Higher spermidine intake is linked to lower mortality: a prospective population-based study

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ABSTRACT

Background: Spermidine administration is linked to increased survival in several animal models.

Objective: The aim of this study was to test the potential association between spermidine content in diet and mortality in humans.

Design: This prospective community-based cohort study included 829 participants aged 45–84 y, 49.9% of whom were male. Diet was assessed by repeated dietitian-administered validated food-frequency questionnaires (2540 assessments) in 1995, 2000, 2005, and 2010. During follow-up between 1995 and 2015, 341 deaths occurred.

Results: All-cause mortality (deaths per 1000 person-years) decreased across thirds of increasing spermidine intake from 40.5 (95% CI: 36.1, 44.7) to 23.7 (95% CI: 20.0, 27.0) and 15.1 (95% CI: 12.6, 17.8), corresponding to an age-, sex- and caloric intake–adjusted 20-y cumulative mortality incidence of 0.48 (95% CI: 0.45, 0.51), 0.41 (95% CI: 0.38, 0.45), and 0.38 (95% CI: 0.34, 0.41), respectively. The age-, sex- and caloric ratio–adjusted HR for all-cause death per 1-SD higher spermidine intake was 0.74 (95% CI: 0.66, 0.83; $P < 0.001$). Further adjustment for lifestyle factors, established predictors of mortality, and other dietary features yielded an HR of 0.76 (95% CI: 0.67, 0.86; $P < 0.001$). The association was consistent in subgroups, robust against unmeasured confounding, and independently validated in the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) Study (age-, sex-, and caloric ratio–adjusted HR per 1-SD higher spermidine intake: 0.71; 95% CI: 0.53, 0.95; $P = 0.019$). The difference in mortality risk between the top and bottom third of spermidine intakes was similar to that associated with a 5.7-y (95% CI: 3.6, 8.1 y) younger age.

Conclusion: Our findings lend epidemiologic support to the concept that nutrition rich in spermidine is linked to increased survival in humans. This trial was registered at www.clinicaltrials.gov as NCT03378843. *Am J Clin Nutr* 2018;108:371–380.

Keywords: polyamines, spermidine, life span, cancer, vascular disease

INTRODUCTION

Spermidine is the most abundant polyamine in a majority of different human tissues, with intracellular spermidine concentrations declining during the natural course of organismal aging (1–3). Conversely, the administration of spermidine is linked to increased survival of yeast, worms, flies, and human immune cells and reduces age-associated mortality in mice (**Supplemental**

This study is part of the excellence initiative [Competence Centers for Excellent Technologies (COMET)] of the Austrian Research Promotion Agency FFG: "Research Center of Excellence in Vascular Ageing—Tyrol, VASCage" (K-Project 843536) funded by the Federal Ministry for Transport, Innovation and Technology (BMVIT), Federal Ministry of Science, Research and Economy (BMWFW), Wirtschaftsagentur Wien, and Standortagentur Tirol. PW is the recipient of an Erwin Schrödinger Fellowship (Austrian Science Fund, J3679-B13). GK is supported by Cancéropôle Ile-de-France, Institut National du Cancer (INCa), the European Research Council (ERC), the LabEx Immuno-Oncology, and the Paris Alliance of Cancer Research Institutes (PACRI). FM has received support from to the Austrian Science Fund (grants P23490-B20, P29262, P24381, P 29203, P 27893, and "SFB Lipotox") as well as from BMWFW (grants "Unkonventionelle Forschung"

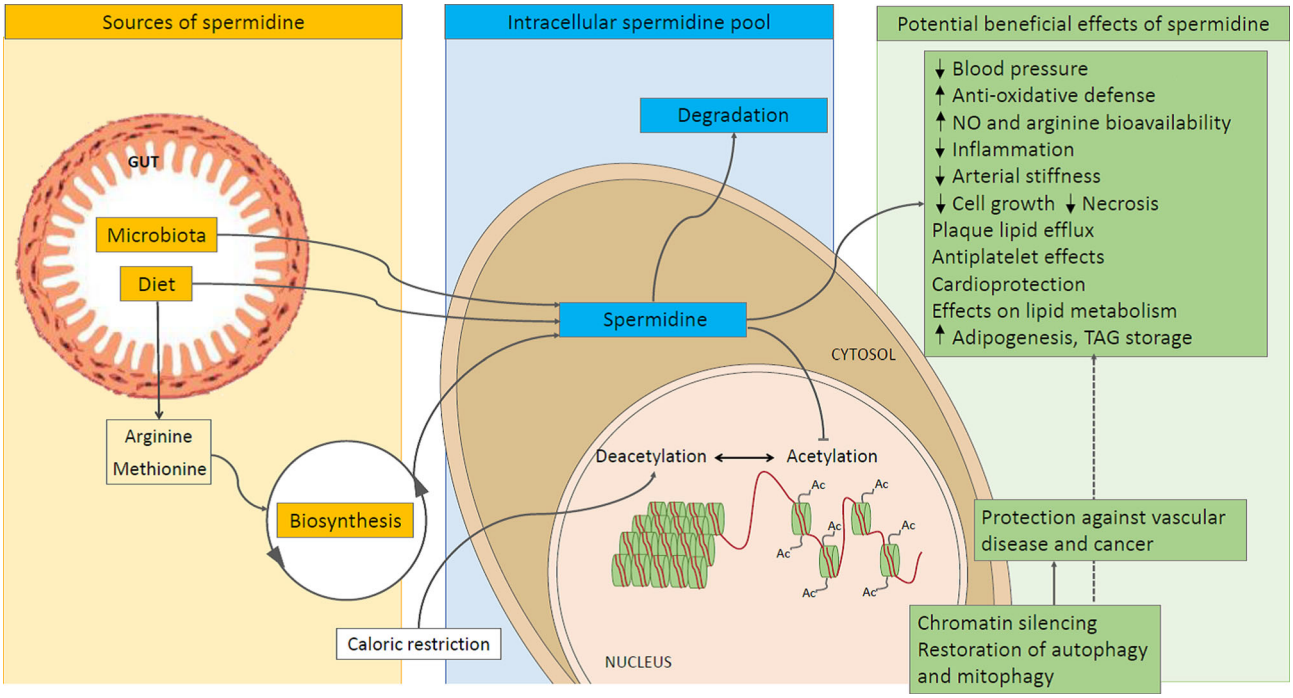

FIGURE 1 Sources of spermidine, spermidine metabolism, and potential lifetime-prolonging effects of spermidine in humans. Spermidine homeostasis is subject of elaborate regulation involving nutritional uptake, intestinal synthesis by the gut microbiota, endogenous biosynthesis, degradation, and active transporter systems between compartments (1, 2, 6, 8). Spermidine and caloric restriction both result in histone hypo-acetylation with chromatin silencing either by sirtuin-mediated activation of histone deacetylases (caloric restriction) or by inhibition of the histone acyl transferase (spermidine) (5, 9–11). Spermidine exerts autophagy-dependent antiaging properties both at the cytosolic and nuclear levels. Spermidine was shown to interfere with lipid metabolism, suppress harmful inflammatory processes, lower blood pressure, confer cardiac protection, and exert antiplatelet effects (4, 12–16). Ac, acetyl; NO, nitric oxide; TAG, triacylglycerol.

Table 1) (2–7). Very high spermidine concentrations in sperm fluid may prevent cell senescence and confer long-term survival to germ cell lines. Spermidine homeostasis is influenced by nutritional uptake, intestinal sources (microbiota) (6), endogenous biosynthesis, degradation, and active transporter systems between compartments (**Figure 1**) (1, 2, 8).

Spermidine is the polyamine most readily absorbed from the human gut. A broad and diverse palette of foods contain high amounts of spermidine (17, 18), such as fresh green pepper, wheat germ, cauliflower, broccoli, mushrooms, and a variety of cheeses, whereas even higher amounts are found in soybean products such as natto, shitake mushrooms, amaranth grain, and durian (19). In the current study, we analyzed the potential association between dietary spermidine intake and mortality in the general community.

METHODS

Study participants

The Bruneck Study is a prospective, population-based cohort study. Its study population was enrolled in 1990 as an age- and sex-stratified random sample of all inhabitants of Bruneck (Bolzano Province, Italy) aged 40–79 y (125 women and 125 men in each of the fifth to eighth decades of life; $n = 1000$) and re-evaluations were scheduled every 5 y since 1990 (20–24). The population is exclusively white and is unique for its participation and in-person follow-up rates >90% (20–24), facilitated by annual population mobility proportions as low as 0.2%. Moreover, for all participants, full medical records from general practitioners and Bruneck Hospital, the only hospital in the region, were available for review. For this analysis, we defined the baseline as the year of the first detailed dietary assessment (1995) involving 829 women and men aged 45–84 y with a follow-up of 20 y (1995–2015; **Supplemental Figure 1**). The study protocol conformed to the Declaration of Helsinki and was approved by the local ethics committees (Bolzano and Verona). Participants gave their written informed consent and did not receive financial compensation. Participant characteristics were assessed by standard procedures (20–24) detailed in the online supporting material (page 3).

and "Flysleep") and for the BioTechMed-Graz flagship project EPIAge. TE is the recipient of an Austrian Programme for advanced Research and Technology (APART) fellowship of the Austrian Academy of Sciences. The funding organizations did not influence the design or conduct of the study; the collection, management, analysis, or interpretation of the data; the preparation, review, or approval of the manuscript; nor the decision to submit the manuscript for publication.

SK and RP contributed equally to this work.

Supplemental Tables 1–9 and Supplemental Figures 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Received December 20, 2017. Accepted for publication April 23, 2018.

First published online June 28, 2018; doi: https://doi.org/10.1093/ajcn/nqy102.

The Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) Study served as a prospective replication cohort. A total of 1770 healthy unrelated participants (663 women and 1107 men aged 39–67 y) were recruited by health-screening programs in large companies in and around the city of Salzburg. The cohort was examined in the years 1999–2002, with follow-up for deaths until September 2013 (median follow-up: 12.8 y) (25, 26). The current trial was registered at www.clinicaltrials.gov as NCT03378843.

Dietary assessment

Dietary intake was evaluated by quinquennial (1995, 2000, 2005, and 2010) dietitian-administered 118-item food-frequency questionnaires (FFQs) on the basis of the gold-standard FFQ by Willett et al. (27) and adapted to the dietary habits in the survey area (for details and validation, see **Supplemental Tables 2–4**). FFQs were available from 829 individuals who provided 2540 individual assessments. For each item in the FFQ, a common unit or portion size was specified, and we instructed participants to customize how often, on average, they had consumed that amount in the past years. The 9 response categories ranged from "never" to "≥6 times/d." We calculated nutritional intake by assigning a weight proportional to the frequency of use for each food (1 time/d equals a weight of 1), multiplying this weight by the nutrient value for the specified size, and summing the contribution of all foods. Nutrient-composition data for foods were based on the USDA Nutrient Database (release 23) (28). We compiled a special nutrient database for polyamines (Supplemental Table 4). Dietitians made use of illustrative photos of foods when exploring aphasic patients and of information provided by spouses, caregivers, and nursing homes. We dissected complex foods into component foods utilizing common recipes. An open-ended section inquired about foods not considered in the FFQ that were consumed ≥1 time/wk and various kinds of nutritional supplements.

To obtain the best estimate of long-term nutritional intakes and to minimize effects of within-person variation, we used the cumulative update method, which takes the average of all previous data (29). Accordingly, dietary spermidine estimated in 1995 was used to predict events occurring between 1995 and 2000, the average of dietary spermidine estimated in 1995 and 2000 was used to predict events occurring between 2000 and 2005, and the average of dietary spermidine estimated in 1995, 2000, and 2005 was used to predict events occurring between 2005 and 2010. Subsidiary analyses that suspended further updating after the manifestation of cancer, vascular disease, or diabetes yielded very similar results. Further sensitivity analyses focused on baseline spermidine intake only or performed a noncumulative update. Estimates of polyamines and other nutrient intake were calorie adjusted unless specified otherwise. For that purpose, we used the residuals obtained by regressing polyamine or other nutrient intake on total energy intake (30).

We also calculated the ratio of calorie intake to energy expenditure, termed "caloric ratio," throughout the article. This ratio reflects caloric excess or restriction, one of few dietary features convincingly linked to health span and possibly life span in humans (3).

The reproducibility and validity of the original FFQ are well documented (27) and extend to its application in the Bruneck Study. A comparison with 9-d diet records is depicted in Supplemental Table 2 and shows a reasonable level of agreement. As expected, dietary patterns remained stable over time (Supplemental Table 3). Accordingly, under most circumstances, point estimates adequately reflect medium-term dietary intake. The average correlation of spermidine intake in the 4 quinquennial assessments was 0.54. Dietary assessment in the SAPHIR study relied on a 69-item FFQ and the Nutrient Database for Germany but otherwise used the same methodologic standards.

Ascertainment of causes of death

In the Bruneck Study, we collected detailed information on the date, causes, and circumstances of death for all study participants who did not survive the entire follow-up period by consulting death certificates, all medical records ever compiled on study participants, and autopsy reports in the rare event of unexpected death. We were able to ascertain 100% of deaths and reliably classify them as vascular deaths, cancer deaths, or deaths from other causes. Classification details are summarized in **Supplemental Table 5**. The experienced researcher who categorized all deaths was unaware of the dietary data. Our focus was on the primary cause of death.

In SAPHIR, ascertainment of death relied on the review of hospital records and information from the Austrian Death Registry (Statistics Austria). Follow-up was complete except for a presumably low proportion of expatriates.

Statistical analysis

We estimated the conditional 20-y cumulative incidence of all-cause death in thirds of spermidine intake with adjustment for age, sex, and caloric intake with the use of parametric survival analysis with inverse probability weighting and present parametric survival curves based on flexible Parmar-Royston spline-based models and nonparametric Kaplan-Meier curves (31, 32). Cox proportional hazards models with time-varying covariates were used to estimate HRs and 95% CIs for the association between spermidine intake and death. No departure from the proportional hazards assumption was detected when inspecting Schoenfeld residuals and checking the parallelity of log-log survival plots. Our primary endpoint was death from any cause. In analyses focusing on cause-specific deaths, participant data were censored if the participant died from other causes. This approach produces HRs for each cause of death that are etiologically interpretable (33, 34). In addition to these cause-specific hazard models, Fine-Gray subdistribution hazard models were fitted (35). Both approaches account for competing risks (36).

We modeled spermidine intake as a categorical or continuous variable (after $\log_e$-transformation) and used restricted cubic splines to detect potential nonlinearities. All of the analyses were adjusted for age, sex, and "caloric ratio." Multivariable analyses additionally included well-established risk factors and determinants of death such as socioeconomic status, BMI, smoking, diabetes, hypertension, and aspirin medication (all with updates every 5 y) and features of lifestyle and diet such as physical activity and alcohol consumption (both with cumulative updates every 5 y).

TABLE 1
Characteristics of the study population according to dietary spermidine intake in the Bruneck Study[1]

Characteristic	Spermidine intake			*P*
	Tertile 1 (<62.2 μmol/d)	Tertile 2 (62.2–79.8 μmol/d)	Tertile 3 (>79.8 μmol/d)	
Age, y	69.0 ± 10.8	66.6 ± 10.3	64.7 ± 10.2	<0.001
Female sex, *n* (%)	322 (38.0)	458 (54.1)	539 (63.6)	<0.001
Caloric ratio,[2] kcal:kcal	1.11 ± 0.40	1.06 ± 0.39	1.05 ± 0.37	0.002
Low social status, *n* (%)	565 (66.7)	482 (57.0)	414 (48.8)	<0.001
Physical activity, MET-h/wk	42.4 ± 35.9	49.9 ± 35.1	53.9 ± 35.8	<0.001
BMI, kg/m^2	25.7 ± 3.8	25.9 ± 4.1	25.8 ± 4.0	0.52
Current smoker, *n* (%)	158 (18.7)	151 (17.9)	96 (11.3)	<0.001
Alcohol intake, g/d	26.7 ± 29.2	20.5 ± 20.6	14.3 ± 16.0	<0.001
Diabetes, *n* (%)	50 (5.9)	62 (7.3)	85 (10.1)	0.002
Hypertension, *n* (%)	434 (51.3)	434 (51.4)	443 (52.4)	0.86
Aspirin medication, *n* (%)	143 (16.9)	162 (19.2)	153 (18.2)	0.46
Red/processed meat, servings/d	0.71 ± 0.46	0.61 ± 0.40	0.54 ± 0.36	<0.001
Fruit/vegetables, servings/d	3.29 ± 1.19	4.46 ± 1.31	6.01 ± 2.06	<0.001
Dairy products, servings/d	2.69 ± 1.60	2.45 ± 1.28	2.45 ± 1.34	<0.001
Glycemic load	137 ± 29	141 ± 25	146 ± 22	<0.001
Alternative Healthy Eating Index	31.2 ± 6.1	36.7 ± 6.6	40.9 ± 7.6	<0.001

[1]Values are means ± SDs unless otherwise indicated. Values for all variables except for age and sex are age and sex standardized. MET, metabolic equivalent of task.

[2]The caloric ratio was calculated as the ratio of caloric intake to energy expenditure (online supporting material, page 3) and reflects caloric excess or restriction.

To substantiate our findings, we conducted a number of subsidiary analyses:

1) We excluded the initial 5 y of follow-up to address the concern of reverse causation.
2) We additionally adjusted for a propensity score that reflected associations of spermidine intake with macronutrients (total fat, carbohydrate, and protein consumption), composite categories of food items (calorie-adjusted intake of red/processed meat, vegetables/fruit, and dairy products), glycemic load, and total fiber.
3) We estimated the confounding effect of individual foods rich in spermidine.
4) To further minimize the potential of confounding by healthy diet and lifestyle, we substituted the ratio of spermidine to overall polyamine intake for spermidine intake and also adjusted for the Alternative Healthy Eating Index (online supporting material, page 4) (37). Because spermidine intake is presumably part of a healthy diet, this analysis is expected to yield conservative risk estimates.
5) We examined the robustness of our results from the influence of unmeasured confounding (38) and corrected for measurement error in spermidine intake and covariates by means of regression calibration and data from our validation study (39).

Finally, we compared the effect size on survival associated with low compared with high spermidine intake (comparison of extreme thirds) and that associated with chronological age by comparing the relevant β coefficients extracted from an age-, sex- and caloric ratio–adjusted model (with CIs calculated by using the bootstrap with 10,000 replications).

All P values are 2-sided, and an α level of 0.05 was used. Analyses were conducted with the use of R 3.2.2 (R Foundation for Statistical Computing) and the packages *survival, flexsurv, obsSens,* and *cmprsk*.

RESULTS

Spermidine intake

Spermidine intake was greater in women than in men and declined with age (**Supplemental Table 6**), but did not change with calendar time independent of age and sex ($P = 0.138$). Spermidine's share in overall polyamine intake amounted to 26.0% (95% CI: 21.2%, 31.1%). The main sources of spermidine intake in the Bruneck Study were whole grains (13.4% of total), apples and pears (13.3%), salad (9.8%), vegetable sprouts (7.3%), and potatoes (6.4%) (**Supplemental Figure 2**). Characteristics of the study population according to spermidine intake (tertile groups) are shown in **Table 1**.

Spermidine intake and mortality

During 13,019 person-years at risk, 341 deaths were recorded (median time to death: 9.8 y): 137 from vascular disease, 94 from cancer, and 110 from other causes. The crude overall rates (95% CIs) of death significantly decreased across tertile groups of increasing spermidine intake: 40.5 (36.1, 44.7), 23.7 (20.0, 27.0), and 15.1 (12.6, 17.8) per 1000 person-years corresponding to an age-, sex- and caloric intake–adjusted 20-y cumulative incidence of death of 0.48 (0.5, 0.51), 0.41 (0.38, 0.45), and 0.38 (0.34, 0.41) (**Table 2**, **Supplemental Figure 3**).

The inverse association between spermidine intake and all-cause mortality remained significant under progressive adjustment: respective HRs (95% CIs) for a 1-SD higher spermidine intake in unadjusted; age-, sex-, and caloric ratio adjusted; and further multivariable adjusted models were 0.62 (0.55, 0.69), $P < 0.001$; 0.74 (0.66, 0.83), $P < 0.001$; and 0.76 (0.67, 0.86), $P < 0.001$] (Table 2). The inverse association was of a linear dose-response type (P-nonlinear = 0.71 by penalized cubic splines).

TABLE 2
Total mortality according to dietary polyamine intake[1]

	Tertile				Entire group	
	Tertile 1 (low intake)	Tertile 2	Tertile 3 (high intake)	*P*	Per 1-SD higher intake	*P*
Spermidine group						
Person-years, *n*	4227	4353	4439	—	—	—
Deaths, *n*	171	103	67	—	—	—
Incidence rate per 1000 person-years	40.5 (36.1, 44.7)	23.7 (20.0, 27.0)	15.1 (12.6, 17.8)	—	—	—
Twenty-year cumulative incidence of death: age-, sex-, and caloric intake–adjusted	0.48 (0.45, 0.51)	0.41 (0.38, 0.45)	0.38 (0.34, 0.41)	—	—	—
Unadjusted HR	1.00	0.57 (0.45, 0.73)	0.37 (0.28, 0.49)	<0.001	0.62 (0.55, 0.69)	<0.001
Age-, sex-, caloric ratio–adjusted HR	1.00	0.77 (0.60, 0.99)	0.56 (0.42, 0.74)	<0.001	0.74 (0.66, 0.83)	<0.001
Age-, sex-, caloric ratio–adjusted HR: baseline spermidine intake	1.00	0.80 (0.62, 1.03)	0.61 (0.47, 0.81)	<0.001	0.75 (0.67, 0.83)	<0.001
Age-, sex-, caloric ratio–adjusted HR: noncumulative update	1.00	0.79 (0.62, 1.01)	0.63 (0.47, 0.83)	<0.001	0.80 (0.71, 0.89)	<0.001
Multivariable-adjusted HR[2]	1.00	0.82 (0.63, 1.05)	0.61 (0.45, 0.83)	0.002	0.76 (0.67, 0.86)	<0.001
Multivariable-adjusted HR: baseline spermidine intake[2]	1.00	0.83 (0.64, 1.08)	0.70 (0.52, 0.94)	0.002	0.78 (0.69, 0.88)	<0.001
Multivariable-adjusted HR: additional adjustment[3]	1.00	0.92 (0.67, 1.25)	0.76 (0.49, 1.18)	0.23	0.73 (0.60, 0.90)	0.004
Multivariable-adjusted HR: first 5 y of follow-up excluded[2]	1.00	0.76 (0.56, 1.02)	0.55 (0.39, 0.79)	0.001	0.72 (0.62, 0.84)	<0.001
Multivariable-adjusted HR: spermidine-to-polyamine ratio[2,4]	1.00	0.64 (0.49, 0.84)	0.61 (0.45, 0.81)	<0.001	0.76 (0.67, 0.87)	<0.001
Multivariable-adjusted HR: spermidine-to-polyamine ratio and AHEI adjustment[2,4]	1.00	0.72 (0.55, 0.95)	0.69 (0.51, 0.93)	0.014	0.83 (0.73, 0.94)	0.004
Spermine group						
Age-, sex-, caloric ratio–adjusted HR	1.00	0.84 (0.65, 1.08)	0.84 (0.65, 1.08)	0.175	0.90 (0.81, 1.00)	0.048
Multivariable-adjusted HR[2]	1.00	0.87 (0.67, 1.12)	0.83 (0.64, 1.09)	0.174	0.89 (0.80, 0.99)	0.039
Putrescine group						
Age-, sex-, caloric ratio–adjusted HR	1.00	0.85 (0.66, 1.11)	1.01 (0.78, 1.31)	0.95	1.01 (0.91, 1.12)	0.90
Multivariable-adjusted HR[2]	1.00	0.81 (0.62, 1.07)	1.01 (0.77, 1.32)	0.90	1.02 (0.91, 1.14)	0.78

[1] Values are HRs (95% CIs) or *n* (%) unless otherwise indicated. Person-years of follow-up for each participant were accrued from the 1995 baseline until death or 31 October 2015; 95% CIs for crude incidence rates were computed as bias-corrected accelerated bootstrap CIs. The 20-y cumulative incidence of all-cause death in thirds of spermidine intake was estimated by using parametric survival analysis, accounting for age, sex, and caloric intake by inverse probability weighting. HRs (95% CIs) were derived from Cox regression analysis with time-varying covariates (updated every 5 y) and calculated for tertile groups (columns 2–4; *P*-trend across tertile groups in column 5) or a 1-SD higher $\log_e$-transformed calorie-adjusted cumulatively updated spermidine intake, which corresponds to a 37.6% higher intake (column 6 with corresponding *P* values in column 7). Sensitivity analyses used a noncumulative update of spermidine intake or focused on baseline spermidine intake only. AHEI, Alternative Healthy Eating Index.

[2] Analyses were adjusted for age, sex, and the caloric ratio (variable reflecting caloric excess or restriction). The multivariable models were additionally adjusted for socioeconomic status, physical activity level, alcohol consumption (grams per day), BMI, smoking, diabetes, hypertension, and aspirin medication. For units of covariates, see Table 1.

[3] Additionally adjusted for a propensity score that reflected associations between spermidine intake and macronutrients (total fat, carbohydrate, and protein consumption expressed as percentage of calorie intake), composite categories of food items (calorie-adjusted intake of red/processed meat, vegetables/fruit, and dairy products), glycemic load, and total fiber.

[4] We substituted the ratio of spermidine to overall polyamine intake for spermidine intake and also adjusted for the AHEI.

Compared with participants in the bottom third of spermidine intake, the age-, sex-, and caloric ratio–adjusted HRs (95% CIs) of participants in the middle and top third were 0.77 (0.60, 0.99) and 0.56 (0.42, 0.74) and corresponding multivariable-adjusted HRs were 0.82 (0.63, 1.05) and 0.61 (0.45, 0.83) (*P*-trend < 0.001 and 0.002). This reduction in mortality risk equaled the effect attributable to a 5.7-y (95% CI: 3.6, 8.1 y) difference in chronological age. **Supplemental Figure 4** shows *P* values for the associations between 146 nutrients and all-cause mortality.

Findings were not appreciably different when focusing on baseline spermidine intake rather than cumulatively updated values (Table 2) and baseline spermidine intake was associated with mortality over the entire follow-up [i.e., age-, sex-, caloric ratio–adjusted HRs (95% CIs) were 0.81 (0.65, 1.01) for deaths occurring between 1995 and 2000, 0.73 (0.59, 0.91) for 2000–2005, 0.69 (0.55, 0.87) for 2005–2010, and 0.72 (0.56, 0.91) for 2010–2015].

Subgroup and sensitivity analyses

The inverse relation between spermidine intake and all-cause mortality was stable in women, men, and numerous

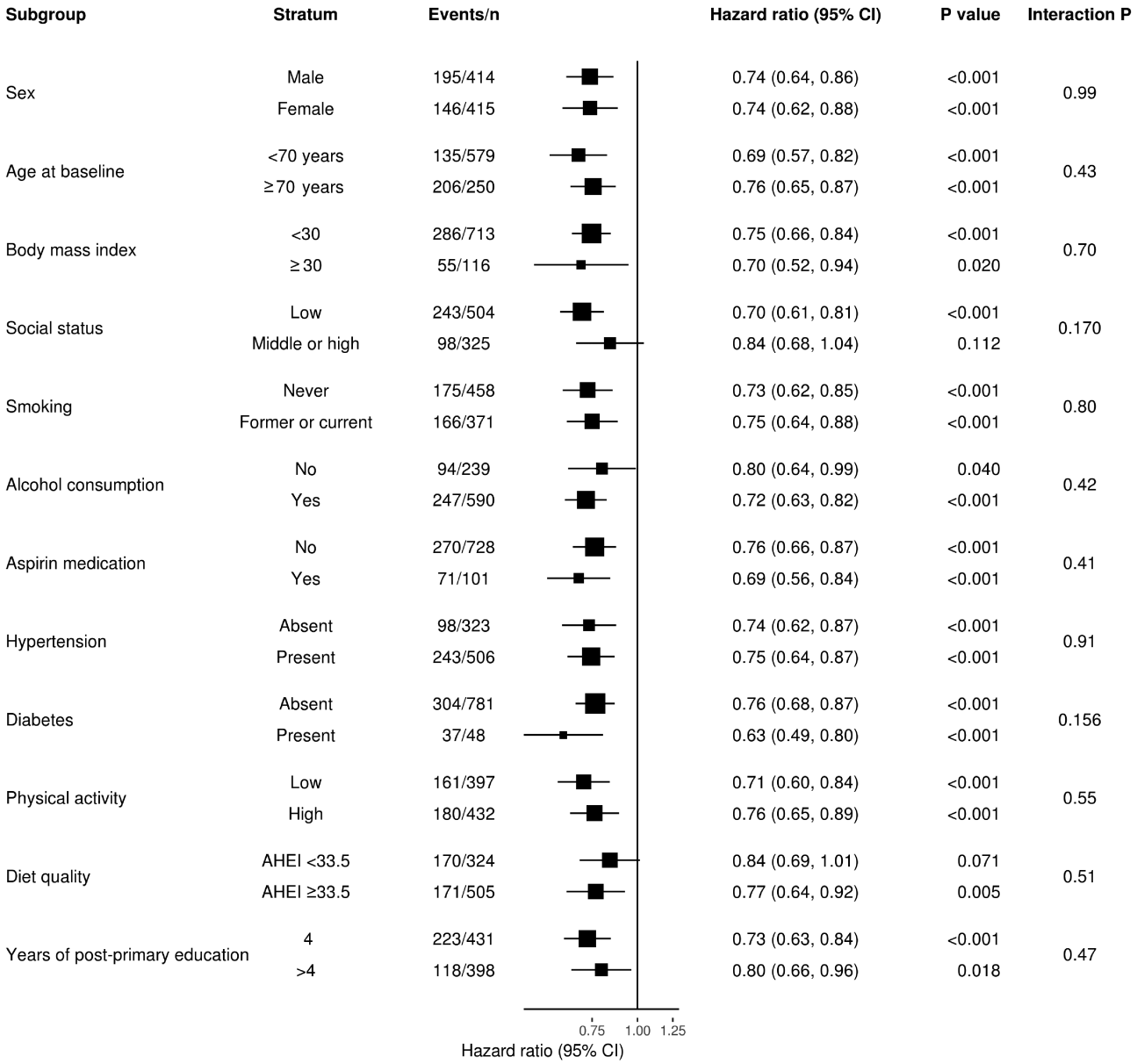

FIGURE 2 HRs for all-cause mortality according to spermidine intake in subgroups. The HRs presented are for a 1-SD higher spermidine intake. Physical activity level was considered high if participants exercised ≥42 metabolic-equivalent hours/wk. The AHEI cutoff was chosen on the basis of the mode of the AHEI distribution. Results were calculated with the use of Cox regression. AHEI, Alternative Healthy Eating Index.

subgroups (**Figure 2**). Analyses excluding the initial 5 y of follow-up or additionally adjusting for a variety of lifestyle and dietary characteristics yielded similar results (Table 2). Moreover, we found no relevant attenuation of the association of spermidine with mortality when adjusting for individual foods that contributed to total spermidine intake, whereas beneficial effects of whole grains and several fruit and vegetables declined under adjustment for spermidine intake (**Supplemental Table 7**). Lower spermidine intake at older ages was, in part, attributable to decreased salad consumption and adjustment for salad also had no relevant effect on the key association (Supplemental Table 7). Moreover, there was no evidence of a differential association between spermidine and mortality dependent on the dietary origin of spermidine (data not shown). Correction for measurement error increased the magnitude of association between spermidine intake and mortality (age-, sex-, and caloric ratio–adjusted HR: 0.60; 95% CI: 0.50, 0.74), whereas the use of the spermidine-to-polyamine ratio and control for the Alternate Healthy Eating Index resulted in weakened, yet still significant, associations (Table 2). Potential effects of a hypothetical unmeasured confounder are shown in **Supplemental Table 8** with the putative confounder characterized by its correlation with spermidine intake ($r = 0.0$–1.0; rows) and association with mortality (HRs: 1.0–0.5; columns). This sensitivity analysis indicates that only a confounder strongly associated with both mortality (e.g., HR = 0.6) and spermidine intake (e.g., $r = 0.4$) could pretend an association of the observed strength in the absence of a true association.

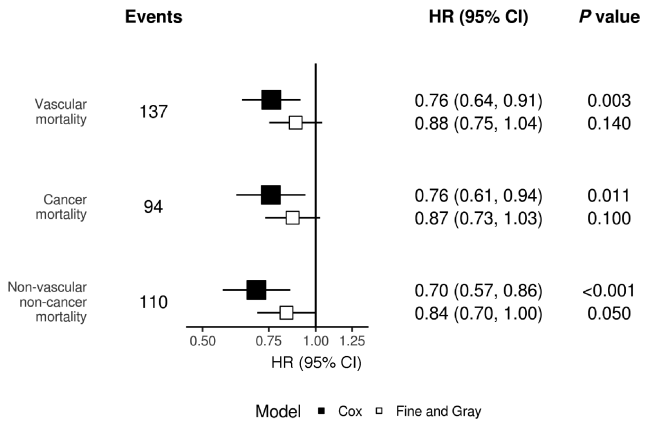

FIGURE 3 HRs for cause-specific mortality according to spermidine intake in the Bruneck Study. HRs (95% CIs) were calculated for a 1-SD higher spermidine intake with adjustment for age, sex, and caloric ratio. Results are from cause-specific hazard models (solid squares) and Fine-Gray subdistribution hazard models (open squares) accounting for competing risks of death. Cause-specific HRs are the preferred choice for studying disease etiology (our main focus), whereas subdistribution HRs are better suited for the prediction of individual risks. The Fine-Gray models rely on baseline spermidine intake only because time-varying variables cannot be modeled in this setting, whereas cause-specific Cox models rely on cumulatively updated spermidine intake.

Spermidine intake was inversely related to all major causes of death; however, more endpoints would be required to draw definite conclusions. **Figure 3** and Supplemental Table 5 depict cause-specific HRs, and Figure 3 also presents data from Fine-Gray subdistribution hazard models.

No associations were observed for putrescine (Table 2) and arginine or methionine intakes (data not shown), which are natural sources (i.e., metabolic precursors) of endogenous polyamine synthesis. Spermine intake showed weak inverse associations and the association for all-cause mortality was significant (Table 2).

External replication

In the SAPHIR study, spermidine intake was quantitatively similar to that observed in the Bruneck Study (geometric mean: 74.5 μmol/d) and higher in women ($P < 0.001$). The contribution of individual foods to spermidine intake in SAPHIR compared with the Bruneck Study was nearly identical for salad (10.8%), potatoes (6.6%), and fruit (24.1%) but lower for whole grains (6.5%) and higher for vegetables other than salad (30.5%). A total of 48 participants died: 7 from vascular disease, 29 from cancer, and 12 from other causes. Spermidine intake was inversely related to mortality with age-, sex-, and caloric ratio–adjusted and multivariable HRs (95% CIs) per 1-SD unit of higher spermidine intake of 0.71 (0.53, 0.95) and 0.74 (0.56, 0.99), which is similar to the estimates generated in the Bruneck Study (**Supplemental Table 9**).

Caloric ratio and mortality

In both the Bruneck and SAPHIR studies, low caloric ratio, a measure of caloric restriction, exhibited significant associations with all-cause mortality [age- and sex-adjusted HRs (95% CIs) for a 1-SD lower caloric ratio: 0.86 (0.78, 0.94) and 0.69 (0.56, 0.85); $P = 0.001$ and $P < 0.001$, respectively], which remained significant after adjustment for spermidine intake.

DISCUSSION

Spermidine and life span

Determinants of increased survival are of great historical and current interest (3) and potential effects of nutritional interventions on survival have been extensively studied in short-lived model organisms and human cell lines, including caloric restriction of ∼30% beneath the level of ad libitum feeding and spermidine supplementation (3). Both interventions similarly reduced the acetylation of multiple cellular proteins including histones (and hence transcriptional programs) and cytoplasmic enzymes (and hence metabolic functions), processes critical for cell homeostasis in aging and starvation (Figure 1) (5, 9–10), and effectively induced autophagy, a cytoprotective self-digestive process and key to longevity (5, 40, 41). Although there is some reason to assume that caloric restriction favors healthy aging in humans (42), a concept also corroborated by the current analyses, no respective data have so far been available for spermidine.

To our knowledge, our study is the first to show an inverse relation between the amount of dietary spermidine intake and all-cause mortality in the general community (Table 2). The association emerged as independent of other determinants of longevity and lifestyle. The survival advantage was driven by a reduced risk of death from all major causes. The key association was highly consistent in subgroups (Figure 2) and successfully replicated in an independent cohort from the same geographical region. It exhibited a linear dose-response type and particular strength. Spermidine showed the strongest inverse relation with mortality among 146 nutrients studied (Supplemental Figure 4). The reduction in mortality risk related to a diet rich in spermidine (top compared with bottom third of spermidine intake) was comparable to that associated with a 5.7-y younger age. All of the findings apply to spermidine from dietary sources and to amounts characteristically found in the Western diet and cannot readily be extrapolated to high-dose spermidine supplementation or extreme diets.

To minimize the risk of residual confounding by lifestyle and dietary patterns related to polyamine intake, additional analyses were carefully adjusted for multiple features of diet (composite categories of food item, macronutrients, individual foods, and indexes of a healthy or unhealthy diet), but none of these approaches attenuated the key association. Moreover, the spermidine-mortality association emerged as robust from the influence of unmeasured confounding and did not extend to putrescine, another major polyamine contained in many healthy foods (see below). Finally, we provide evidence against reverse causation and corrected for measurement error.

Our human data are appealing in view of the rapidly expanding knowledge about potential favorable effects of spermidine and the solid experimental evidence linking spermidine intake with a longer life span in model organisms, including mammals (Supplemental Table 1). Increased survival after lifelong and late-in-life oral supplementation of spermidine has recently been

shown in mice (4). Previous experiments in mice achieved increased survival by food supplementation with probiotics that amplified gut microbiota polyamine synthesis or reduced midlife mortality by a polyamine-rich diet in short-lived mouse strains (6, 7).

Potential mechanistic links

Spermidine may be linked to survival by its capacity to restore or induce efficient autophagy (5) and by other mechanisms (Figure 1). With regard to vascular disease, autophagy assists in recycling damaged and potentially harmful cellular material by sequestration within autophagosomes and lysosomal digestion (43). It was reported to enhance resistance of cells to stress conditions, clear dysfunctional mitochondria, curtail inflammation, restore nitric oxide bioavailability (12), and reduce arterial deposition of advanced glycation end-products in mice (12). Other reports suggested antiplatelet effects of spermidine in rabbits (13) and potential roles in antioxidative defense in human endothelial cells (14) and lipid metabolism (15), including lipid efflux from advanced plaques in mice (16). A recent landmark study found blood pressure–lowering and multiple cardioprotective effects of spermidine in mice and rats, potentially mediated by enhanced global arginine bioavailability and improved cardiac autophagy and mitophagy (4). Spermidine supplementation delayed the development of hypertensive heart disease and protected from hypertension-associated renal damage in this study (4).

With regard to cancer, tumor-suppressive effects of autophagy involve genomic stabilization, limitation of inflammation, and facilitation of adequate immune responses against cancer cells (43); and spermidine supplemented in drinking water was shown to enhance anticancer immuno-surveillance in mice in an autophagy-dependent manner (44). Initial research in knockout and transgenic mice suggested that amplification of polyamine synthesis is capable of promoting carcinogenesis (43); however, current research in aged mice showed no elevated risk of cancer upon spermidine feeding, but even lower rates of colon and some other (6, 44, 45) tumors and consistent human data have been published recently (46).

Moreover, polyamine production by the microbiota was shown to suppress low-grade inflammation in the colon, restore colonic barrier function, and protect against age-dependent memory impairment in mice (6, 47). Oral administration of spermidine protected flies against age-induced memory impairment (48, 49) and alleviated experimental autoimmune encephalitis in a mouse model of multiple sclerosis (50).

Other polyamines

The key association in our study applies to spermidine and, to a lesser extent, to spermine, which may be converted into spermidine or vice versa, synthesized from spermidine by regulatory circuits involving the enzyme spermine synthase (Table 2), but not to putrescine. In this context, it has to be emphasized that experiments convincingly linked spermidine (and spermine) to increased survival but not putrescine (4). Moreover, spermidine is the polyamine most readily absorbed from human gut without intestinal metabolism (40–80%), whereas putrescine is almost entirely metabolized. Finally, there is strong evidence for separate regulation of each of the 3 polyamines (1, 2, 51).

Merits and limitations

Strengths of our study include its population-based design, long-term virtually complete follow-up, repeated and validated high-quality assessment of diet (assisted by dietitians), careful ascertainment of causes of death, and replication in an independent cohort. There are limitations as well. Despite the considerable efforts, bias due to complex measurement error and residual confounding cannot be ruled out. Moreover, constraints inherent to nutritional epidemiology apply to our study as well. These include measurement error due to participants' self-report of diet and the nonconsideration of storage conditions and food preparation for each food, which would be beyond the scope of FFQs but may affect spermidine content. Finally, all of the participants were white, and findings do not necessarily apply to other ethnicities with different socioeconomic backgrounds and demographic characteristics.

Conclusions

In summary, this study provides the first evidence, to our knowledge, for an association between nutrition rich in spermidine and increased survival in humans. These data add to experimental findings that suggest longevity-inducing and health-promoting effects of spermidine in model organisms and human cell lines (2, 3, 5–7). If confirmed in future intervention trials, our study may have implications for health education at a population level advocating high spermidine content as a novel feature of a healthy diet.

The authors' responsibilities were as follows— SK, JW, BP, HT, and FM: provided essential reagents or provided essential materials; RP and SK: analyzed data or performed statistical analysis; SK and RP: wrote the manuscript; JW, SK, and FM: had primary responsibility for final content; SK, RP, P Willeit, MN, KW, P Werner, BP, BI, LK, MC, CR, SW, BM, MG, SS, HS, FO, GK, MM, TE, HT, FM, and JW: contributed to data acquisition and critical revision of the manuscript for important intellectual content; and all authors: conducted research (hands-on conduct of the experiments and data collection) and read and approved the final manuscript. All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). FM, TE, and SS have equity interests in TLL, a company founded in 2016 that will develop natural food extracts. All of the other authors declared no financial relationships with any company or organization that might have an interest in the submitted work in the previous 3 y, and no other relationships or activities exist that could appear to have influenced the submitted work.

REFERENCES

1. Igarashi K, Kashiwagi K. Modulation of cellular function by polyamines. Int J Biochem Cell Biol 2010;42:39–51.
2. Minois N, Carmona-Gutierrez D, Madeo F. Polyamines in aging and disease. Aging (Albany NY) 2011;3:716–32.
3. de Cabo R, Carmona-Gutierrez D, Bernier M, Hall MN, Madeo F. The search for antiaging interventions: from elixirs to fasting regimens. Cell 2014;157:1515–26.
4. Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, Harger A, Schipke J, Zimmermann A, Schmidt A et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat Med 2016;22:1428–38.

5. Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L et al. Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 2009;11:1305–14.
6. Matsumoto M, Kurihara S, Kibe R, Ashida H, Benno Y. Longevity in mice is promoted by probiotic-induced suppression of colonic senescence dependent on upregulation of gut bacterial polyamine production. PLoS One 2011;6:e23652.
7. Soda K, Dobashi Y, Kano Y, Tsujinaka S, Konishi F. Polyamine-rich food decreases age-associated pathology and mortality in aged mice. Exp Gerontol 2009;44:727–32.
8. Tabor CW, Tabor H. Polyamines. Annu Rev Biochem 1984;53:749–90.
9. Marino G, Pietrocola F, Eisenberg T, Kong Y, Malik SA, Andryushkova A, Schroeder S, Pendl T, Harger A, Niso-Santano M et al. Regulation of autophagy by cytosolic acetyl-coenzyme A. Mol Cell 2014;53: 710–25.
10. Morselli E, Marino G, Bennetzen MV, Eisenberg T, Megalou E, Schroeder S, Cabrera S, Benit P, Rustin P, Criollo A et al. Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. J Cell Biol 2011;192:615–29.
11. Pietrocola F, Lachkar S, Enot DP, Niso-Santano M, Bravo-San Pedro JM, Sica V, Izzo V, Maiuri MC, Madeo F, Marino G et al. Spermidine induces autophagy by inhibiting the acetyltransferase EP300. Cell Death Differ 2015;22:509–16.
12. LaRocca TJ, Gioscia-Ryan RA, Hearon CM Jr., Seals DR. The autophagy enhancer spermidine reverses arterial aging. Mech Ageing Dev 2013;134:314–20.
13. de la Pena NC, Sosa-Melgarejo JA, Ramos RR, Mendez JD. Inhibition of platelet aggregation by putrescine, spermidine, and spermine in hypercholesterolemic rabbits. Arch Med Res 2000;31: 546–50.
14. Yang H, Lee SE, Kim GD, Park HR, Park YS. Hemeoxygenase-1 mediates an adaptive response to spermidine-induced cell death in human endothelial cells. Oxid Med Cell Longev 2013;2013: 238734.
15. Minois N. Molecular basis of the "anti-aging" effect of spermidine and other natural polyamines—a mini-review. Gerontology 2014;60:319–26.
16. Michiels CF, Kurdi A, Timmermans JP, De Meyer GR, Martinet W. Spermidine reduces lipid accumulation and necrotic core formation in atherosclerotic plaques via induction of autophagy. Atherosclerosis 2016;251:319–27.
17. Atiya AM, Poortvliet E, Stromberg R, Yngve A. Polyamines in foods: development of a food database. Food Nutr Res 2011;55:5572.
18. Zoumas-Morse C, Rock CL, Quintana EL, Neuhouser ML, Gerner EW, Meyskens FL Jr. Development of a polyamine database for assessing dietary intake. J Am Diet Assoc 2007;107:1024–7.
19. Nishimura K, Shiina R, Kashiwagi K, Igarashi K. Decrease in polyamines with aging and their ingestion from food and drink. J Biochem 2006;139:81–90.
20. Willeit P, Willeit J, Mayr A, Weger S, Oberhollenzer F, Brandstatter A, Kronenberg F, Kiechl S. Telomere length and risk of incident cancer and cancer mortality. JAMA 2010;304:69–75.
21. Willeit P, Kiechl S, Kronenberg F, Witztum JL, Santer P, Mayr M, Xu Q, Mayr A, Willeit J, Tsimikas S. Discrimination and net reclassification of cardiovascular risk with lipoprotein(a): prospective 15-year outcomes in the Bruneck Study. J Am Coll Cardiol 2014;64:851–60.
22. Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G et al. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. Circulation 2014;129:1821–31.
23. Kiechl S, Wittmann J, Giaccari A, Knoflach M, Willeit P, Bozec A, Moschen AR, Muscogiuri G, Sorice GP, Kireva T et al. Blockade of receptor activator of nuclear factor-kappaB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. Nat Med 2013;19:358–63.
24. Kiechl S, Lorenz E, Reindl M, Wiedermann CJ, Oberhollenzer F, Bonora E, Willeit J, Schwartz DA. Toll-like receptor 4 polymorphisms and atherogenesis. N Engl J Med 2002;347:185–92.
25. Langley SR, Willeit K, Didangelos A, Matic LP, Skroblin P, Barallobre-Barreiro J, Lengquist M, Rungger G, Kapustin A, Kedenko L et al. Extracellular matrix proteomics identifies molecular signature of symptomatic carotid plaques. J Clin Invest 2017;127:1546–60.
26. Melmer A, Lamina C, Tschoner A, Ress C, Kaser S, Laimer M, Sandhofer A, Paulweber B, Ebenbichler CF. Body adiposity index and other indexes of body composition in the SAPHIR study: association with cardiovascular risk factors. Obesity (Silver Spring) 2013;21:775–81.
27. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
28. Gebhardt SE, Lemar LE, Pehrsson PR, Exler J, Haytowitz DB, Showell BA, Nickle MS, Thomas RG, Patterson KK, Bhagwat SA et al. USDA national nutrient database for standard reference, release 23. Available from: http://www.ars.usda.gov/nutrientdata. (accessed 20 December, 2017).
29. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol 1999;149:531–40.
30. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
31. Cole SR, Hernan MA. Adjusted survival curves with inverse probability weights. Comput Methods Programs Biomed 2004;75:45–9.
32. Royston P, Parmar MK. Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects. Stat Med 2002;21:2175–97.
33. Seshasai SR, Kaptoge S, Thompson A, Di AE, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I et al. Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med 2011;364:829–41.
34. Pintilie M. Analysing and interpreting competing risk data. Stat Med 2007;26:1360–7.
35. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509.
36. Austin PC, Lee DS, Fine JP. Introduction to the analysis of survival data in the presence of competing risks. Circulation 2016;133:601–9.
37. McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, Spiegelman D, Hunter DJ, Colditz GA, Willett WC. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. Am J Clin Nutr 2002;76:1261–71.
38. Lin DY, Psaty BM, Kronmal RA. Assessing the sensitivity of regression results to unmeasured confounders in observational studies. Biometrics 1998;54:948–63.
39. Keogh RH, White IR. A toolkit for measurement error correction, with a focus on nutritional epidemiology. Stat Med 2014;33:2137–55.
40. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153:1194–217.
41. Madeo F, Pietrocola F, Eisenberg T, Kroemer G. Caloric restriction mimetics: towards a molecular definition. Nat Rev Drug Discov 2014;13:727–40.
42. Willcox BJ, Willcox DC. Caloric restriction, caloric restriction mimetics, and healthy aging in Okinawa: controversies and clinical implications. Curr Opin Clin Nutr Metab Care 2014;17:51–8.
43. Rubinsztein DC, Marino G, Kroemer G. Autophagy and aging. Cell 2011;146:682–95.
44. Pietrocola F, Pol J, Vacchelli E, Rao S, Enot DP, Baracco EE, Levesque S, Castoldi F, Jacquelot N, Yamazaki T et al. Caloric restriction mimetics enhance anticancer immunosurveillance. Cancer Cell 2016;30:147–60.
45. Soda K, Kano Y, Chiba F, Koizumi K, Miyaki Y. Increased polyamine intake inhibits age-associated alteration in global DNA methylation and 1,2-dimethylhydrazine-induced tumorigenesis. PLoS One 2013;8:e64357.
46. Vargas AJ, Ashbeck EL, Wertheim BC, Wallace RB, Neuhouser ML, Thomson CA, Thompson PA. Dietary polyamine intake and colorectal cancer risk in postmenopausal women. Am J Clin Nutr 2015;102:411–9.
47. Kibe R, Kurihara S, Sakai Y, Suzuki H, Ooga T, Sawaki E, Muramatsu K, Nakamura A, Yamashita A, Kitada Y et al. Upregulation of colonic luminal polyamines produced by intestinal microbiota delays senescence in mice. Sci Rep 2014;4:4548.
48. Gupta VK, Scheunemann L, Eisenberg T, Mertel S, Bhukel A, Koemans TS, Kramer JM, Liu KS, Schroeder S, Stunnenberg HG et al. Restoring

polyamines protects from age-induced memory impairment in an autophagy-dependent manner. Nat Neurosci 2013;16:1453–60.
49. Gupta VK, Pech U, Bhukel A, Fulterer A, Ender A, Mauermann SF, Andlauer TF, Antwi-Adjei E, Beuschel C, Thriene K et al. Spermidine suppresses age-associated memory impairment by preventing adverse increase of presynaptic active zone size and release. PLoS Biol 2016;14:e1002563.
50. Yang Q, Zheng C, Cao J, Cao G, Shou P, Lin L, Velletri T, Jiang M, Chen Q, Han Y et al. Spermidine alleviates experimental autoimmune encephalomyelitis through inducing inhibitory macrophages. Cell Death Differ 2016;23:1850–61.
51. Larque E, Sabater-Molina M, Zamora S. Biological significance of dietary polyamines. Nutrition 2007;23:87–95.