). SSAT can further acetylate N^1-acetylspermine to N^1,N^{12}-diacetylspermine, which in turn can be processed by APAO to form N^1-acetylspermidine (64, 114). Of note, spermine can be directly reconverted to spermidine by spermine oxidase (170). Besides N^1-acetylspermidine, N^8-acetylspermidine, another acetylated spermidine variant, can be formed via nuclear spermidine N^8-acetyltransferase (37). N^8-acetylspermidine can be hydrolyzed back to spermidine. Although specific cytosolic N^8-acetylspermidine deacetylase activity has been characterized in vivo (84), the actual enzyme remains to be identified. Finally, a diacetylated form of spermidine (N^1,N^8-diacetylspermidine) has been identified (57).

These acetylation reactions not only serve intracellular PA interconversions but can also directly and indirectly regulate the actual degradation and secretion of spermidine (**Figure 4**). For instance, the acetylated products of the SSAT reactions can be directly exported to the extracellular space (92). The characterization of this process (and of mammalian PA transporters in general) remains inconclusive, but there is some evidence that the amino acid transporter SLC3A2 is involved. In particular, SSAT seems to be associated with SLC3A2 in the plasma membrane, which may catalyze the export of acetylated PAs and putrescine through an Arg antiporter mechanism (160).

Thus, increased generation of putrescine via sequential SSAT and APAO reactions on spermidine may indirectly contribute to the export of cellular spermidine. In the same line, conversion of spermidine to putrescine enhances the substrate preference (lowered Michaelis constant) for diamine oxidase, thus allowing effective degradation by this enzyme (36, 96). Importantly, the oxidase reactions catalyzed by APAO and spermine oxidase also generate reactive aldehydes (3-acetoaminopropanal and 3-aminopropanal, respectively) and hydrogen peroxide, which can cause cellular damage. This may be especially relevant for spermine oxidase, which in contrast to APAO does not reside in peroxisomes (112).

The main excretory pathway for spermidine (and PAs in general) is urinary secretion. Acetylputrescine and N^1- and N^8-acetylspermidine together account for more than 90% of the PAs excreted in human urine (69). The levels of diacetylated PA species (N^1,N^{12}-diacetylspermine and N^1,N^8-diacetylspermidine) are low (0.5% and 1.4%, respectively), but variations between individuals seem to be small and their excretory share is strictly regulated (57). Whereas the intracellular generation of acetylated spermidine and spermine species has been largely characterized (see above), it remains unclear how acetylputrescine is produced before or during renal excretion.

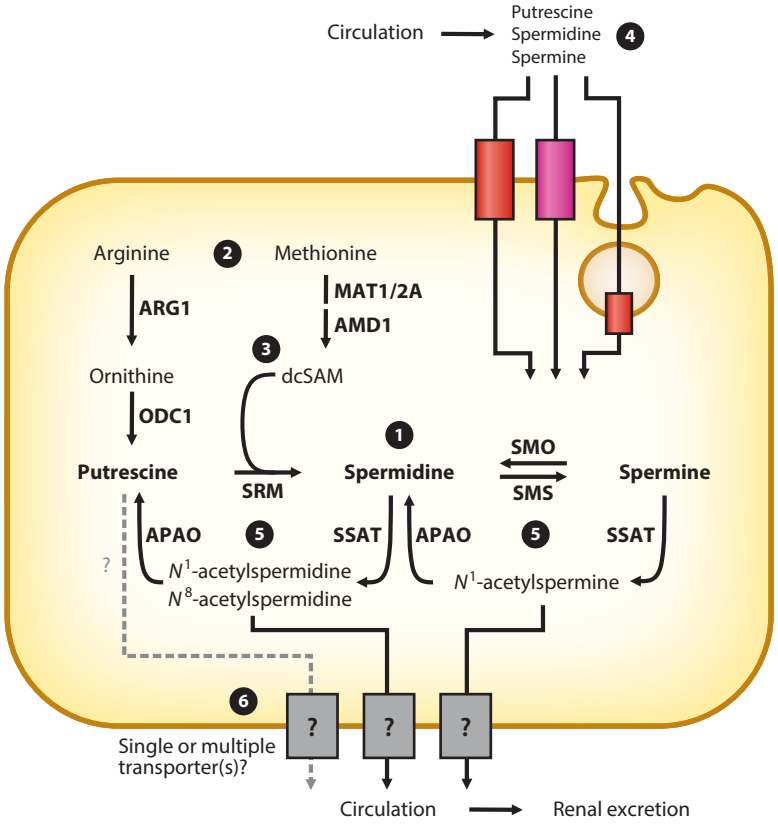

Figure 4

Cellular diamine and polyamine metabolism. (❶) Intracellular de novo synthesis of putrescine, spermidine, and spermine involves the amino acids arginine, ornithine, and methionine. (❷) Arginine is hydrolyzed to ornithine, which is then decarboxylated to putrescine; methionine is metabolized to SAM and subsequently to dcSAM and serves as an aminopropyl group donor to produce spermidine from putrescine. (❸) dcSAM also functions as an aminopropyl group donor to generate spermine from spermidine. (❹) In addition, extracellular putrescine, spermidine, and spermine may enter the cell via specific transporters or by endocytosis. (❺) Interconversion reactions between putrescine, spermidine, and spermine further modulate their intracellular levels. (❻) Cellular secretion is accomplished via acetylated forms of spermidine and spermine. Putrescine has also been suggested to undergo active secretion, although the process of putrescine secretion is not yet understood. Abbreviations: AMD1, *S*-adenosyl-L-methionine decarboxylase 1; APAO, acetyl polyamine oxidase; ARG1, arginase 1; dcSAM, decarboxylated *S*-adenosylmethionine; MAT1/2A, methionine adenosyltransferase 1/2A; ODC1, ornithine decarboxylase 1; SAM, *S*-adenosylmethionine; SMO, spermine oxidase; SMS, spermine synthase; SRM, spermidine synthase; SSAT, spermidine/spermine N^1-acetyltransferase.

Importantly, circulating spermidine and spermine can be oxidized by serum amine oxidase to putrescine and acrolein, a toxic by-product of PA metabolism, respectively. Both putrescine and acrolein levels increase in the plasma of patients with chronic renal failure (60). Therefore, dietary supplementation with spermidine in individuals with renal failure should be scrutinized for potential adverse side effects.

PAs can also be eliminated through fecal excretion. This route is tightly connected to the gut microbiota, which produces a diverse array of metabolites, including spermidine. The levels of

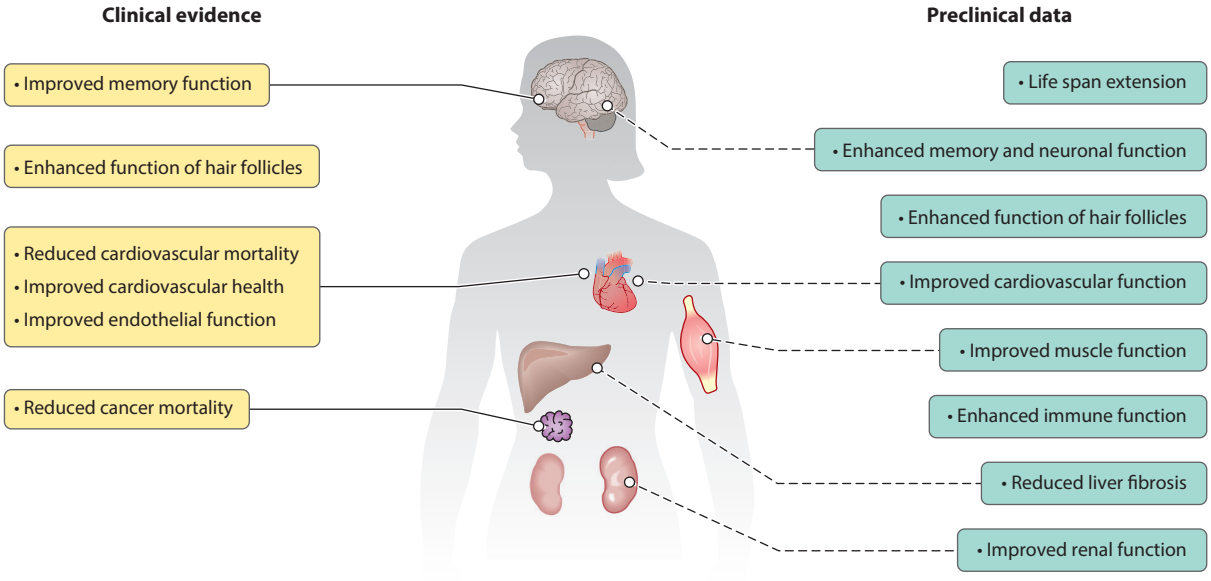

Figure 5

Proposed health benefits of dietary spermidine in humans according to epidemiological and clinical data, highlighting possible future avenues based on preclinical studies. (*Left*) Health benefits proposed in clinical and epidemiological studies: improved memory function (173), enhanced function of hair follicles (129), reduced cardiovascular mortality (71), improved cardiovascular health (32) and endothelial function (87), and reduced cancer mortality (71). (*Right*) Diverse beneficial effects of spermidine administration proposed in preclinical, nonhuman studies: extended life span (32, 145, 178), enhanced memory and neuronal function (50–52, 140, 169, 180), enhanced function of hair follicles (126, 128), improved cardiovascular function (23, 32, 76, 174, 179) and muscle function (26, 39, 47), enhanced immune function (122, 176), reduced liver fibrosis (178), and improved renal function (32, 73, 145).

PAs in the intestinal tract can originate from dietary intake, but it is thought that the largest contribution comes from synthesis by intestinal microbiota (125). Accordingly, the administration of prebiotics and probiotics may affect PA levels in the intestine, as previously shown in rodents (70, 88, 103). The level of PAs in the intestine is strongly influenced by portal circulation and biliary excretion (109), but elimination by the feces may play a role as well (86).

9. CLINICAL TRIALS

The prospective Bruneck Study (enrollment of a study population in 1990 as an age- and sex-stratified random sample of all inhabitants of Bruneck, Bolzano Province, Italy) involved a detailed dietary assessment using dietitian-administered, scientifically validated (171) FFQs (2,540 assessments) repeated over 20 years. The analysis involved 829 participants aged 45–84, in which 341 deaths occurred during the follow-up between 1995 and 2015 (71). The analysis of this study revealed a correlation between high dietary intake of spermidine and low mortality from one of three categories of death (vascular deaths, cancer deaths, or deaths from other causes) (71) and from fatal heart failure specifically (32) (**Figure 5**). This correlation withstood correction for possible confounding factors, including alcohol and aspirin consumption, quality of diet, level of physical activity, and socioeconomic status, among others. Instead, health-related effects of the dietary intake of the spermidine precursors putrescine, Arg, and methionine were not observed, while the spermidine-related and interconvertible PA spermine showed similar, but weaker, associations

(71). These epidemiological associations based on the FFQs, in view of the accumulating, broad, and supporting preclinical data, principally substantiate the concept that the PAs spermidine and spermine may promote survival in an evolutionarily conserved manner. The main sources of spermidine intake in the Bruneck Study were whole grains (13.4%), apples and pears (13.3%), green salad (9.8%), vegetable sprouts (7.3%), and potatoes (6.4%) (**Figure 2**). Importantly, the authors did not find any association between the effect on mortality and the dietary origin of spermidine (71). The analysis revealed a sex- and age-dependent correlation with spermidine intake: Women showed higher intake, and increased age was connected to reduced consumption (71). Taken together, the findings from the Bruneck Study give important insights into the possible impact of dietary PAs on humans, albeit in a locally restricted population. The study showed positive correlations of spermidine/spermine intake levels with diverse health aspects (e.g., reduced risk of cardiovascular disease) and an inverse association with general (including cancer-related) mortality. Although the favorable associations withstood extensive corrections for possible confounding factors, the data from the Bruneck Study have their limitations given their basis in FFQs, and future clinical trials with targeted dietary supplementation with spermidine are required to confirm the causality of spermidine in humans.

The Bruneck Study was replicated in the SAPHIR Study, in which a second, independent cohort of 1,770 healthy unrelated participants (663 women and 1,107 men aged 39–67) was examined from 1999 to 2002, with follow-up for deaths until 2013 (71). Compared with that in the Bruneck Study, the contribution of individual foods to spermidine intake in the SAPHIR Study was nearly identical for green salad (10.8%) and potatoes (6.6%) but lower for whole grains (6.5%). The contribution from fruit (24.1%) and vegetables other than green salad (30.5%) was comparably high. Again, women showed a higher intake of spermidine. Most importantly, the SAPHIR Study corroborated the finding from the Bruneck Study that spermidine-rich nutrition is associated with increased survival: Spermidine showed the most prominent inverse correlation with mortality for the 146 macronutrients and micronutrients that were identified in the nutrition of the subjects (71).

This observation aligns with the fact that spermidine (and spermine) levels decrease during aging (102, 116, 134, 168). Long-lived people (nonagenarians and centenarians) maintain whole-blood levels of PAs similar to those of middle-aged individuals (121), corroborating the potential importance of spermidine and spermine for longevity. Accordingly, a PA-rich diet has been connected to a low incidence of cardiovascular disease and a long life span (14, 146). Thus, one may argue that as a person ages, spermidine levels decrease to a detrimental extent and thus need to be replenished through dietary or pharmacological supplementation. In this respect, spermidine might reach vitamin status for aged individuals (81).

Despite the potential of spermidine to protect against cancer as suggested by the Bruneck and the SAPHIR studies, PAs have been connected to procarcinogenic activities. Specifically, an upregulation in biosynthesis and thus in PA levels has been reported for skin, breast, colon, lung, and prostate cancers (107). Increased levels of acetylated PAs in urine or blood have been described as biomarkers for different tumor types (reviewed in 21). One study suggested that increased dietary intake of PAs might exacerbate the risk of developing colorectal adenoma (167), but in a follow-up work, the same authors could not confirm this proposed connection (166).

Nutritional modulation of spermidine levels may be achieved not only by directly ingesting PA-rich foods but also by boosting PA production through dietary regulation of intestinal microbiota. A clinical trial has recently explored this potential (87). A yogurt preparation with *Bifidobacterium animalis* subsp. *lactis* (Bifal) and free Arg was administered to 18 subjects (100 g of yogurt once a day, after lunch, for 12 weeks). All subjects were nonsmokers (10 men, 8 women), aged 30–65 years, and had a body mass index less than 30. A corresponding placebo group ($n = 16$) was used as a

control. Arg is a precursor of putrescine, and the microbial hybrid putrescine biosynthesis system, which involves different types of bacteria (among them *B. animalis* subsp. *lactis)*, can promote intestinal production of putrescine from free Arg (but not from dietary protein-bound Arg) (74). The ingestion of the Bifal-Arg yogurt resulted in positive effects (87). The reactive hyperemia index, a measure of endothelial responsiveness, was increased, suggesting reduced risk of cardiovascular conditions. The Bifal-Arg group exhibited diminished systolic and diastolic blood pressure and enhanced high-density lipoprotein–cholesterol levels in serum, which are correlated with a reduced risk for cardiovascular disease (124) (**Figure 5**). Moreover, the Bifal-Arg group exhibited reduced platelet and serum triglyceride concentrations, two factors connected to atherosclerosis (104, 154). Finally, the Bifal-Arg group showed increased putrescine levels in feces (in accord with the fact that Arg is a precursor of putrescine) and elevated concentrations of spermidine in serum (with no changes in the fecal concentration). No alterations in fecal or serum spermine levels were detected. The authors suggest that treatment with Bifal-Arg induces microbial production of putrescine, which after intestinal absorption is used to biosynthesize spermidine to exert protective effects on endothelial function (87).

An alternative strategy for increasing spermidine intake consists of direct supplementation with enriched foods or food extracts. A recent report (172) used spermidine-rich wheat germ extract, which had been previously validated for safety and tolerability in older adults (135). The study was designed as a pilot trial (3 months) and tested the memory performance of a cohort of individuals ($n = 30$, aged 60–80) with SCD. Therefore, these subjects represent a high-risk group that may benefit from early spermidine-based intervention. Specifically, the authors evaluated mnemonic discrimination, the ability to differentiate among highly similar remembrances, as well as memory and executive functions. Application of spermidine-rich extract enhanced both memory performance and mnemonic discrimination (172) (**Figure 5**). Currently, a second trial (12 months, $n = 100$) is ongoing: The recruitment finished in March 2019 and the last follow-up check is planned for September 2020 (173). This study will extend the previous pilot trial and assess memory performance, as well as changes in neurocognitive, behavioral, and physiological parameters, in subjects with SCD. A recent study demonstrated that higher levels of spermidine in serum weakly, but significantly, correlate with improved cognitive performance of elderly people taking the Mini-Mental State Exam (116).

Another interesting benefit for dietary supplementation with spermidine is its impact on hair growth and hair follicles (127). Supported by several preclinical in vivo and in vitro studies (40, 126–128), a recent clinical study examined the effects of a spermidine-based nutritional supplement (which was not further defined) on hair follicles in healthy humans. After three months, the supplement-treated group showed a prolonged anagen phase (active growth phase of hair follicles) compared with the placebo group (129).

Future clinical trials will further elucidate how dietary modulation of spermidine influences human health. Therefore, it will be important to include laboratory assessments that analyze the causal relationship between protective effects and pathways ignited by spermidine, including autophagy.

10. FUTURE DIRECTIONS AND CONCLUSION

In this review, we summarize the field of dietary PAs, especially spermidine, in human nutrition and health. Accumulating data from numerous preclinical studies, as well as a growing number of clinical studies, substantiate the hypothesis that PAs are important determinants of healthy human nutrition. Overall, spermidine, among other diamines and polyamines, plays crucial roles in diverse physiological and disease-related scenarios. However, we do not yet fully understand

the mechanistic relationship between spermidine, health, and disease. This is also outlined by the observation that increased levels of spermidine occur in disease-specific contexts (24, 110, 133), whereas preclinical data suggest potent protective effects of spermidine in such pathologies. Whether altered levels of spermidine in disease contexts indicate a homeostatic cellular stress response or reflect metabolic alterations causally linked to disease progression remains to be solved.

While the cornerstones of the complex interplay between dietary PAs and health are gradually outlined by ongoing preclinical and clinical studies, it will be crucial to explore in more detail PA stability during food cultivation, processing, and storage. Ultimately, refining nutritional databases to estimate PAs and examining the exact PA contents in standardized meals prepared for trial purposes will help define the role of food-derived PAs in human health. In fact, a deeper understanding of dietary PAs will help shape future standards of a healthy diet and guide the development of dietary plans that favor life span and health span.

DISCLOSURE STATEMENT

D.C.-G., G.K., and F.M. are the scientific cofounders of Samsara Therapeutics, a company that develops novel pharmacological autophagy inducers. T.P. has equity interests in Samsara Therapeutics. F.M. and D.C.-G. have equity interests in The Longevity Labs (TLL), a company founded in 2016 that develops natural food extracts. T.E. has equity interests in and conducts paid consultancies for TLL. G.K. holds research contracts with Bayer Healthcare, Genentech, GlaxoSmithKline, Institut Mérieux, Kaleido Biosciences, Lytix Biopharma, NuCana, Oncolinx, PharmaMar, Samsara Therapeutics, SOTIO, and Tioma Therapeutics. G.K. is on the Board of Directors of the Bristol Myers Squibb Foundation France. G.K. is a scientific cofounder of everImmune, a biotech company that develops immunostimulatory bacteria, and Therafast Bio.

ACKNOWLEDGMENTS

We thank Thomas Kuenzer for technical assistance with compiling **Figure 2** and Stefan Kiechl, as well as Raimund Pechlaner, for critical discussions about and assistance with developing **Figure 2**. F.M. is grateful to the Austrian Science Fund FWF (SFB-LIPOTOX F3007 & F3012, W1226, P29203, P29262, P27893, and P31727); the Austrian Federal Ministry of Education, Science and Research; and the University of Graz for grants Unkonventionelle Forschung-InterFast and flysleep (BMWFW-80.109/0001-WF/V/3b/2015) and the field of excellence program BioHealth. We acknowledge support from NAWI Graz, the BioTechMed-Graz flagship project EPIAge, and the field of excellence program BioHealth. G.K. is supported by the Ligue Contre le Cancer Comité de Charente-Maritime (Equipe Labellisée); the Agence National de la Recherche (ANR)–Projets blancs; the ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; the Association pour la Recherche sur le Cancer (ARC); the Cancéropôle Île-de-France; the Institut National du Cancer (INCa); the Institut Universitaire de France; the Fondation pour la Recherche Médicale (FRM); the European Commission (ArtForce); the European Research Council (ERC); the Leducq Foundation; the LabEx Immuno-Oncology; the Recherche Hospitalo-Universitaire Torino Lumière; the Site de Recherche Intégrée e sur le Cancer (SIRIC) Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); and the Paris Alliance of Cancer Research Institutes (PACRI).

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