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Dietary polyamine intake and all-cause and cause-specific mortality in Japanese adults in the Takayama study

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Abstract

Epidemiological studies on the potential health effects of dietary polyamines are scarce. The present study aimed to estimate habitual intake of polyamines (putrescine, spermidine and spermine) and examine whether spermidine intake is inversely associated with all-cause and cause-specific mortality in a population-based cohort study in Japan. The study included 13 355 men and 15 724 women aged 35 years and older. Diet was assessed via a validated FFQ at the baseline in 1992. The intake of polyamines was estimated mainly using databases of polyamine content in foods consumed among Japanese population. Sex-specific hazard ratios (HR) and 95 % CI for all-cause and cause-specific mortality were estimated according to polyamine quartiles. During 16 years of follow-up, 2901 deaths in men and 2438 in women occurred. The intake of any polyamine was not significantly associated with all-cause or cause-specific mortality after controlling for covariates in men and women. There was a suggestive positive association between spermidine intake and cancer mortality in women: HR for the highest *v.* lowest quartile were 1·38 (95 % CI (0·99, 1·93); $P_{trend} = 0{\cdot}02$). Our results did not provide support for the notion that dietary spermidine has beneficial effects on mortality. Further studies on dietary polyamines and longevity, as well as the morbidity of specific diseases, including cancer, are needed across populations with different dietary habits.

Keywords: **Polyamines: Spermidine: Mortality: Japanese**

Polyamines, such as putrescine, spermidine and spermine, are aliphatic amines that are ubiquitous in all living cells[1]. Polyamines participate in several biological processes including proliferation and differentiation, protein synthesis, RNA transcriptions, the stabilisation of negative changes of DNA and cell apoptosis[2,3]. Their autophagy and their antioxidant and anti-inflammatory effects have attracted attention, and the potential benefits of polyamines in the prevention of chronic diseases have been expected[2]. In particular, spermidine has been shown to have cardioprotective and lifespan-extending effects[4,5]. Lifespan extension has been demonstrated in yeast, worms, flies and human immune cells[6]. Regarding cancer, previous findings have been contradictive. Numerous studies have shown that polyamine levels are elevated in cancer cells, and a major target of anti-tumour therapy has been polyamine biosynthesis[7]. Therefore, there has been a concern that dietary polyamines may raise the risk of cancer. However, in contrast to this potential procarcinogenic properties of polyamines, spermidine supplementation was reported to reduce tumourigenesis[8,9] and improve anti-tumour immunity[10] in mice. Food is an important source of polyamines. However, epidemiological studies on the potential health effects of dietary polyamine intake are scarce. To our knowledge, only two studies have prospectively examined the association between polyamine intake and all-cause mortality[11,12]. Even studies on polyamine intake and the risk of any chronic diseases are very few[13–16].

Polyamines can be found in all types of foods in a wide range of concentrations[17]. To estimate the dietary intake of polyamines, studies reporting the polyamine content in foods are essential. The review of polyamine content data in previous literature[17] showed that the polyamine content in many food items has been measured by Japanese researchers. Some foods typical to the Japanese diet, such as fermented soyabeans, mushrooms and fermented pickles, have been reported to have high concentrations of spermidine. Therefore, it should be advantageous for us to utilise the Japanese databases based on foods consumed among the Japanese population. The present study aimed to estimate the intake of polyamines (putrescine, spermidine and spermine) in a usual diet at baseline (1992) and to examine whether spermidine intake is inversely associated with subsequent risk of all-cause and cause-specific mortality in a population-based cohort of Japanese men and women (the Takayama study).

Abbreviations: HR, hazard ratio; ICD, International Classification of Diseases.

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Methods

The Takayama study

The Takayama study, a population-based cohort study, was established in 1992 in Japan with the objective of studying associations between dietary and lifestyle factors and morbidity from chronic diseases. Details of the Takayama study have been described elsewhere[18]. A total of 31 552 residents aged 35 years and older in Takayama, Gifu Prefecture, Japan, returned a baseline self-administered questionnaire that included questions on demographic characteristics, smoking habits, diet, physical activity and medical and reproductive histories, yielding a participation rate of 85·3 %. We excluded individuals if they reported having or having had cancer (186 men and 540 women) and stroke or CHD (886 men and 861 women) on the baseline questionnaire. Therefore, a total of 29 079 participants (13 355 men and 15 724 women) were available for the present analysis. Details of the exclusion process are given elsewhere[19]. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethical Committee of Gifu University Graduate School of Medicine (no. 19-45, 22-186, 26-277).

Dietary assessment

We used a validated, self-administered, semiquantitative FFQ to assess dietary polyamines and other nutrients at baseline[20]. The participants were asked how often on average during the previous year they had consumed each of the items listed (169 items of foods and dishes), and the usual portion size for each item was specified. Some of the items for mixed dishes were predefined into several individual foods. Thus, a total of 520 different foods were covered by the FFQ. There are four studies reporting the polyamine content in foods consumed in Japan[21–24]. To estimate the intake of polyamines (putrescine, spermidine, spermine), we first chose the database by Nishimura *et al.*[23], because as many as 227 kinds of food and drink were measured. In their study, polyamine concentrations in foods were determined by use of a Toyo Soda HPLC system. Details of the measurements are given elsewhere[23]. The database showed that 1 nmol/g of polyamines was measurable. When polyamine concentrations were reported as <1 nmol/g or not detected, we assigned the value 0·5 or 0 nmol/g, respectively, for such foods. When polyamine concentrations were given as the range, the mid-point value of the range was assigned for such foods. For mixed dishes, the values based on the polyamine content of each component food were assigned. Although the polyamine content in salt, sugars and oils was not reported in the database, the values for these foods were regarded as 0 as reported by other researchers, including another Japanese research group[17,24,25]. These assignments covered 82·6 % of the cumulative total number of foods (including repetition) on the FFQ, and these foods accounted for 94·6 % of the total energy in men and 93·9 % of that in women. In the next step, for the foods not measured by Nishimura *et al.*[23] but measured by other Japanese researchers, we assigned new values after considering the differences between the studies: seaweeds, confectionaries, five kinds of vegetables, one kind of mushroom, one kind of fish and ketchup were measured by Nishibori *et al.*[24]. The values from the report by Nishibori *et al.*[24] were assigned to these foods after calculating the conversion factors based on the comparison of the values of sixty-seven foods measured by both studies[23,24]. The value for oolong tea was similarly determined using data reported by Okamoto *et al.*[21]. After these processes, the assignments covered 86·8 % of the cumulative total number of foods (including repetition) on the FFQ, and these foods accounted for 97·1 % of the total energy in men and 97·6 % of that in women. For the remaining unmeasured fishes, the average values in Nishimura's data were assigned according to the order of fish. Similarly, for the remaining fruits, the average values for those belonging to the same family were assigned. Finally, for the remaining foods, we assigned the average values according to food group. For mustard and mustard sauce, the values reported by Cipolla *et al.*[25] were assigned. For white pepper, value 0 reported by Cipolla *et al.*[25] was assigned. Intakes of other nutrients and food groups were estimated on the information using the Japanese Standard Tables of Food Composition, 5th revised and enlarged edition[26]. The validity of the polyamine estimation has been checked in a subsample of this population by comparing the intakes from the FFQ and 12 1-d diet records kept over a 1-year period[20]. Spearman's correlation coefficients between the FFQ and the diet records for intakes of putrescine, spermidine and spermine were 0·73, 0·52 and 0·32, respectively, in men and 0·42, 0·55 and 0·32, respectively, in women. A detailed description of the FFQ, along with its reliability and validity for estimation of other nutrient and food group intakes, was published previously[20].

Physical activity was assessed by asking the average hours per week spent performing various kinds of activities during the past year. Weekly metabolic equivalents were estimated multiplying the reported duration of activity by its correspondent energy expenditure requirements. The details including its validity are described elsewhere[27].

Follow-up and endpoints

Information concerning subjects who died or moved away from Takayama City between the baseline (1 September 1992) and 1 October 2008, was obtained from residential registers or family registers. The mean duration of follow-up was 14·1 years. Causes of death were identified from death certificates provided by the Legal Affairs Bureau. We classified deaths using the International Classification of Diseases (ICD), 10th Revision. We examined death due to all-causes, all cancer (ICD-10: C00–D48), CVD (ICD-10: I00–I99) and all other causes (non-cancer, non-CVD). During the study period, 941 (6·5 %) men and 971 (5·7 %) women moved out of Takayama City. They were censored at the time of move out of the city. The date of moving was unknown for 104 (0·7 %) men and 147 (0·9 %) women. They were censored at the latest date when they were known to reside in the city.

Statistical analyses

Dietary intakes of polyamines were adjusted for total energy intake using the residual method[28]. Baseline characteristics of

participants according to quartile of each of energy-adjusted polyamine intake were evaluated using regression technique or the χ^2 test when appropriate. For each participant, person-years of follow-up were calculated from the date of response to the baseline questionnaire to the date of death, the date of emigration out of Takayama or the end of follow-up (1st October 2008), whichever occurred first. Multivariable Cox regression models were used to estimate sex-specific hazard ratios (HR) and 95 % CI of all-cause and cause-specific mortality by quartiles of polyamine intakes using the lowest quartile as the reference category. In multivariable analysis, we adjusted for age, total energy, marital status (married or not married), level of education (≤11, 12–14, ≥15 years or missing), BMI (in quartile and missing), physical activity (metabolic equivalents-h/week), alcohol consumption (in quartile for men, and non-drinkers and drinkers below or above the median alcohol level for women), smoking status (never, former, current with ≤30 years of smoking, current with >30 years of smoking or missing for men and never, former, current or missing for women), histories of diabetes and hypertension (yes, no), coffee (0, 1–6, 7 cups/week), menopausal status (premenopausal or postmenopausal, women only) and intakes of polyunsaturated fat, glycaemic load, salts and vegetable (energy-adjusted). Tests of linear trends were conducted by modelling polyamine intake as continuous variable in the model with the median value of each category. The proportional hazards assumption was evaluated by entering interaction terms between exposures and the natural logarithm of the time into the model. We conducted separate analyses during the first and second halves of the follow-up period (8 years) for spermidine intake and non-cancer, non-CVD mortality in women that did not satisfy proportional hazards assumption ($P = 0{\cdot}02$).

Sensitivity analyses were performed when the assignment of polyamine values was done for 82·6 % or 86·8 % of the foods as mentioned above (the value was considered to be 0 for the remaining foods). Additional sensitivity analysis was conducted by excluding deaths during the first 3 years of the follow-up period. All the statistical analyses were performed using SAS programs. Power calculations showed that the sample size and number of total deaths were sufficiently large to detect a HR of 1·2 (or 0·83) for the highest quartile of intake compared with the lowest, with a statistical power of 80 % and significance level of 5 %.

Results

Intakes of putrescine, spermidine and spermine (mean) were 210·7 (SD 143·9), 95·7 (SD 56·6) and 55·9 (SD 42·1) μmol/d in men and 162·7(SD 111·5), 97·6 (SD 56·3) and 55·7 (SD 42·9) μmol/d in women, respectively. The main dietary sources of polyamines in men and women were as follows: putrescine – alcoholic beverages (34·4 %), vegetables (26·2 %) and fruits (19·3 %) in men and vegetables (37·3 %), fruits (28·8 %) and alcoholic beverages (10·7 %) in women; spermidine – vegetables (37·9 %), legumes (16·2 %) and mushrooms (12·3 %) in men and vegetables (39·8 %), legumes (15·8 %) and mushrooms (12·9 %) in women; spermine, meats (36·0 %), vegetables (31·2 %) and fish and shellfish (14·8 %) in men and vegetables (41·6 %), fish and shellfish (26·9 %) and meats (11·7 %) in women. Baseline characteristics of study population by quartile of spermidine intake are shown in Table 1. Men with higher intake of spermidine intake were more likely to be older, married, less educated, never-smokers, less obese and physically less active and to have reported a history of hypertension and diabetes. Women with higher intake of spermidine intake were more likely to be older, not married, less educated, never-smokers and postmenopausal and to have reported a history of hypertension and diabetes. They (men and women) were also likely to have lower intake of alcohol, total energy, glycaemic load and coffee and to have higher intake of polyunsaturated fat, vegetables and salt. Baseline characteristics according to the quartiles of putrescine and spermine intake are shown in online Supplementary Tables 1–2. Although we presented the mean values for dietary intakes in the table, some of them including dietary polyamines may be overestimated by our questionnaire because the mean values estimated from the FFQ were generally higher than those estimated from twelve 1-d diet records[20].

After a follow-up period, we recorded 2901 male deaths and 2438 female deaths among 29 079 participants. There were no significant associations between any polyamine intake and all-cause or cause-specific mortality in men after controlling for covariates (Table 2). In women, although the highest quartile of spermidine intake was not significantly associated with an increased risk of cancer mortality, a significant positive trend was observed ($P_{trend} = 0{\cdot}04$) (Table 3). Additional adjustment for soya foods did not alter the results; the HR for the highest *v.* lowest quartiles of spermidine intake in women were 1·35 (95 % CI (0·97, 1·89), $P_{trend} = 0{\cdot}04$) for cancer mortality. The sum of putrescine, spermidine and spermine intake was not associated with all-cause or cause-specific mortality in both men and women (data not shown).

Because of the significant trend for the association between spermidine intake and cancer in women, we assessed associations between spermidine intake and the major causes of cancer deaths including stomach cancer (ICD-10: C16, *n* 106), lung and bronchus cancer (ICD-10:C33–C34, *n* 81) and colorectal cancer (ICD-10: C18–C21, *n* 110) in women. Somewhat risk increase was observed for stomach and colorectal cancer mortality associated with spermidine intake; the HR for the highest *v.* lowest quartiles of intake were 1·85 (95 % CI (0·80, 4·30); $P_{trend} = 0{\cdot}08$) and 2·00 (95 % CI (0·88, 4·30); $P_{trend} = 0{\cdot}08$), respectively.

The sensitivity analysis based on measured foods covering 82·6 % of the total number of foods on the FFQ revealed that the results were not substantially altered, for example, in women, the HR of cancer mortality for the highest *v.* lowest quartile of spermidine intake were 1·36 (95 % CI (0·98, 1·88); $P_{trend} = 0{\cdot}02$). Exclusion of deaths during the first 3 years did not alter the results substantially; the HR of cancer mortality for the highest *v.* lowest quartile of spermidine intake were 1·36 (95 % CI (0·96, 1·94); $P_{trend} = 0{\cdot}04$) in women.

Discussion

In spite of increasing biological mechanism data on the beneficial effects of spermidine for lifespan extension and

Table 1. Baseline characteristics of study participants according to the quartile of spermidine intake* in the Takayama cohort

	Men					Women				
Basic characteristics	Q1	Q2	Q3	Q4	P_{trend}	Q1	Q2	Q3	Q4	P_{trend}
Men										
Spermidine range, μmol/d	<50·3	50·3–68·1	68·2–89·4	>89·4		<75·6	75·6–93·2	93·3–112·2	>112·2	
n	3339	3339	3339	3338		3931	3931	3931	3931	
Age, years	49·3	52·2	55·5	58·9	<0·0001	50·8	54·3	57·2	58·3	<0·0001
	%	%	%	%		%	%	%	%	
Married	89·9	90·7	91·8	93·4	<0·0001	77·6	75·9	73·3	74·0	<0·0001
Years of education										
≦11	55·3	54·4	56·6	62·2		61·9	64·0	68·7	70·1	
12–14	33·6	34·5	30·7	25·3		33·3	31·0	26·8	25·5	
≧15	11·1	11·1	12·7	12·6	<0·0001	8·9	5·0	4·5	4·4	<0·0001
Smoking										
Never	12·5	16·1	17·6	21·0		76·7	81·6	85·4	86·6	
Former	21·6	26·2	30·6	34·5		4·6	4·4	4·4	4·1	
Current	65·9	57·8	51·8	44·5	<0·0001	18·7	14·0	10·3	17·1	<0·0001
History of hypertension	17·4	17·5	18·8	21·9	<0·0001	12·6	16·9	19·7	20·2	<0·0001
History of diabetes mellitus	4·4	5·4	6·0	8·0	<0·0001	1·3	2·2	3·3	3·9	<0·0001
BMI, kg/m^2	22·7	22·5	22·4	22·4	<0·0001	22·0	21·9	21·9	22·1	0·06
Alcohol intake, g/d	63·8	40·1	32·0	32·0	<0·0001	12·7	7·3	5·2	5·7	<0·0001
Exercise, MET-h/week	30·7	26·4	25·3	25·9	<0·0001	18·8	18·5	18·4	19·6	0·22
Daily dietary intake										
Total energy, kcal	2997	2478	2314	2697	<0·0001	2495	1948	1835	2252	<0·0001
Polyunsaturated fat, g	15·0	13·8	14·2	18·7	<0·0001	14·7	12·6	12·7	16·8	<0·0001
Glycaemic load	263·2	223·4	199·6	210·9	<0·0001	241·9	179·8	162·9	181·8	<0·0001
Vegetables, g	263·9	283·8	338·1	603·5	<0·0001	295·8	297·5	352·3	632·1	<0·0001
Salts, g/1000 kcal	13·2	12·4	13·1	18·1	<0·0001	12·4	11·3	11·9	16·7	<0·0001
Coffee, cups/d	1·08	0·91	0·78	0·65	<0·0001	0·90	0·71	0·56	0·52	<0·0001

MET, metabolic equivalent; Q, quartile.
* Spermidine intake is adjusted for total energy.

Table 2. Hazard ratios (HR) with 95 % CI for total and cause-specific mortality in men by the quartiles of polyamine intake

		Quartile 2		Quartile 3		Quartile 4		
	Quartile 1	HR	95 % CI	HR	95 % CI	HR	95 % CI	P_{trend}
All-cause mortality								
Putrescine								
Median*	60·0	117		166·4		260·5		
No. of deaths	694	800		826		581		
Age-adjusted HR	1·0	0·95	0·86, 1·05	1·01	0·91, 1·12	0·96	0·86, 1·07	0·73
Multivariable HR†	1·0	0·98	0·88, 1·09	1·06	0·95, 1·18	1·05	0·94, 1·18	0·93
Spermidine								
Median*	38·2	59·6		77·5		110·3		
No. of deaths	464	613		817		1007		
Age-adjusted HR	1·0	0·98	0·86, 1·10	0·98	0·87, 1·10	0·97	0·87, 1·09	0·66
Multivariable HR†	1·0	0·97	0·85, 1·11	0·98	0·86, 1·13	0·99	0·85, 1·17	0·91
Spermine								
Median*	17·9	32·4		43·7		63·7		
No. of deaths	518	633		778		972		
Age-adjusted HR	1·0	0·92	0·82, 1·04	0·99	0·89, 1·11	1·01	0·90, 1·13	0·43
Multivariable HR†	1·0	0·92	0·81, 1·04	1·01	0·88, 1·15	1·04	0·90, 1·19	0·22
Cancer mortality								
Putrescine								
No. of deaths	254	250		271		199		
Age-adjusted HR	1·0	0·85	0·71, 1·01	0·96	0·81, 1·14	0·89	0·74, 1·07	0·45
Multivariable HR†	1·0	0·89	0·74, 1·07	0·99	0·81, 1·20	0·88	0·71, 1·10	0·42
Spermidine								
No. of deaths	177	213		263		321		
Age-adjusted HR	1·0	0·94	0·77, 1·15	0·93	0·76, 1·13	0·94	0·78, 1·14	0·64
Multivariable HR†	1·0	0·97	0·78, 1·21	0·97	0·77, 1·23	1·01	0·77, 1·32	0·85
Spermine								
No. of deaths	194	222		243		315		
Age-adjusted HR	1·0	0·92	0·76, 1·12	0·91	0·75, 1·10	1·00	0·83, 1·20	0·80
Multivariable HR†	1·0	0·94	0·76, 1·15	0·96	0·77, 1·20	1·06	0·84, 1·33	0·41
Cardiovascular mortality								
Putrescine								
No. of deaths	177	231		206		161		
Age-adjusted HR	1·0	1·03	0·85, 1·26	0·95	0·77, 1·16	1·04	0·84, 1·29	0·88
Multivariable HR†	1·0	0·96	0·78, 1·18	0·84	0·66, 1·06	1·00	0·78, 1·30	0·96
Spermidine								
No. of deaths	102	157		226		290		
Age-adjusted HR	1·0	1·09	0·85, 1·41	1·13	0·89, 1·44	1·15	0·91, 1·45	0·28
Multivariable HR†	1·0	1·05	0·80, 1·37	1·06	0·80, 1·41	1·07	0·77, 1·48	0·76
Spermine								
No. of deaths	127	164		209		275		
Age-adjusted HR	1·0	0·93	0·74, 1·18	1·01	0·81, 1·27	1·06	0·85, 1·31	0·36
Multivariable HR†	1·0	0·86	0·67, 1·10	0·92	0·71, 1·19	0·95	0·72, 1·23	0·92
Non-cancer, non-cardiovascular mortality								
Putrescine								
No. of deaths	263	318		348		221		
Age-adjusted HR	1·0	0·97	0·83, 1·15	1·10	0·94, 1·30	0·96	0·81, 1·15	0·97
Multivariable HR†	1·0	1·02	0·85, 1·21	1·17	0·97, 1·41	1·07	0·86, 1·32	0·40
Spermidine								
No. of deaths	183	243		328		396		
Age-adjusted HR	1·0	0·96	0·79, 1·16	0·94	0·78, 1·13	0·91	0·76, 1·09	0·28
Multivariable HR†	1·0	0·96	0·78, 1·18	0·97	0·78, 1·21	0·96	0·75, 1·25	0·87
Spermine								
No. of deaths	197	246		325		382		
Age-adjusted HR	1·0	0·92	0·76, 1·11	1·05	0·88, 1·26	0·99	0·83, 1·18	0·81
Multivariable HR†	1·0	0·93	0·76, 1·14	1·11	0·90, 1·36	1·08	0·87, 1·35	0·27

* Adjusted for total energy.
† Adjusted for age, BMI, physical activity, smoking status, alcohol consumption, education, marital status, histories of diabetes and hypertension and intakes of polyunsaturated fat, glycaemic load, vegetables, salt and coffee.

cardioprotection, we did not observe the expected inverse associations of spermidine intake with all-causes and CVD mortality in both men and women. So far, only two studies have prospectively examined the associations between spermidine intake and all-cause mortality. In the Bruneck study among 829 Italian men and women, a high intake of spermidine was significantly associated with a decreased risk of all-cause and non-cancer, non-CVD mortality[11]. In the US National Health and Nutrition survey among 23 894 participants, spermidine intake was significantly associated with a

Table 3. Hazard ratios (HR) with 95 % CI for total and cause-specific mortality in women by the quartiles of polyamine intake

		Quartile 2		Quartile 3		Quartile 4		
	Quartile 1	HR	95 % CI	HR	95 % CI	HR	95 % CI	P_{trend}
All-cause mortality								
Putrescine								
Median*	94·9	137·8		168·4		229·3		
No. of deaths	441	667		739		591		
Age-adjusted HR	1·0	1·13	1·00, 1·27	1·13	1·01, 1·27	0·99	0·87, 1·12	0·43
Multivariable HR†	1·0	1·09	0·96, 1·24	1·09	0·95, 1·26	1·00	0·86, 1·17	0·63
Spermidine								
Median*	62·4	85·2		101·6		132·7		
No. of deaths	374	587		750		727		
Age-adjusted HR	1·0	0·93	0·84, 1·04	1·02	0·92, 1·13	1·00	0·90, 1·11	0·61
Multivariable HR†	1·0	1·00	0·87, 1·15	1·08	0·93, 1·26	1·05	0·88, 1·25	0·56
Spermine								
Median*	30·6	45·9		56·3		77·7		
No. of deaths	404	566		730		738		
Age-adjusted HR	1·0	1·04	0·92, 1·18	1·15	1·02, 1·30	1·02	0·90, 1·15	0·99
Multivariable HR†	1·0	1·02	0·89, 1·17	1·12	0·97, 1·29	1·05	0·90, 1·21	0·60
Cancer mortality								
Putrescine								
No. of deaths	135	166		193		152		
Age-adjusted HR	1·0	1·05	0·83, 1·31	1·14	0·92, 1·43	0·93	0·73, 1·17	0·50
Multivariable HR†	1·0	1·01	0·79, 1·29	1·08	0·83, 1·40	0·79	0·59, 1·05	0·07
Spermidine								
No. of deaths	117	133		198		198		
Age-adjusted HR	1·0	0·90	0·70, 1·16	1·19	0·95, 1·50	1·11	0·88, 1·40	0·15
Multivariable HR†	1·0	0·99	0·75, 1·30	1·39	1·04, 1·84	1·38	0·99, 1·93	0·02
Spermine								
No. of deaths	120	153		177		196		
Age-adjusted HR	1·0	1·09	0·86, 1·38	1·17	0·93, 1·48	1·15	0·92, 1·45	0·24
Multivariable HR†	1·0	1·15	0·89, 1·49	1·27	0·96, 1·66	1·26	0·95, 1·66	0·13
Cardiovascular mortality								
Putrescine								
No. of deaths	137	259		279		228		
Age-adjusted HR	1·0	1·31	1·06, 1·61	1·25	1·02, 1·61	1·16	0·94, 1·44	0·54
Multivariable HR†	1·0	1·22	0·97, 1·53	1·18	0·92, 1·51	1·22	0·93, 1·59	0·32
Spermidine								
No. of deaths	106	227		293		277		
Age-adjusted HR	1·0	1·25	0·99, 1·57	1·29	1·03, 1·61	1·15	0·92, 1·44	0·66
Multivariable HR†	1·0	1·17	0·91, 1·51	1·25	0·95, 1·63	1·19	0·88, 1·62	0·46
Spermine								
No. of deaths	127	205		286		285		
Age-adjusted HR	1·0	1·12	0·89, 1·39	1·26	1·02, 1·56	1·10	0·89, 1·36	0·56
Multivariable HR†	1·0	1·05	0·83, 1·34	1·18	0·92, 1·51	1·08	0·84, 1·39	0·64
Non-cancer, non-cardiovascular mortality								
Putrescine								
No. of deaths	169	242		267		210		
Age-adjusted HR	1·0	1·04	0·85, 1·26	1·03	0·85, 1·25	0·89	0·73, 1·09	0·18
Multivariable HR†	1·0	1·02	0·83, 1·27	1·01	0·80, 1·28	0·97	0·75, 1·26	0·74
Spermidine								
≤8 years of follow-up								
No. of deaths	52	87		115		85		
Age-adjusted HR	1·0	0·96	0·68, 1·36	0·99	0·71, 1·38	0·72	0·51, 1·02	0·03
Multivariable HR†	1·0	0·86	0·58, 1·26	0·84	0·55, 1·28	0·68	0·41, 1·13	0·13
>8 years of follow-up								
Median*	62·0	84·5		101·0		132·8		
No. of deaths	93	139		149		168		
Age-adjusted HR	1·0	0·99	0·76, 1·29	0·82	0·63, 1·06	0·84	0·65, 1·08	0·10
Multivariable HR†	1·0	0·96	0·72, 1·28	0·84	0·62, 1·15	0·85	0·59, 1·22	0·33
Spermine								
No. of deaths	157	207		267		257		
Age-adjusted HR	1·0	0·95	0·77, 1·17	1·02	0·84, 1·25	0·86	0·70, 1·05	0·11
Multivariable HR†	1·0	0·90	0·71, 1·12	0·96	0·76, 1·22	0·87	0·68, 1·11	0·33

* Adjusted for total energy.

† Adjusted for age, BMI, physical activity, smoking status, alcohol consumption, education, marital status, histories of diabetes and hypertension, menopausal status and intakes of polyunsaturated fat, glycaemic load, vegetables, salt and coffee.

decreased risk of all-cause and CVD mortality[12]. Putrescine (diamine) differs from spermidine (triamine) in its genesis and metabolic reactions[29]. We did not observe any significant association of putrescine intake with all-cause and cause-specific mortality, which was in line with the Bruneck study[11].

The apparent discrepancy in the results on all-cause and CVD mortality between previous studies and ours may be related to different distributions of food sources of spermidine. Foods with high spermidine content, such as fermented soya, fermented vegetables and shiitake mushrooms, are much consumed among the Japanese population. On the other hand, cereals and cheeses, which are also abundant in spermidine, are consumed less in Japan than in Western countries. Although some of these spermidine-rich food groups themselves have been reported to be inversely associated with all-cause or CVD mortality[30–33], such associations may be not due to spermidine but to other components of the foods. The results from our study based on total spermidine intake estimated from the diet covering food sources different from those in the previous studies would be informative to infer the relationship between spermidine itself and mortality. The lack of significant associations between spermidine intake and all-cause and CVD mortality in our study would not deny the possibility that spermidine intake is unrelated to the risk of all-cause or CVD mortality. There is also the possibility that exposure to polyamines is not reflected in dietary intake. Although a long-term intake of spermidine rich food (fermented soyabeans) raised the blood level of spermidine in healthy Japanese men[34], Vargas *et al.* reported that dietary polyamines were not associated with urinary polyamines from 24-h urine samples in men after controlling for total energy[35].

We observed a suggestive positive association between spermidine intake and cancer mortality in women. This may be relevant to that cancer cells require polyamines for growth and proliferation[7]. However, in the Bruneck study[11], there was a suggestive inverse association between spermidine intake and cancer mortality risk ($P = 0{\cdot}05$). So far, only one study, the Women's Health Initiative Observational Study, has prospectively assessed the association between dietary polyamine (the sum of putrescine, spermidine and spermine) and colorectal cancer incidence and mortality in postmenopausal US women[13]. There was no significant positive association between dietary polyamine and colorectal cancer incidence and mortality. Instead, an inverse association was suggested in women with BMI ≤ 25 or fibre consumption above the median. Another study included colorectal adenomatous polyps as an outcome disease and polyamine intake (the sum of putrescine, spermidine and spermine) was prospectively positively associated with the risk of colorectal adenomatous polyps[36]. There have been no epidemiological studies on polyamine intake and the risk of other types of cancer. Further studies on polyamine intake and cancer are needed, especially in light of the potential requirement of polyamines for cancer cell growth.

Our study has several strengths: a population-based cohort, the use of polyamine content data for Japanese foods and a validated dietary questionnaire, information on potential confounders and a high rate of follow-up. However, there are some limitations. Although we utilised multiple databases to estimate polyamine intake, data were still missing for some foods. For these foods, we had to assign the values of similar foods or the same food groups. Measurement data themselves are subject to measurement errors. If there is systematic errors in measuring or estimating polyamine intake, such errors can bias associations. However, it is unlikely that such measurement errors are dependent on outcomes. We used conventional statistical approach (Cox proportional hazard model) in our study including multiple endpoints such as cause-specific mortality. We cannot deny that these endpoints are competing events for each other. Diet was only collected at baseline, and dietary changes may have occurred during the follow-up period. Although we have included many potential confounders, the results may be subjective to the effects of residual confounding or unknown confounders.

Conclusions

We observed no significant inverse associations between spermidine intake and all-cause and CVD mortality in both men and women. The results did not provide support for the notion that dietary spermidine has beneficial effects on mortality. On the contrary, there was a suggestive positive association for cancer mortality in women. Overall, there were no consistent associations between dietary polyamines and mortality. However, as epidemiological data addressing these issues are yet scarce, further studies on dietary polyamines and longevity, as well as the morbidity of specific diseases, including cancer, are needed across populations with different dietary habits.

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The authors have no conflict of interest to declare.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114523002465

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