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Polyamine-rich food decreases age-associated pathology and mortality in aged mice

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ABSTRACT

The purpose of this study was to test whether oral intake of foods rich in polyamines (spermine and spermidine) suppresses age-associated pathology in aged mice. Synthetic polyamines were mixed into experimental chows, and 24-week-old Jc1:ICR male mice were fed one of three chows containing differing polyamine concentrations. The spermine and spermidine concentrations in the low, normal, and high polyamine chows were 143 and 224 nmol/g, 160 and 434 nmol/g, and 374 and 1540 nmol/g, respectively. An increase in concentration of polyamine in the blood was found only in mice fed the high polyamine chow at 50 weeks of age. While the body weights of mice in all three groups were similar, the survival rate of mice fed high polyamine chow was significantly higher than those in the other two groups ($p = 0.011$). Mice fed the high polyamine chow analyzed at 88 weeks of age, corresponding to the end of the study, demonstrated lower incidence of glomerulosclerosis and increased expression of senescence marker protein-30 in both kidney and liver compared to those fed the low polyamine chow. As these pathological changes are associated with senescence, oral polyamine appears to inhibit the progression of age-associated pathologies.

1. Introduction

Lifestyle, especially food habits, is believed to inhibit the progression of age-associated diseases and prolong the lifespan of humans. Epidemiologic studies have suggested the relation between several foods and prolonged longevity (Hu and Willett, 2002; Renaud and Lanzmann-Petithory, 2001). Eating beans such as soy may help decrease the incidence of age-associated diseases, such as atherosclerotic plaque in arteries, and prolong longevity (Papanikolaou and Fulgoni, 2008; Sacks et al., 2006).

Beans, especially soybeans, have the highest amount of the polyamines (spermine and spermidine) present in natural foods (Bardócz et al., 1993; Okamoto et al., 1997). Because spermine and spermidine are not enzymatically degraded in the alimentary tract, oral spermine and spermidine are absorbed quickly from intestinal lumen and distributed to all organs and tissues (Bardocz et al., 1990, 1995). And, we recently found that long-term intake of polyamine-rich foods gradually increases blood polyamine levels in humans and animals (Soda et al., 2009).

Polyamines are indispensable for cell growth and differentiation and have important roles in cellular physiology. In addition, previous studies have demonstrated that the polyamines, spermine and spermidine, suppress inflammatory mediators, such as pro-inflammatory cytokines, both *in vitro* and *in vivo* (Soda et al., 2005; ter Steege et al., 1999; Zhang et al., 1997). And they also suppress the expression of lymphocyte function-associated antigen-1 (LFA-1) on the surface of peripheral blood mononuclear cells (PBMCs) in humans and inhibit LFA-1 associated cellular functions (Kano et al., 2007; Soda et al., 2005). The increase in expression of LFA-1, an adhesion molecule with a crucial role in inflammation (Wacholtz et al., 1989), on human PBMCs with aging may contribute to the progression of age-associated diseases (Franceschi et al., 2000; Soda et al., 2005). Recent studies have shown the involvement of inflammation in the pathogenesis of many age-associated diseases (Franceschi et al., 2000). Anti-inflammatory substances and drugs seem to inhibit pathogenesis of these diseases (Demierre et al., 2005). In this study, we examined the effect of oral polyamines on age-associated pathology and the longevity of mice.

2. Materials and methods

2.1. Animals

The experimental design was approved by the Institutional Review Board of Jichi Medical University, and all procedures performed on animals followed the Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985). Eight-week-old

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male Jc1:ICR mice, purchased from Saitama Doubutsu (Saitama, Japan), were used. The mice (4–5 per cage) were fed Rodent Laboratory Chow (Nippon Bio-Supply, Tokyo, Japan) and water *ad libitum* and allowed to adapt to the laboratory environment for approximately 2 weeks prior to initiation of experiments. The animals were then randomly divided into three groups (fed chow containing either high polyamine, normal polyamine, or low polyamine), housed in a temperature-controlled (22 °C) cage supplied with high efficiency particulate-arresting (HEPA; 0.3 μm) filtered air, and maintained under a 12 h light/dark diurnal cycle. They were fed *ad libitum* Rodent Laboratory Chow until 24 weeks of age, and fed thereafter the same chow supplemented with one of three different polyamine concentrations.

For the measurement of blood polyamine levels, 30 mice per group were bred. Survival was measured and pathology examinations conducted in approximately 53 mice per group. One mouse in the low polyamine group died before the switch to experimental chow; therefore, there were 52, 53, and 53 mice in the low, normal, and high polyamine groups, respectively.

2.2. Chows

Ingredients of the experimental chows were prepared and mixed by Nippon Clea Co. (Tokyo, Japan) based on formulations used for standard laboratory chow (CE-2). In order to decrease polyamine content in the low polyamine chow from the existing standard chow, soy products in which polyamine concentration is high and that exist in large amounts in standard chow, were replaced by ingredients with relatively low polyamine concentration. Thus, casein was used instead of soy cake and lard was used instead of soybean oil. The composition and raw materials of the low polyamine chow are shown in Table 1.

The levels of polyamines, spermine and spermidine, were selected on the basis of the polyamine content in foods and subacute toxicity of polyamine (Bardócz et al., 1993; Okamoto et al., 1997; Sousadias and Smith, 1995; Til et al., 1997). High polyamine chow was prepared by adding synthetic spermine (Wako Pure Chemical Industries Ltd., Osaka, Japan), spermidine (Sigma Chemical. Co., St. Louis, MO), and putrescine, a diamine with two amino groups, to low polyamine chow (final concentration [w/w]: 0.015%, 0.06%, and 0.015%, respectively). Normal polyamine chow was prepared by adding spermine, spermidine, and putrescine to low polyamine chow (final concentration [w/w]: 0.002%, 0.008%, and 0.002%, respectively). Ingredients were mixed at 60 °C to prevent evaporation of polyamines. The mixture was pelleted, and the polyamine concentrations in the three chows were confirmed by high performance liquid chromatography (HPLC).

Table 1
Ingredients and nutrients of the experimental chows.[a]

	Low polyamine chow	Normal polyamine chow	High polyamine chow
Moisture (w/w, %)	7.6	7.6	7.6
Protein (w/w, %)	26.4	26.4	26.4
Fat (w/w, %)	10.2	10.2	10.2
Fiber (w/w, %)	2.5	2.5	2.5
Ash (w/w, %)	6.0	6.0	6.0
Nitrogen free extract (w/w, %)	47.3	47.3	47.3
Calorie (kcal/100 g)	397	397	397
Putrescine (nmol/g)	496	625	1075
Spermidine (nmol/g)	224	434	1540
Spermine (nmol/g)	143	160	374

Fat provides 23.1% of the total calories.
Polyamine concentration was measured by HPLC.
[a] Ingredients of the experimental chows were: milk casein, white-fish meal, yeast, wheat germ, lard, wheat bran, defatted rice bran, alfalfa meal, wheat meal, maize, milo, vitamin mixture (Retinol 0.81 mg, Vitamin B1 1.71 mg, Vitamin B2 1.30 mg, Vitamin B6 1.35 mg, Vitamin B12 7.65 μg, total Vitamin C 17 mg, Vitamin E 6.15 mg, Pantothenic acid 2.70 mg, Niacin 17.95 mg, Folic acid 0.26 mg, Choline 0.17 g, Biotin 45.4 μg, Inositol 547 mg in 100 g of chow), and mineral mixture (Ca 1.05 g, P 1.06 g, Mg 0.35 g, K 1.28 g, Mn 10.15 mg, Fe 30.08 mg, Cu 0.75 mg, Zn 5.18 mg, Na 0.45 g in 100 g of chow).

2.3. Data collection

For each mouse, body weight was determined every other week at 10 am, and survival was checked every other day. Food consumption was measured by subtracting the amount remaining from the amount provided three times a week, and total consumption was calculated at age 38, 44, and 50 weeks old.

Blood sampling was performed in mice for the determination of blood polyamine levels at the 8th, 16th, and 26th week after start of feeding with experimental chow. At the time of blood sampling, any diseased mice, such as those that appeared debilitated or with obvious tumor(s) on the body surface or in the organs and tissues, were excluded. Blood was drawn under anesthesia with pentobarbital (60 μg/g body weight) from the right atrium with a 1-ml syringe fitted with a 26 G needle. Whole blood samples were kept in EDTA-coated tubes at −80 °C until the assay was performed.

When mice reached 88 weeks of age, the survival study was concluded, and animals were euthanized to obtain organs and tissues. Only mice with no apparent tumors or pathological abnormalities on the body surface or in the viscera were selected, and six mice each from the high polyamine chow group and the low polyamine chow group were used for the histopathological and immunohistochemical evaluations. The kidney, liver, pancreas, and gastrocnemius muscle were evaluated histologically and immunohistochemically.

2.4. Determination of polyamine concentrations

Whole blood samples in EDTA-coated tubes were stored at −80 °C. To measure polyamine, each sample was twice thawed and sonicated for 5 min and centrifuged at 18,000*g* for 10 min. Each of the resulting supernatant (100 μl) was transferred to a new microfuge tube containing 20% trichloroacetic acid (100 μl) with 20 μM *N*-(3-aminopropyl) cadaverine as an internal standard. After centrifugation at 18,000*g* for 10 min, each supernatant was collected and stored at −20 °C until measurement by HPLC. The HPLC conditions were as follows: column, cation exchange resin, JEOL LC-R-2, 4.6 mm × 8 cm; elution buffer, a mixture of 12.5% (v/v) of methanol and 87.5% (v/v) of 0.28 M sodium citrate buffer, pH 5.5, containing 2.0 M sodium chloride; flow rate 0.5 ml/min; column temperature, 65 °C; post-column reagent solution, 6 mM *o*-phthaldialdehyde in 0.4 M potassium borate buffer, pH 10.4, containing 0.2% mercaptoethanol, 0.1% Brij 35; flow rate, 0.25 ml/min at 65 °C; fluorescence detection, excitation 345 nm, emission 450 nm.

2.5. Histological and immunohistochemical examinations

The tissues were collected and fixed in 10% formaldehyde, embedded in paraffin, and cut into serial 3-μm sections. One of the sections was stained with hematoxylin and eosin (H&E). Histological features in the high polyamine group were compared to those in the low polyamine groups and those in young mice (age, 20 weeks).

Sections were stained with a rabbit polyclonal antibody to rat SMP-30 (1:150; Senescence Marker Protein-30; Shima Laboratories, Tokyo, Japan), and the staining was visualized using a Chem-Mate Evision/peroxidase complex kit (DAKO Japan, Kyoto, Japan). Intensity of immunohistochemical staining was graded relative

to the level of background staining and quantified on a scale of 0–5 (Table 4).

The histological and immunohistochemical examinations were conducted by two veterinary pathologists, and their evaluations were peer reviewed.

2.6. Statistical analysis

Data are expressed as means ± SD. Comparisons of three groups of data were analyzed by the Kruskal–Wallis test, and comparisons among two groups of data were analyzed by the Mann–Whitney test. A p value less than 0.05 was considered to be statistically significant. The survival times were estimated using the Kaplan–Meier method. Comparison of survival between groups was tested using a generalized Wilcoxon analysis.

3. Results

3.1. Food consumption and changes in body weight

The amount of consumption of chow was different among the three groups of mice ($p = 0.043$, Kruskal–Wallis test). Average food consumption of mice fed the low polyamine chow was similar to that of mice fed normal polyamine chow (6.37 ± 1.08 g/day/mouse [low polyamine] vs. 6.94 ± 0.30 g/day/mouse [normal polyamine]; $p = 0.354$ by Mann–Whitney test). Mice in the high polyamine group (7.70 ± 1.11 g/day/mouse) consumed significantly more chow ($p = 0.031$: vs. low polyamine chow group, and $p = 0.047$: vs. normal polyamine chow group).

Nevertheless, the body weight in all three groups was similar (Fig. 1) from age 11 to 55 weeks.

3.2. Polyamine levels in mouse whole blood

After 8 and 16 weeks of consuming the experimental chows, the mean spermine concentrations in the blood from six mice per group did not significantly differ between the three groups (Table 2). On the 26th week of feeding high polyamine chow (when the mice were 50 weeks old), the mean blood spermine concentration increased with very wide inter-individual variability (range = 3.4–22.7 μmol/L). The mean spermine concentration from the high polyamine chow group tended to be higher, but not statistically different, from the normal polyamine group ($p = 0.085$) and the low polyamine group ($p = 0.058$) (Table 2).

Similar to blood spermine concentrations, the mean blood spermidine concentrations from six mice per group did not differ between the three groups after 8 and 16 weeks of consuming the experimental chows. On the 26th week of feeding, consumption of high polyamine chow increased blood spermidine concentrations in some animals and increased the variability of spermidine concentration (range = 29.1–100.3 μmol/L). The mean blood spermidine concentration in the high polyamine group was higher than that in the normal polyamine group ($p = 0.038$) and the low polyamine group ($p = 0.004$) (Table 2).

Table 2
Whole blood polyamine concentrations in mice after 8, 16, and 26 weeks of feeding with experimental chow.

Test diets	Low polyamine	Normal polyamine	High polyamine
Spermine concentration			
8 weeks ($n = 6$)	3.6 ± 1.2	4.3 ± 1.3	4.0 ± 2.0
16 weeks ($n = 6$)	4.9 ± 1.6	4.3 ± 1.1	5.2 ± 1.6
26 weeks ($n = 9$)	4.7 ± 1.5	5.2 ± 2.8	10.1 ± 7.1
Spermidine concentration			
8 weeks ($n = 6$)	33.8 ± 6.9	33.3 ± 9.1	41.5 ± 10.0
16 weeks ($n = 6$)	34.8 ± 6.4	34.6 ± 4.7	39.7 ± 5.1
26 weeks ($n = 9$)	31.2 ± 6.5	34.1 ± 6.6	52.7 ± 24.2

Blood polyamine concentrations were measured by HPLC. Data are presented as means ± SD of mice. n indicate the number of mice tested.

3.3. Survival

Mice in each group started to die around 50 weeks of age. The cause of death was not identified in all mice; however, high polyamine chow did not shorten survival (Fig. 2). Mice in the high polyamine group lived longer than mice in the other two groups ($p = 0.011$). Survival was significantly longer in the high polyamine group than in the low polyamine group ($p = 0.032$) and the normal polyamine group ($p = 0.003$). However, survival in the normal polyamine and low polyamine groups was similar ($p = 0.432$).

3.4. Histological and immunohistochemical findings

Pathological changes were found more predominantly in the experimental groups of mice than in younger mice (Table 3). When comparing the high polyamine group and the low polyamine

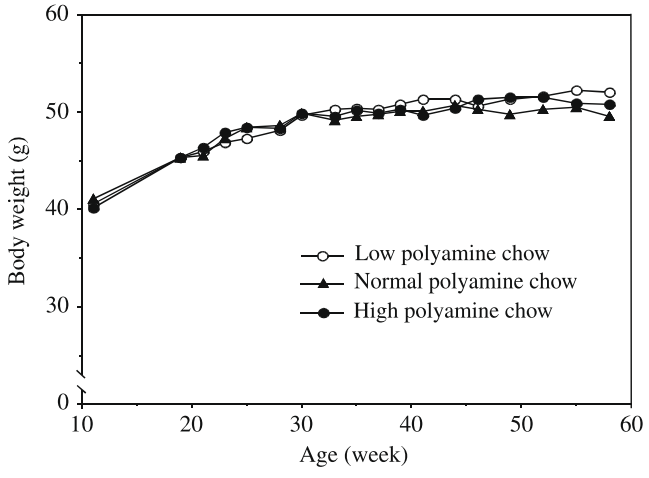

Fig. 1. Body weights of mice were similar among the three groups of mice. Mice were fed regular chow until they were 24 weeks old, and one of the experimental chows with three different polyamine concentrations thereafter. Each value indicates the mean body weight of mice. When mice reached 58 weeks of age, at which point some started to die, measurement of body weight was terminated in order to reduce strain on the mice.

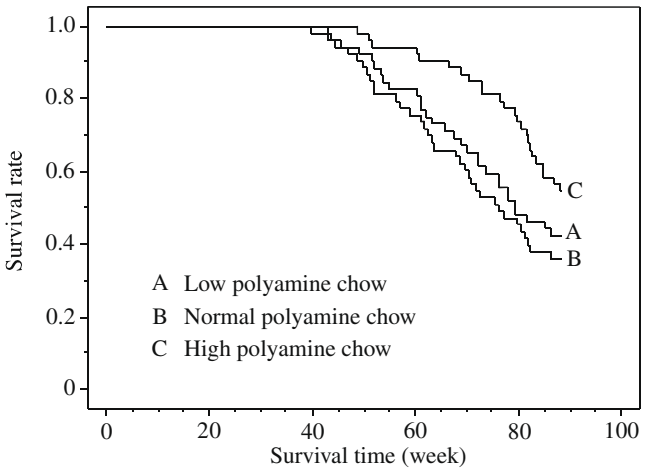

Fig. 2. High polyamine chow prolonged murine longevity. After age around 50 weeks, survival was significantly higher in the high polyamine group than in the other two groups.

Table 3
Histological evaluations of organs and tissues.

Experimental group	Low polyamine	High polyamine	Young (20 weeks old)
Pathological changes			
Kidney			
Glomerular atrophy	4/6	0/6	0/6
Interstitial fibrosis	4/6	5/6	1/6
Liver			
Fatty change	2/6	3/6	0/6
Spotty necrosis	1/6	2/6	0/6
Congestion	2/6	0/6	0/6
Cellular atrophy	4/6	6/6	0/6
Pancreas			
Periductal fibrosis	6/6	6/6	2/6
Fatty change	3/6	4/6	0/6
Langerhans proliferation	6/6	5/6	0/6
Muscle			
Fatty change	3/6	5/6	2/6
Fiborosis	2/6	1/6	0/6

Numerators indicate the number of mice with apparent respective pathology. Denominators indicate the total number of mice examined.

group, a significant difference was found in the progression of the glomerulosclerosis in the kidney. Global glomerular atrophy was found in the low polyamine group but not in the high polyamine group (Fig. 3).

Immunohistochemical examination showed SMP-30 expression mainly in the proximal tubular epithelium and to a lesser extent in the distal and collecting tubular epithelium of the kidney. In the liver, SMP-30 was mainly present in hepatocytes of the centrilobular and midlobular areas. SMP-30 expressed in the liver and kidney was less in the experimental groups than in young mice. SMP-30 tended to be expressed more strongly in the high polyamine group compared to the low polyamine group. The most significant difference in SMP-30 staining was observed in the nucleus of the distal collecting tubular epithelium ($p = 0.047$) and to a lesser extent, in the peri-lobular area of liver ($p = 0.056$) (Table 4 and Fig. 3).

4. Discussion

The enzymatic activities of spermine/spermidine synthases (which catalyze the synthesis of spermine from spermidine and

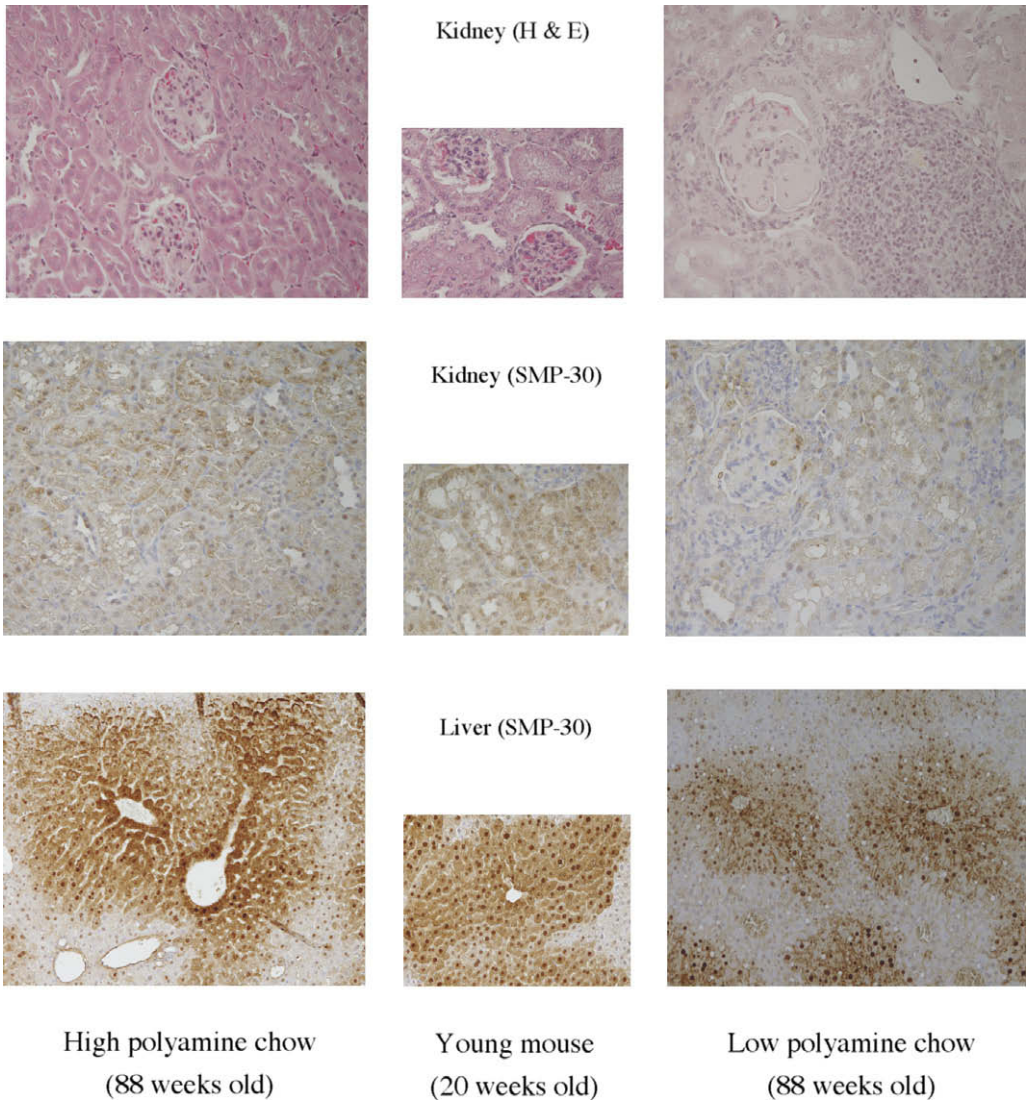

Fig. 3. Histological features. Upper: (Kidney, H&E stain) Glomerulosclerotic changes were more prominent in kidneys in the low polyamine group than the high polyamine group and were absent in young mice (age, 20 weeks). Middle: (Kidney, SMP-30) The intensity of cytoplasmic staining of SMP-30 protein (brown) in the proximal and distal tubules was weaker in old mice and stronger in mice fed high polyamine chow than those fed low polyamine chow. Lower: (Liver, SMP-30) The intensity of SMP-30 staining in the liver was less in the low polyamine group than in the high polyamine group, but high in young mice. Original magnification 50×.

Table 4
Immunohistochemical staining of SMP-30 in kidney and liver.

Animal group	Low polyamine	High polyamine	Young (20 weeks old)
Kidney			
Distal collecting tubular			
Cytoplasm	2.17 ± 0.98	3.00 ± 1.10	3.00 ± 1.10
Nucleus	1.00 ± 0.63	2.67 ± 1.51	4.17 ± 0.41
Liver			
Peri-lobular area	1.50 ± 0.55	2.00 ± 0.00	2.67 ± 1.21
Central vein area	4.00 ± 0.89	4.33 ± 0.52	4.57 ± 0.52

The intensity of SMP-30 staining was quantified as follows: very strong, 5; strong, 4; moderate, 3; weak, 2; very weak, 1; no staining, 0. Values are means ± SD of six mice.

spermidine from putrescine) decrease gradually with aging and are not under regulatory and rate limiting control (Morgan, 1998). Therefore, intracellular *de novo* synthesis of polyamines, spermine and spermidine, decreases with aging (Das and Kanungo, 1982; Morgan, 1998). However, polyamines in foods have been shown to be absorbed quickly from the intestinal lumen and distributed to organs and tissues in the body (Bardocz et al., 1990; Uda et al., 2003). Our previous study on healthy volunteers has shown that polyamine-rich foods seem to have a greater influence over blood polyamine concentrations in the elderly compared to the young (Soda et al., 2009). In this study, the high polyamine rich chow slowly and gradually increased blood polyamine concentration as the mice aged. Spermine and spermidine are indispensable for cell growth and differentiation, and are involved in diverse functions such as DNA synthesis and stability, regulation of transcription, ion channel regulation, and protein phosphorylation (Childs et al., 2003; Kutuzov et al., 2005; Thomas and Thomas, 2001; Wallace et al., 2003; Williams, 1997). In addition, extracellular spermine and spermidine exhibit anti-inflammatory properties (Soda et al., 2005; Zhang et al., 1997). Inflammation is considered to be a factor accelerating the progression of age-associated pathologies (Franceschi et al., 2000). Thus, as we expected, spermine and spermidine from foods maintain cellular function in aged animals and helped decrease age-associated pathology and prolong longevity.

Increases in polyamine concentrations in the body are closely related to neoplastic growth (Russell, 1983). The overexpression of ornithine decarboxylase (ODC), the key enzyme for polyamine biosynthesis, and the resultant increase in polyamine concentrations have been shown to accelerate tumor promotion (Clifford et al., 1995; Hibshoosh et al., 1991; O'Brien et al., 1997). In animals bearing tumors or that have been initiated for neoplastic growth, polyamine intake accelerates tumor growth, and conversely, the restricted polyamine intake decelerates tumor growth and results in prolonged survival in animals with tumors (Quemener et al., 1994; Sarhan et al., 1992). However, most of these studies were conducted to test the effects of polyamines on existing tumors or on the growth of tumors after initiation of neoplastic growth. It has been shown that oncogenic transformation does not occur when normal cells are targeted for ODC overexpression (Clifford et al., 1995; Hibshoosh et al., 1991; O'Brien et al., 1997), and we have not found evidence that increased polyamine intake can promote oncogenic transformation in normal untreated animals. In the present study, we did not confirmed the exact numbers of neoplastic growths in mice. However, if polyamine-rich food increases neoplastic growths in mice, the survival rate of the high polyamine group would have been lower than that of the other two groups. In fact, increased polyamine intake did not shorten their survival time.

Increased caloric intake and overweight shorten lifespan and promote and accelerate age-associated diseases (Krauss et al., 1996; Vasselli et al., 2005). In our study, the composition of the experimental chows was similar to that of normal chows, and fat in the experimental chows supplied only 23.1% of total calories. Although they ate more food during the latter half of their lifespan, mice fed high polyamine chow lived longer and had less age-associated pathology. The increased food consumption may be a consequence of sustained physical activity of aged mice in the high polyamine group, because mice in this group had thick and healthy fur coats and appeared more active than the other groups of mice, especially during the final period of observation.

One of the most typical pathological changes seen in senescence is glomerulosclerosis (Anderson and Brenner, 1986; Bolton and Sturgill, 1980). And SMP-30, a protein expressed in multiple organs and tissues including liver and kidney, seems to protect organs from oxidative stress during aging, and its tissue levels decrease with aging (Fujita et al., 1992; Sato et al., 2008). Therefore, both the lower incidence of glomerulosclerosis and the preservation of SMP-30 staining indicate that the progression of age-associated pathologies is attenuated in mice fed the high polyamine chow.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.exger.2009.08.013.

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