Review

Basic Knowledge of Polyamines That Researchers Must Know before Starting Research, and the Relationship of Polyamines to Aging, Health and Disease

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Basic Knowledge of Polyamines That Researchers Must Know before Starting Research, and the Relationship of Polyamines to Aging, Health and Disease

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Abstract: The human polyamines spermine and spermidine are essential for cell function, and their properties have been extensively studied. However, research results of experiments conducted without understanding the properties of polyamines have been evaluated and published by reviewers who appear to have little knowledge of the properties of polyamines. It is sad that some studies repeat what was done more than half a century ago before polyamines were characterized. First, I explain the basic properties of polyamines that researchers need to know. Then, the relationship between changes in polyamine levels in the body and age- and lifestyle-related diseases is discussed. In addition, the epigenetic mechanism of action of polyamines in relation to healthy longevity and, conversely, age- and lifestyle-related diseases and the progression of senescence are discussed. Spermine, which increases with a polyamine-rich diet, acts to suppress aberrant gene methylation and proinflammatory status associated with aging. Meanwhile, chronic inflammation associated with aging induces the enzyme activity of spermine oxidase, which breaks down spermine and produces acrolein, resulting in reduced spermine levels. Acrolein, a toxic aldehyde that increases with age- and lifestyle-related diseases, is thought to accelerate these diseases.

Keywords: polyamine; spermine; spermidine; lifespan extension; polyamine-rich food; age-related disease; inflammation; gene methylation; LFA-1

1. Introduction

The history of the discovery and study of the existence of polyamines is extremely long. There are many findings that have accumulated during this long period of research, especially the properties of polyamines, which have been clarified by many researchers, especially in the last half century or so. Our group was the first in the world to report the health and longevity effects of a diet high in polyamines, having discovered physiological activities that may contribute to healthy longevity through basic research based on the established properties of polyamines [1]. However, given the current situation, it seems that many researchers do not understand these basic characteristics and are therefore repeating the same mistakes that were made more than half a century ago when these findings were made. For example, following our report, polyamines have been reported to prolong lifespan [2–5], but some of them propose as a mechanism of longevity activities that are not originally possessed by polyamines themselves, which were obtained in *in vitro* experiments conducted without understanding the properties of polyamines. One of the reasons for this, I suspect, is that the results of experiments performed by researchers who have made no effort to learn the basic properties of polyamines are evaluated by reviewers with the same limited knowledge. It is unacceptable to be asked questions by reviewers about the very basic knowledge that one should understand before starting research on polyamines.

In order not to waste the research results that polyamine researchers have accumulated over the past half century, and not to waste more effort, time, and resources, I will devote much of the first half of this review to a detailed description of the properties of polyamines. These are things that every polyamine researcher should naturally understand before starting research. Then, I will review recent reports on the relationship between human health and age- and lifestyle-related diseases and

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polyamines, especially their concentrations, and explain the mechanisms of polyamine concentration changes based on the basic background of polyamines.

2. Polyamines

Natural polyamines are low molecular weight aliphatic polycations found in the cells of all living organisms and their intracellular concentrations are very high, ranging from micromolar to low millimolar. They are known to be essential for cell growth and differentiation and are synthesized in cells on demand. In addition to the *de novo* intracellular synthesis, cells can take up polyamines from the environment. Polyamines (spermine and spermidine) and their precursor, putrescine, contain multiple amino groups (-NH_2). The molecular weights of spermine (SPM) with four amino groups and spermidine (SPD) with three amino groups are about 140 g/mol and 200 g/mol, respectively. Putrescine (PUT) with two amino groups is a precursor of polyamines and is called a diamine. In eukaryotes, polyamine synthesis is initiated from ornithine, which is synthesized from arginine. Ornithine decarboxylase (ODC) is the rate-limiting enzyme in polyamine synthesis and decarboxylates ornithine. SPD and SPM are then synthesized by the sequential addition of aminopropyl groups donated by decarboxylated S-adenosylmethionine (dcSAM), which is converted from *S*-adenosylmethionine (SAM) by the enzymatic activities of adenosylmethionine decarboxylase (AdoMetDC). The addition of aminopropyl groups to PUT and SPD is catalyzed by spermidine synthase and spermine synthase, respectively (Figure 1).

Polyamines and putrescine are universally required for cell growth and differentiation. However, their importance has been found to vary from organism to organism, as summarized in my previous review [6]. For example, according to a study by Inoue et al, Miyamoto et. al., and Wortham et. al., putrescine is essential for cell growth in lower organisms such as bacteria and fungi, whereas spermine is not present in the cell (as cited in [6]). Hamasaki-Katagiri et. al. and Gordon et. al. reported that spermine levels in yeast and nematode bodies are low and not essential for growth (as cited in [6]). All of this suggests that these are inconsequential roles for spermine in cell growth and differentiation, as well as cell function, in lower primitive organisms. Spermine is considered more important in highly developed animals. For example, a decrease in the spermine levels due to a deficiency in spermine synthase has serious consequences in humans [7].

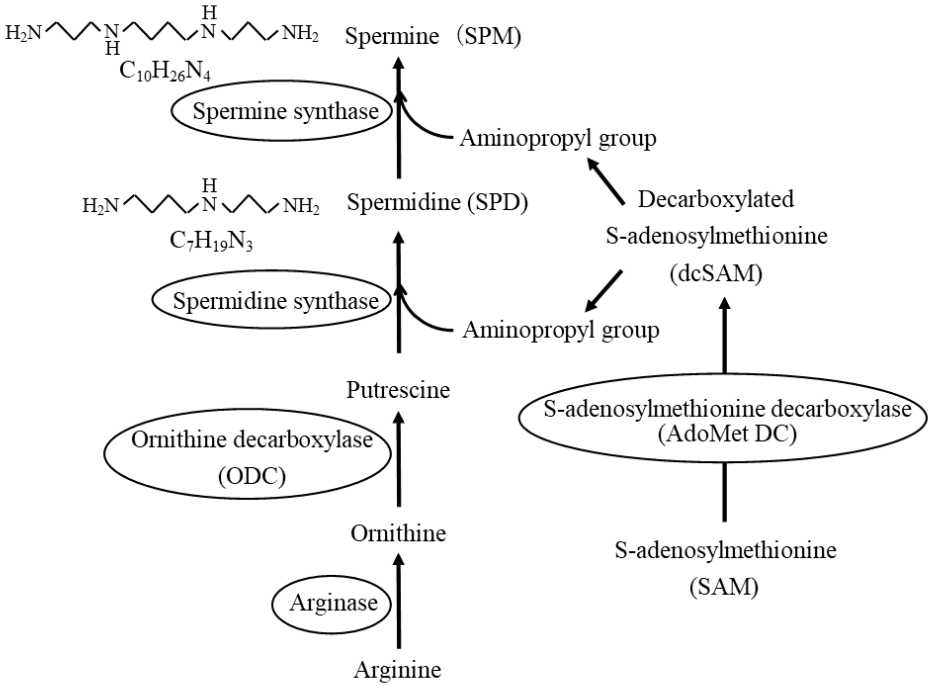

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Figure 1. Polyamine Synthesis Pathway. Ornithine, which is synthesized from arginine via the urea cycle, is a raw material for polyamine synthesis. Ornithine decarboxylase (ODC), rate-limiting enzyme in polyamine synthesis, decarboxylates ornithine to synthesize putrescine. Spermidine synthase and spermine synthase catalyze the addition of aminopropyl groups to putrescine and spermidine to synthesize spermidine and spermine, respectively. Aminopropyl groups are donated by decarboxylated S-adenosylmethionine (dcSAM), which is converted from *S*-adenosylmethionine (SAM) by the enzymatic activities of adenosylmethionine decarboxylase (AdoMetDC).

The degradation pathway of polyamines is also elucidated (Figure 2). Spermidine/spermine N-(1)-acetyltransferase (SSAT) and N1-acetylpolyamine oxidase (APAO) are enzymes that break down SPM and SPD in cells. SSAT is a highly inducible enzyme that catalyzes the transfer of acetyl groups from acetyl-coenzyme A to the terminal amines of SPM and SPD. APAO catalyzes the oxidation of N1-acetylspermine and N1-acetylspermidine produced by SSAT activity, releasing aldehyde and hydrogen peroxide to produce SPD and PUT, respectively. The resulting aldehyde, 3-acetamidopropanal, has been shown to have no cytotoxic activity [8]. This degradation pathway, along with a mechanism for transporting polyamines across the cell membrane called the polyamine transporter, exists to maintain homeostasis, which keeps the intracellular concentration of polyamines constant.

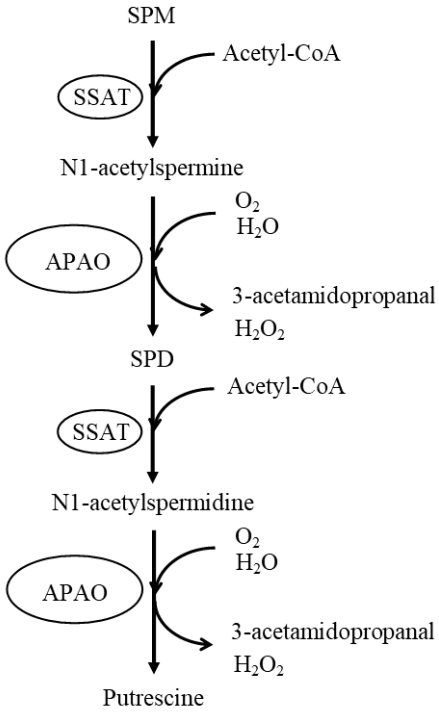

Figure 2. Polyamine Degradation Pathway Under Normal Conditions. SPM and SPD are converted to N1-acetylspermine and N1-acetylspermidine, respectively, by the enzymatic activity of SSAT. N1-acetylpolyamine oxidase (APAO) preferentially catalyzes the oxidation of N1-acetylspermine and N1-acetylspermidine to SPD and putrescine, respectively. This degradation process produces non-toxic 3-acetamidopropanal. Abbreviations used in the Figure 2, SPM; spermine, SPD; spermidine, SSAT; spermidine/spermine N-(1)-acetyltransferase, APAO; N1-acetylpolyamine oxidase.

The alternative polyamine degrading enzyme, spermine oxidase (SMO), can directly convert SPM back to SPD (Figure 3). SMO, a highly inducible enzyme, specifically oxidizes spermine. The enzymatic activity of SMO degrades spermine, producing 3-aminopropanal (3-AP) as a by-product. Produced 3-AP spontaneously deaminates to form acrolein [9]. Unlike the metabolite (3-acetamidopropanal) produced by the enzymatic activities of SSAT and APAO, both 3-AP and

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acrolein are substances with potent cytotoxic activities. In fact, SMO activation in the presence of SPM has been shown to cause severe damage to cells, supporting the strong cytotoxic activity of these two substances produced by SMO [10,11]. And, several pathologies in which SMO is activated have been reported [12–15], and acrolein is detected in the blood in such pathologies [16], indicating that enzymatic activities of SMO are activated in such conditions. Conversely, suppression of SMO activity can ameliorate disorders caused by pathological conditions [17].

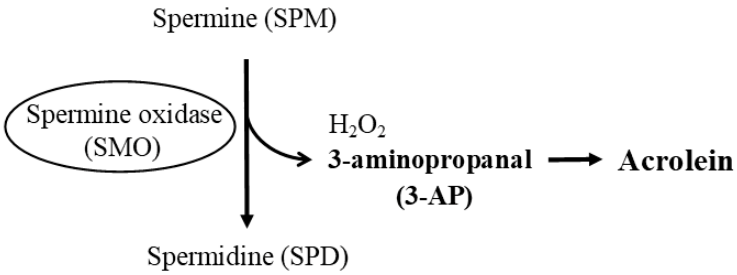

Figure 3. Polyamine Degradation by Spermine Oxidase (SMO). SMO, a highly inducible enzyme expressed in macrophages and epithelial cells, directly converts SPM back to SPD. SMO degrades SPM and produces 3-AP as a by-product. Generated 3-AP is spontaneously deaminated to form acrolein. Both 3-AP and acrolein are highly toxic and exhibit cytotoxic activity. Abbreviations used in the Figure 3, SPM; spermine, SPD; spermidine, SMO; spermine oxidase, 3-AP; 3-aminopropanal.

3. The Most Basic and Important Aspects of Conducting Polyamine Research

Recently, several researchers have postulated that spermidine activates autophagy function and that its bioactivity is the mechanism by which increased polyamine intake promotes longevity [2–5]. However, these studies used medium supplemented with fetal bovine serum (FBS) to perform polyamine experiments. These research methods were conducted without a good understanding of the properties of polyamines due to a lack of awareness on the part of the researchers, and many similar research results and misinterpretations have been seen in the past. Importantly, ruminant serum contains a copper-containing amine oxidase called bovine serum amine oxidase (BSAO). This enzymatic activity is not observed in humans or non-ruminants.

It has been known for about 70 years that polyamines are cytotoxic when added to cell cultures mixed with FBS [18]. Cytotoxic substances are produced during the conversion of spermine and spermidine catalyzed by copper-containing amine oxidase in FBS, i.e., BSAO. This enzymatic activity does not disappear even in serum deactivated by heat treatment. BSAO catalyzes the oxidation of SPM and SPD to produce SPD and putrescine, respectively, while simultaneously producing acrolein as a by-product (Figure 4). As described above, the cytotoxic activities of acrolein are very potent. Therefore, when cells are cultured with polyamines, even at low concentrations, in culture medium supplemented with FBS, acrolein provokes strong cytotoxic activity and kills cultured cells or at least impairs cellular function.

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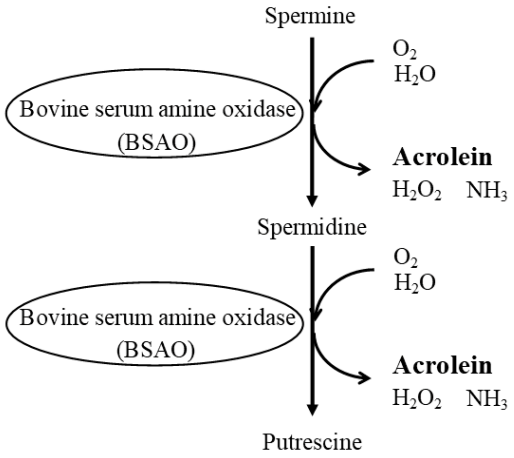

Figure 4. Polyamine Degradation Pathway by Fetal Bovine Serum Amine Oxidase. BSAO in ruminant sera such as bovine serum catalyzes the oxidation of spermine and spermidine to spermidine and putrescine, respectively. The acrolein produced in this process is very toxic and can cause cell damage and death. Abbreviation used in the Figure 4, BSAO; bovine serum amine oxidase.

This means that SPD is degraded to produce acrolein in the culture medium, but the production of acrolein by SPD degradation has not been confirmed *in vivo*. In addition, while SMO breaks down one molecule of SPM to produce one molecule of acrolein, BSAO converts one molecule of SPM to one molecule of acrolein and one molecule of SPD, which in turn breaks down the SPD produced to produce one molecule of acrolein. Simply put, SPM should be at least twice as cytotoxic as SPD in FBS-supplemented medium. And, it has been reported that treatment with SPM in culture supernatant supplemented with BSAO is more toxic than SPD treatment [19]. Therefore, when cultured with SPD, the autophagic function is activated by relatively mild cytotoxic activity and the cells do not die. However, it is easy to speculate that the addition of SPM at the same concentration as SPD will kill most of the cultured cells due to increased cytotoxic activity, thus eliminating the means to determine the activation of intracellular autophagy. I suspect this is the reason why autophagy activation has been reported by SPD, which has lower biological activities in humans, but not by SAPM, which has strong biological activities and plays important roles in humans.

At this time, when the existence of enzymes that degrade SPD to produce acrolein is unknown, it can be said that at least an examination of the biological activity of SPD in culture medium mixed with FBS containing BSAO is not an examination of the biological activity that occurs *in vivo* in humans. Applying the reaction that occurs under these culture conditions to polyamine activity in vivo, the phenomenon is attributed to SPM degradation due to SMO activation induced by certain pathological conditions or the presence of inflammation (Figure 5). The finding of significant age-related increases in autophagy markers in the aged kidney suggests a compensatory activation of basal autophagy in response to the increased cytotoxic activities with aging [20] and supports an increase in various stimuli, such as chronic inflammation, with aging and the activation of autophagy responses induced by them. Spermine and spermidine themselves do not activate autophagy, indicating that the increase in autophagy markers upon addition of SPD to cultured cells is only observed in the presence of BSAO [19]. Scientists need to know what is described above in order to study the activity of polyamines *in vitro*. We have constantly used SAO-free proteins such as human serum in our experiments. Alternatively, when using a culture medium mixed with bovine serum, researchers should mix a compound that inhibits SAO activities and study the function of polyamines [19]. In this case, however, it is important to remember that the activity of the compound itself must also be considered.

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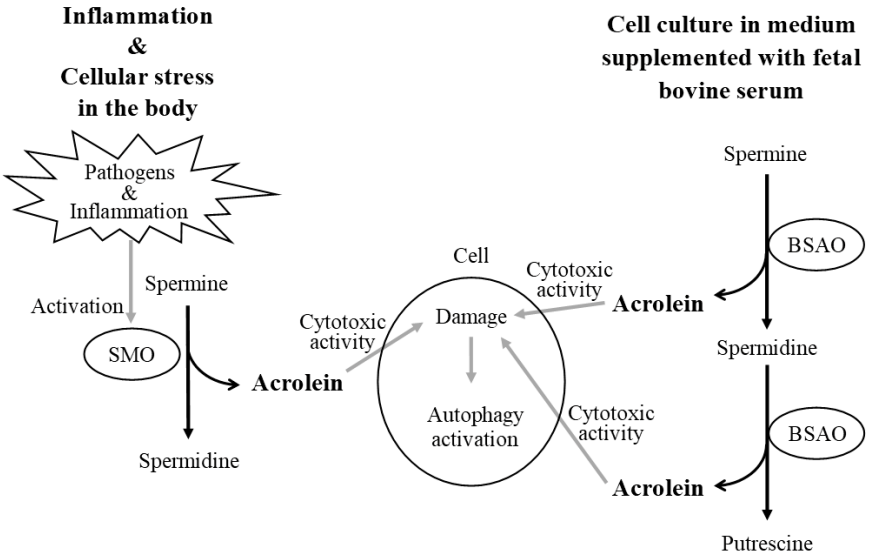

Figure 5. Similarities between cells cultured in bovine serum medium *in vitro* (figure right) and cells under cytotoxic stress such as inflammation *in vivo* (figure left) **Figure Left:** BSAO in bovine serum degrades both spermine and spermidine in the culture medium to produce acrolein. Heat treatment to deactivate the serum does not eliminate the enzymatic activity of BSAO. In response to the potent cytotoxic activity of acrolein, cells have been shown to activate autophagy. Spermine and spermidine themselves have no cytotoxic activity and therefore do not activate autophagy. **Figure Right:** Inflammation and certain pathogens activate SMO, and SMO exclusively breaks down spermine and produces acrolein via the production of 3-AP. The background of autophagy activation observed in cell cultures mixed with bovine serum containing BSAO is similar to the background of autophagy activation observed in cells from the elderly and patients suffering from age-related diseases induced and promoted by chronic inflammation. Abbreviations used in the Figure 5, SMO; spermine oxidase, BSAO; bovine serum amine oxidase, 3-AP; 3-aminopropanal.

Polyamines are involved in various cellular functions such as transcription, RNA modification, protein synthesis, and regulation of enzyme activity, and exist in association with DNA, RNA, and various protein molecules. As summarized in my previous review article [6], a high percentage of all polyamines are bound by ionic interactions to nucleic acids, proteins, and other negatively charged molecules in the cell. Therefore, very few polyamines are free in the body. According to a study by Igarashi et.al., only 2-5% of SPM and 7-15% of SPD are present as free polyamines in tissues and organs (as cited in [6]). In addition, the majority of polyamines in circulating blood are found in blood cells, particularly in red and white blood cells. In plasma, Cooper et.al. reported that SPM and SPD are both present at concentrations of only about 1.0% of whole blood (as cited in [6]). When measuring polyamine concentrations in serum or plasma by HPLC, polyamine peaks, especially SPM peaks, can be difficult to detect due to the very low levels [21]. If the concentration of polyamines, especially SPM, in serum or plasma is not high, HPLC may detect only a shimmer of the baseline or no peaks at all. Therefore, determining polyamine concentrations from an uncertain peak is difficult to measure accurately, especially with respect to SPM concentrations. Many recent papers often measure serum or plasma concentrations and do not mention or describe well-considered SPM concentrations, suggesting that this is due to the above problems preventing adequate studies. In addition, it is important to remember that any amount of hemolysis will release large amounts of polyamines present in the blood cells into the fluid component of the blood, which will significantly distort the measurements.

We have measured polyamine levels in whole blood for the above reasons. Measuring polyamines in whole blood requires instrumental adjustments and a somewhat complicated procedure, but we believe it is essential for accurate concentration measurements.

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4. Age-related Changes in Polyamine Concentrations

The relationship between aging and polyamine levels has already been summarized in my previous review article [6]. During fetal and developmental stages, polyamine synthase is highly activated, but its activity gradually declines with age (as cited in [6]). From this, it can be inferred that polyamine levels decrease with age. In fact, as described primarily in the titles and abstracts of the papers, when the relationship between age and polyamine concentrations is examined in all age groups, including children, blood polyamine levels decrease with age [22].

However, the age-related decline in polyamine concentrations is observed during early life (fetal or developmental period). According to a study by Nishimura et.al., polyamine levels in various tissues and organs of the mice did not differ between 10-week-old and 26-week-old mice, except in the skin (as cited in [6]). In human autopsied brain, Morrison et.al. reported that both putrescine and SPM did not change with age (from 1 day to 103 years), whereas SPD increased markedly from birth, reached a maximum at 40 years of age, and was maintained into old age (as cited in [6]). In addition, Dezortova et.al. and Rui et.al. reported that, in animal and human semen and some organs, SPD levels do not tend to decrease and have been reported to increase with age (as cited in [6]). Blood circulating throughout the body reflects polyamine concentrations in organs and tissues, but there does not appear to be a decrease in blood polyamine concentrations with aging. According to a study by Elworthy et al, blood polyamine concentrations did not show significant age-related changes (as cited in [6]). Similarly, no age-related decline in plasma polyamine concentrations has been reported by Chaisiri et.al (as cited in [6]). We also found that whole blood polyamine levels do not change with age [23,24]. Urinary polyamine excretion, which reflects blood polyamine levels, also does not change with age in adults. According to a study by van den Berg, an age-dependent decrease in polyamine excretion was observed during the first year of life, but no age-dependent decrease in urinary excretion of SPD was observed in older age groups (as cited in [6]). Similarly, Yodfat et.al. reported that there was no age-related decrease in polyamine excretion in 171 male and 166 female healthy volunteers ranging in age from 14 days old to 84 years old (as cited in [6]).

Although there is no decrease in polyamine levels in the body with age, it is noteworthy that there are large individual differences in blood polyamine levels [23,24]. It is not clear what the biological basis is for the large individual differences in blood polyamine levels. However, this large individual variation in polyamine concentrations is an aspect that makes the clinical application of polyamines difficult. It is well known that in cancer patients, polyamines, which are synthesized in large amounts by cancer cells, are transferred into the blood, resulting in elevated blood polyamine levels. Therefore, attempts have been made to diagnose the presence of cancer based on differences in polyamine levels, but large individual differences have made clinical application difficult. When considering the clinical application of polyamine concentration as an indicator, it is essential to study it in many clinical cases. Otherwise, large individual differences in polyamine concentrations would result in very different analysis results depending on case selection. For example, one group of investigators reported that cognitive decline correlated with low serum SPD levels [22], while another group reported that the more severe the cognitive decline, the higher the serum SPD [25].

5. Age- and Disease-related Changes in the Ratio of Spermine to Spermidine

Changes in polyamine concentrations with age are not pronounced and do not decrease with age. On the other hand, the SPM/SPD ratio tends to decrease because SPM concentration tends to decrease with age [23,26,27]. And this decline tends to be more pronounced in patients with age- and lifestyle-related diseases [23,26]. In particular, it has been reported to be more pronounced in patients with renal failure. SPD concentrations in erythrocytes were found to be significantly higher in patients with advanced renal failure who were not on hemodialysis compared to healthy subjects. In these patients, erythrocyte SPM concentrations were unchanged as compared to healthy subjects, resulting in a lower SPM/SPD ratio [28].

Similar changes in polyamine levels, i.e. a decrease in the SPM/SPD ratio and/or an increase in SPD levels, have been reported in other age-related diseases such as cerebral infarction, neurodegenerative diseases and sarcopenia [26,27,29,30]. In patients with neurodegenerative

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diseases such as Alzheimer's disease, SPD levels were elevated in the frontal and parietal lobes of the brain [31]. Plasma concentrations of putrescine and SPD increased in stroke patients, while SPM concentrations remained unchanged, resulting in a significant decrease in the SPM/SPD ratio [32]. Similarly, it has been reported that the blood SPM/SPD ratio of Parkinson's disease patients is lower than that of healthy subjects, and that the age-related decline in the SPM/SPD ratio occurs at a younger age and is more pronounced than in healthy subjects [26]. We also found that whole blood SPD levels were higher and SPM/SPD ratios were lower in sarcopenic patients than in non-sarcopenic subjects. And the SPM/SPD ratio tended to decrease with age in sarcopenic patients, while no such decrease was observed in non-sarcopenic elderly [23].

Chronic inflammation has been implicated in the background of these age-related chronic diseases [33]. This means that in patients with these diseases, inflammation-induced increases in SMO activity and 3-AP or acrolein levels should be noted. In fact, urinary acrolein levels were significantly higher in patients with diabetes mellitus than in those without [34]. An increase in plasma acrolein concentration and SMO activity has been observed in patients with chronic renal failure, such as diabetic nephropathy, chronic glomerulonephritis and nephrosclerosis [35]. In patients with cerebrovascular disease, SMO was activated [17,32] and plasma acrolein levels were elevated [36,37]. Plasma acrolein levels were higher in patients with rheumatoid arthritis than in healthy individuals [38]. At the same time, in patients with brain damage, increases in plasma acrolein concentrations were associated with increases in interleukin-6 and C-reactive protein [37], suggesting a close relationship between inflammation, SMO activation and acrolein production.

Chronic inflammation has been implicated in the onset and progression of several age- and lifestyle-related diseases as well as protein-energy depletion leading to cardiovascular disease and sarcopenia [39–41]. In addition, the presence of chronic inflammation was a strong predictor of poor outcomes in dialysis patients [42]. In light of these scientific facts, the cytotoxic activity of acrolein, which results from the degradation of SPM by activated SMO in the presence of inflammation, may contribute to the development and progression of these diseases and the prognosis of patients. [43,44]

As noted above, in diseases with a background of chronic inflammation, SPM is degraded, resulting in a lower SPM/SPD ratio. Therefore, the SPM/SPD ratio may have clinical application as a predictor of the onset and progression of age- and lifestyle-related diseases. At the same time, however, the SPM/SPD ratio in the blood, as well as the concentrations of SPM and SPD, vary widely among individuals, and it may be difficult to determine the risk of disease development and progression with a single blood test. However, it may be possible to assess the risk of disease onset and determine disease severity by tracking the SPM/SPD ratio over time in the same individual.

6. Polyamines as a Nutritional Aspect

Considering that the main source of polyamines is thought to be the gastrointestinal tract, i.e. polyamines in food and polyamines synthesized by intestinal bacteria are important, and that cells can take up extracellular polyamines, it is likely that polyamine levels in the body are affected by food intake and the state of intestinal bacteria. In fact, many studies have shown that reducing polyamine intake, as well as inhibiting the activity of gut bacteria with antibiotics, reduces blood polyamine levels [45,46]. Conversely, there has been little experimentation on how polyamine delivery to the gastrointestinal tract affects polyamine levels in the body, but we were the first to find that high polyamine intake increases blood polyamine levels [47,48]. However, it has also been found that a diet high in polyamines does not affect body concentrations in the short term [24,48,49].

When SPD was mixed with drinking water and administered to mice, an increase in blood SPD levels was reported [2]. SPM concentrations also appear to have increased in the figure, but specific data are not shown. Interestingly, our first preliminary experiments with a small number of mice also showed a significant increase in SPD. However, this is not a study that followed changes in concentration in individual animals, but rather a population study with a small number of animals, so it is difficult to deny that the study captured differences due to chance selection of cases. In fact, the standard deviation of the mean blood concentration was extremely large, and the individual differences in concentration appeared to be extremely large.

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We found that when mice were fed a diet containing synthetic polyamines and a polyamine concentration about three times higher than that of soybeans for a long period of time, the blood SPM concentration gradually increased, with a significant difference at the 25th week of feeding [1]. Blood SPD levels also increased in some animals, resulting in a slight increase in mean SPD, but individual differences in SPD concentrations were increased and were not significant. The effects of large differences in polyamine concentrations have a common background in that the choice of cases to study in relation to the disease can yield quite opposite results [22,25].

The effects of a high polyamine diet have also been studied in humans. However, no short-term studies have confirmed changes in blood polyamine levels with a high polyamine diet. Results of studies using high polyamine supplements have also been reported [49]. There is a 12-month study of supplementation in elderly patients between the ages of 60 and 90. This study showed a 10-20% increase in polyamine intake over normal dietary polyamine intake, yet blood SPD levels did not change at all [49]. Thus, if there are clinical changes after a high SPD diet, it is not at all clear whether they are due to SPD or to other components taken at the same time [50]. Specific and detailed data on changes in SPM concentrations are not clear in the report, but the figure in the paper shows a slightly increasing trend in SPM concentrations.

We conducted a study of long-term, high-concentration polyamine diets in humans [24]. The results showed that SPM levels gradually increased, with a significant difference in SPM levels after one year. In this study, natto (fermented soybeans), a traditional Japanese food, was used and the subjects were given almost the same amount of polyamines as found in their regular diet. In other words, the amount of polyamine intake was almost double that of the regular diet. And our reported bioactivity of spermine, i.e., suppression of lymphocyte function-associated antigen 1 (LFA-1) expression on immune cells, was confirmed in association with changes in SPM concentration [24]. Namely, changes in each individual's blood SPM concentration were negatively correlated with changes in LFA-1 expression. The relationship between polyamines and LFA-1 is discussed in the section "7. Biological Activity of Polyamines in Human Health and Disease".

Because polyamines are absorbed from the gastrointestinal tract without being broken down, and because many foods generally contain more SPD than SPM, it was thought that a diet high in polyamines would increase blood SPD. However, although limited research can be confirmed, there is very little evidence that continuous consumption of a high polyamine diet increases blood levels of SPD. Instead, the concentration of SPM seems to increase after long-term continuous consumption of a high polyamine diet, although there are individual differences.

7. Biological Activity of Polyamines in Human Health and Disease

Polyamines have been reported to be associated with a variety of pathologies, while they are known to possess many biological activities that may counteract age-related conditions and senescence [6]. For example, they have anti-inflammatory and antioxidant properties [51,52] and protect cells and genes from damaging stimuli such as ionizing radiation, ultraviolet rays, toxic chemicals, and other stresses [6]. And some researchers, including us, have reported that increasing polyamine intake extends the lifespan of animals [1,5,53]. However, polyphenols, which have bioactivities similar to polyamines, such as protection of cells and genes from harmful stimuli, anti-inflammatory effects, antioxidant activity, etc., have not been shown to extend the life span of animals.

Anti-inflammatory effects of polyamines include suppression of proinflammatory cytokine production by immune cells upon stimulation and suppression of LFA-1 expression on the cell membrane [51,52]. Increased LFA-1 protein causes immune cells to respond to even minor stimuli, triggering the production of proinflammatory cytokines and provoking inflammation. SPM has strong physiological activity and therefore shows anti-inflammatory activity over a range of physiological concentration changes. SPD also has similar biological activity to SPM, but required a concentration change well beyond the physiological concentration change to confirm the effect [51,52]. Furthermore, the suppression of LFA-1 expression on immune cells by SPM is specific, and

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in our study, of the many cell membrane molecules we examined, including adhesion molecules, most cell membrane molecules were unaltered, but LFA-1 was suppressed [51].

The amount of LFA-1 has been found to be related to the methylation status of the ITGAL where the gene for LFA-1 is encoded. Increased levels of LFA-1 protein on immune cells with aging are associated with the progressive demethylation of ITGAL [54,55]. And, in our *in vitro* experiments, demethylation of ITGAL was associated with an increase in the amount of LFA-1 protein on immune cell membranes, and conversely, hypermethylation of ITGAL was associated with a decrease in LFA-1 protein levels [56].

Gene methylation is a change that occurs only in cytosine, one of the four bases that make up a gene information, and is a mechanism that alters the reading of genetic information by adding or removing methyl groups from cytosine. In front of the genetic information, there is a site called the CpG land, which contains repeated sequences of cytosine and guanine. Methylation of cytosines within the CpG island results in decreased transcription and consequently decreased production of the protein encoded by the gene. Conversely, when cytosines within the CpG island are demethylated, transcription is more likely to occur, resulting in increased synthesis of the protein encoded by the gene. DNA methylation is regulated by DNA methyltransferases (DNMTs). DNMTs control the methylation state of cytosines by using methyl groups provided by SAM (Figure 6). The methyl group donor, SAM, on the other hand, is converted to decarboxylated S-adenosylmethionine (dcSAM) by S-adenosylmethionine decarboxylase (AdoMetDC). In the synthesis of SPD and SPM, aminopropyl groups are provided by dcSAM. dcSAM is a potent inhibitor of DNMT, and as dcSAM increases, DNMT activity decreases [57,58]. It has also been reported that there is an inverse correlation between the dcSAM/SAM ratio and DNMT activity [59]. Reduced DNMT activity results not only in demethylation due to a decreased ability to donate a methyl group to cytosine, but also in progressive demethylation at one site and hypermethylation at another, resulting in aberrant methylation of the entire genome [56,60,61]. We found that a decrease in DNMT activity increases demethylation of the ITGAL region, but at the same time creates a genome-wide condition called aberrant methylation, in which demethylation and methylation occur at different sites in the gene. Conversely, when DNMT is activated, the entire genome is no longer aberrantly methylated and ITGAL is now highly methylated [56] (Figure 6).

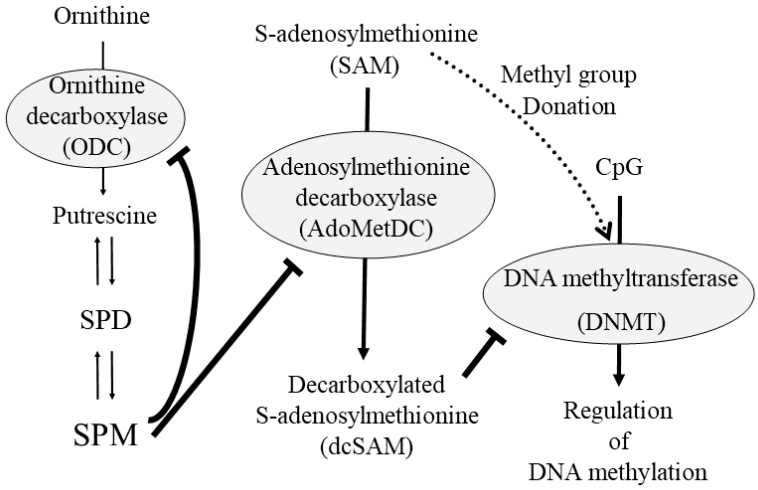

Figure 6. Close Relationship Between Polyamine Metabolism and Gene Methylation. SPM inhibits the polyamine synthesis enzymes, ODC and AdoMetDC. dcSAM inhibits DNMT activity. When dcSAM is reduced, the inhibitory effect of dcSAM on DNMT is reduced and DNMT is activated to regulate gene methylation. Abbreviations used in the Figure 6, SPM; spermine, SPD; spermidine, ODC; Ornithine decarboxylase, AdoMetDC; Adenosylmethionine decarboxylase, dcSAM; decarboxylated S-adenosylmethionine, DNMT; DNA methyltransferase.

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The decrease in the activity of ODC due to aging and the decrease in concentration of SPM due to degradation by chronic inflammation cause the activation of AdoMetDC, resulting in the production of more dcSAM and the decrease in the activity of DNMT, which is repressed by dcSAM, leading to aberrant methylation of the entire gene and demethylation of ITGAL. The degradation of SPM by SMO leads to the formation of acrolein, which has potent cytotoxic activity, and at the same time, coupled with increased LFA-1 protein levels due to demethylation of ITGAL in association with aberrant methylation of the entire genome, induces a proinflammatory state and induces inflammation and cellular damage. Conversely, SPM, which is increased by a diet high in polyamines, suppresses the activity of AdoMetDC and increases the supply of methyl groups to genes due to its strong physiological activity (negative feedback mechanism). As a result, DNMT is activated and the methylation status of the entire genome is regulated. At the same time, ITGAL is hypermethylated, and LFA-1 protein levels decrease. That is, the increased polyamine intake acts to inhibit aberrant methylation of the entire genome, protecting cells and genes from harmful stimuli, and suppressing proinflammatory state. The opposite occurs in diseases caused by chronic inflammation.

8. Conclusions of the Review and Issues to be Addressed

In the previous half of this review, I provided basic notes on polyamine research that researchers need to understand. In addition, I outlined the possibility that changes in blood polyamine concentrations, particularly the SPM/SPD ratio, may be an indicator of the onset and progression of age- and lifestyle-related diseases, as well as the physiological activity of polyamines that may underlie the effect of increased SPM concentration on lifespan extension due to continuous consumption of a high polyamine diet. (Summery Figure).

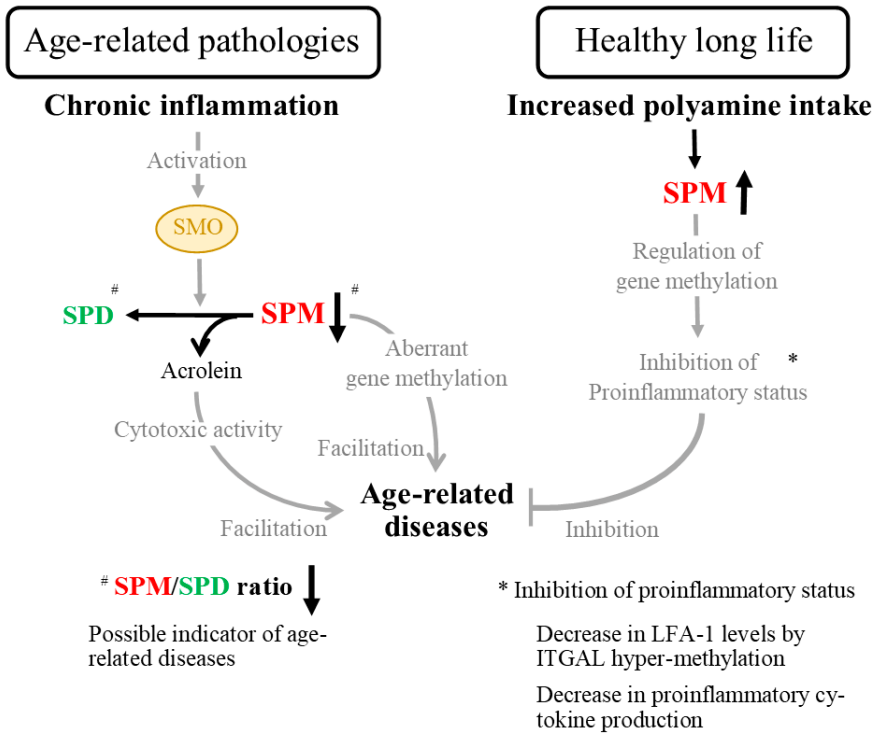

Figure 7. Polyamines and the Background of Healthy Longevity and Age-Related Diseases **Figure Left:** Chronic inflammation, thought to be a major underpinning of age- and lifestyle-related diseases, activates a highly inducible enzyme, SMO. SMO breaks down SPM but not SPD. Thus, in an inflammatory environment, SPM decreases. Because SPM has stronger bioactivities than SPD, the result is a loss of methylation control, leading to aberrant methylation of the entire genome. In addition to the loss of inhibitory effects of SPM on inflammatory cytokine production, aberrant gene methylation is associated with increased de-methylation of ITGAL, a LFA-1 protein gene.

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Demethylation of ITGAL increases LFA-1 protein levels, resulting in an increased proinflammatory status. In addition, a highly toxic substance, acrolein, produced by the enzymatic activity of SMO, exerts strong cytotoxic activity, and consequently impairs cellular function. **Figure Right:** On the other hand, blood SPM is increased by continuous consumption of a high polyamine diet. SPM has a strong effect on the regulation of gene methylation, thereby inhibiting aberrant gene methylation. Controlling the methylation status of the entire genome is associated with increased methylation of the LFA-1 promoter region, resulting in a decreased LFA-1 protein levels. In addition to the inhibition of proinflammatory cytokine production by SPM, decreased LFA-1 protein suppresses the age-related increase in proinflammatory status. Based on the accumulated evidence, the blood SPM/SPD ratio may be an indicator of healthy longevity and the onset and progression of age-related diseases. I believe that regulation of gene methylation and suppression of chronic inflammation are key to controlling age-related diseases. Abbreviations used in the Summery Figure, SPM; spermine, SPD; spermidine, SMO; spermine oxidase, LFA-1; lymphocyte function-associated antigen 1.

There are several things that need to be resolved in this area. One is the cause of individual differences in polyamine concentrations. Intracellular polyamine concentrations are strongly influenced by extracellular supply. The largest source of this supply is the gastrointestinal tract. Therefore, it is necessary to investigate which elements of the gastrointestinal tract cause differences in polyamine supply. Second, it is primarily spermine that is increased by a polyamine-rich diet, even though the diet normally contains high levels of spermidine. It is necessary to investigate which factors in the intestinal environment promote spermine synthesis as a possible cause of individual differences in polyamine concentrations. It would be necessary to investigate which factors in the intestinal environment increase spermine supply, as well as the causes of individual differences in polyamine concentrations. It is interesting to note that most intestinal bacteria can only synthesize up to spermidine. We consider the regulation of gene methylation and the associated anti-inflammatory effects to be the background of polyamine-induced healthy longevity, but what other mechanisms might contribute?

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