

Dietary polyamine intake and colorectal cancer risk in postmenopausal women^{1,2}

Ashley J Vargas,^{3*} Erin L Ashbeck,⁶ Betsy C Wertheim,⁶ Robert B Wallace,⁷ Marian L Neuhauser,⁸ Cynthia A Thomson,^{3,5,6} and Patricia A Thompson^{3,4,6,9}

Departments of ³Nutritional Sciences, ⁴Molecular and Cellular Biology, and ⁵College of Public Health, The University of Arizona, Tucson, AZ; ⁶The University of Arizona Cancer Center, Tucson, AZ; ⁷University of Iowa College of Public Health, University of Iowa, Iowa City, IA; and ⁸Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, WA

ABSTRACT

Background: Putrescine, spermidine, and spermine (i.e., polyamines) are small cationic amines synthesized by cells or acquired from the diet or gut bacteria. Polyamines are required for both normal and colorectal cancer (CRC) cell growth.

Objective: We investigated the association between dietary polyamines and risk of CRC incidence and mortality.

Design: The study was a prospective analysis in 87,602 postmenopausal women in the Women's Health Initiative Observational Study. Multivariate Cox regression was used to calculate HRs and 95% CIs.

Results: Total dietary polyamine intake (mean \pm SD: 289.2 \pm 127.4 μ mol/d) was not positively associated with CRC in fully adjusted models. Instead, intake \geq 179.67 μ mol/d was associated with reduced risk of CRC [HR (95% CI): 0.82 (0.68, 1.00), 0.81 (0.66, 0.99), 0.91 (0.74, 1.12), and 0.80 (0.62, 1.02) for quintiles 2–5, respectively, compared with quintile 1]. Reduced risk was not significant across all quintiles. Polyamines were not significantly associated with CRC-specific mortality in fully adjusted models. When stratified by risk factors for CRC, only body mass index (BMI) and fiber intake significantly modified the association between polyamine intake and CRC. In women with BMI (in kg/m²) \leq 25 or fiber consumption above the median, polyamine intake was associated with significantly lower risk of CRC.

Conclusions: No positive association between dietary polyamines and CRC or CRC-specific mortality risk in women was observed. Instead, a protective effect of dietary polyamines was suggested in women with some CRC risk-lowering behaviors in particular. These results are consistent with emerging evidence that exogenous polyamines may be beneficial in colon health and warrant additional study. *Am J Clin Nutr* 2015;102:411–9.

Keywords: colon cancer, colorectal cancer, diet, dietary polyamines, dietary putrescine, polyamines, putrescine, Women's Health Initiative

INTRODUCTION

The cationic diamine putrescine and its triamine derivatives spermidine and spermine play an essential role in tumor growth, thereby serving as a rationale for targeting the inhibition of intracellular polyamine biosynthesis for the treatment and prevention of cancer (1–4). Relative to adjacent normal tissue and tissues undergoing normal rates of turnover, these polyamines are present

at high concentrations in tumor cells (5–7). Pharmacologic targeting of intracellular polyamines via the inhibition of ornithine decarboxylase, which is the rate-limiting enzyme that converts ornithine to putrescine, has been extensively investigated as a drug-based strategy to suppress tumor growth (1). Although of limited efficacy for advanced cancers (8), an irreversible inhibitor of ornithine decarboxylase (oral difluoromethylornithine) has demonstrated growth suppressive activities, on colorectal adenomatous polyps [i.e., premalignant precursor of colorectal cancer (CRC)] in animal (9) and human (10) studies. Thus, pharmacological studies have established evidence of a growth-promoting role of intracellular polyamines in early colorectal tumorigenesis and as a target for chemoprevention in high-risk populations.

Polyamines, from de novo synthesis, the diet, and products of gut microbiome metabolism, are made available to cells via active transport (11, 12). These extracellular sources of polyamines are essential for normal mammalian embryogenesis (13) and play a key role in the normal development and maintenance of the gastrointestinal tract (14). Polyamines are essential to normal cellular homeostasis (15). Inhibition studies showed that polyamines have important roles in the macromolecular synthesis of proteins and nucleic acids, regulation of gene and protein expression, and regulation of ion channels. More recent system-wide studies identified polyamines as immunomodulatory molecules and as important regulators in adult stem cell populations (16). The ability of probiotics to extend the life span in mice depends on bacterial-derived putrescine to inhibit colonic cell senescence (17).

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² Supplemental Tables 1–3 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

⁹ Present address: Basic Sciences Tower, Level 9, Stony Brook School of Medicine, Stony Brook, NY 11794-8691.

*To whom correspondence should be addressed. E-mail: ashley.vargas@nih.gov.

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These findings are consistent with a pro-growth effect on healthy colonic cells. Despite the recognized role of polyamines in both normal cellular homeostasis and intestinal tumor growth, there have been no studies, to our knowledge, that tested the association between dietary polyamines and human CRC.

The development of American (18) and Swedish (19, 20) dietary polyamine databases has facilitated the estimation of polyamine intake from foods for application to population studies. With these instruments, daily intake of polyamines (primarily from produce, fermented, and aged foods and beverages) has been estimated at $\sim 300 \mu\text{mol}$ in the United States (4, 18). With the use of the American database, we reported that dietary polyamine intake above the population median ($>289 \mu\text{mol/d}$) was associated with a significant 30% increase in odds of developing colorectal adenomatous polyps in patients with a history of forming colorectal adenomas (i.e., high-risk patient population for CRC) (4). Similarly, Raj et al. (21) reported that high polyamine intake reduced the efficacy of difluoromethylornithine when combined with sulindac for the prevention of colorectal adenomas in high-risk polyp formers enrolled in the difluoromethylornithine and sulindac trial previously mentioned (10). Despite these promising observations in patients at above-average risk of developing CRC, to our knowledge, no studies have investigated whether dietary polyamine intake influences carcinogenesis in a general population or in those with average risk of CRC.

Therefore, we sought to test the hypothesis that dietary polyamines increase risk of CRC and CRC-specific mortality in an average-risk cohort. In addition, because dietary polyamines may act as tumor promoters only in a high-risk background (4), we explored whether risk factors that increase the likelihood of having initiated colorectal epithelial cells (e.g., family history of CRC and older age) modify the effect of dietary polyamines on CRC. With the use of data from the WHI (Women's Health Initiative), which is a longitudinal cohort of postmenopausal women with dietary data, adjudicated outcomes, and extended follow-up, we provide the first study to our knowledge of dietary polyamine exposure and CRC risk in average-risk women.

METHODS

Study population

A total of 93,676 postmenopausal women aged 50–79 y were enrolled in the WHI Observational Study from 1993 to 1998 (22). Briefly, women throughout the United States who were either not eligible for or not interested in WHI clinical trials were invited to participate in the Observational Study. Women were ineligible if they had a life expectancy <3 y. Our study excluded participants without follow-up data ($n = 473$), with reported daily energy intakes <600 or >5000 kcal/d ($n = 3534$), or with an incomplete or missing food-frequency questionnaire ($n = 94$) at baseline. Participants who used a Hawaii-specific food-frequency questionnaire ($n = 1973$) that was not compatible with the dietary polyamine database were also excluded. A total of 87,602 women were available for the analysis, with a mean follow-up of 12.4 y; 1.4% of them experienced a CRC event while on study.

Dietary data

Average daily dietary intake over the preceding 3 mo was estimated at baseline by using the 122 line-item WHI food-frequency

questionnaire (23). Dietary polyamine (putrescine, spermidine, and spermine) estimates were calculated by the Fred Hutchinson Cancer Research Center Nutrition Assessment Shared Resource by adapting the Zoumas-Morse (18) dietary polyamine database for use with the WHI food-frequency questionnaire in conjunction with the Nutrient Data System for Research (software version 2005) developed by the Nutrition Coordinating Center, University of Minnesota Food and Nutrient Database. Before ranking the top contributors to total dietary polyamines, food items were grouped into distinct categories (e.g., all poultry together and all cheeses together).

Nondietary exposure data

At baseline, multiple questionnaires that detailed medical, demographic, dietary supplement, and family histories (first-degree relative) were administered (22). Exposure variables used in analyses from these questionnaires are defined in Statistical methods.

Outcome measures

CRC and CRC-specific mortality outcomes were reported to the WHI via mail or telephone annually, and cases were adjudicated on the basis of medical records, pathology reports, and an additional blinded review as previously described (24). Bowel screening for CRC in the study was wholly at the discretion of the participant and her physician. Our analysis includes outcome data through 17 September 2012. There were 1245 adjudicated cases of CRC (1133 cases with a known stage at diagnosis) during follow-up, of which 1047 cases were documented to be of colonic origin, and 157 were documented to be of rectal origin. Of 11,607 deaths during the observational period, 335 deaths were attributed to CRC.

Statistical methods

Associations between dietary nutrients related to CRC or polyamines were calculated by using Pearson's correlation. The percentage of contribution of distinct food categories to total dietary polyamine exposure was calculated at the person level and then ranked by the mean percentage. Cox regression was used to calculate HRs and 95% CIs for the association between quintiles of dietary polyamines and CRC. Only participants with CRC were at risk of CRC-specific death, and non-CRC-specific death is a competing risk of CRC-specific death. Thus, risk of CRC death was analyzed only in participants who had invasive, Surveillance, Epidemiology, and End Results-staged (24) CRC (local, regional, or distant but not in situ) by using a competing risk model to calculate a sub-HR, 95% CI, and *P* value as outlined by Fine and Gray (25) and adjusted for the stage at diagnosis and all model 3 covariates.

Statistical models were adjusted for potential confounders of the polyamine-CRC risk association or strong predictors of CRC on the basis of previous literature. Model 1 was adjusted only for age (continuous) (4, 26). Model 2 was adjusted as for model 1 and for total estimated dietary energy, household income ($<\$10,000$ – $\$19,999$, $\$20,000$ – $\$49,999$, $\$50,000$ – $\$99,999$, or $>\$100,000$), smoking pack-years (continuous) (27, 28), recreational physical activity (continuous metabolic equivalent task hour per week) (29), race-ethnicity (non-Hispanic white, black or African American, Hispanic or Latino, or other) (30), hormone-replacement therapy use (ever or never) (31), history of colonoscopy or sigmoidoscopy

(yes or no) (32), and BMI (in kg/m²; continuous) (33). Model 3 was adjusted as for model 2 and for continuous dietary exposures including total fiber and calcium because they are present in foods with a high polyamine content (4, 18, 19) and are related to CRC risk (34, 35). Arginine (36–38), dietary fat (37, 39), and folate equivalents (40) were also added to model 3 because of their putative effects on polyamine metabolism, presence in high-polyamine foods (4, 18–20), and association with CRC risk (37, 41–44). The association between CRC and dietary putrescine alone and, separately, spermidine plus spermine was evaluated under model 3 conditions. Schoenfeld's residuals were tested, and there was no evidence that proportional hazards assumptions were violated. Likelihood ratio

tests were conducted to analyze the overall effect of adding dietary polyamines (quintiles) to each Cox model.

Potential effect modifiers of the dietary polyamine–CRC association were tested by using a likelihood ratio test that compared model 3 to a model that included an interaction term. The following potential modifiers were evaluated because they are indicative of an initiated colorectal epithelium where polyamines may have a different effect than in a noninitiated colorectum (4, 13, 21, 45, 46): personal history of colorectal polyp removed, family history of CRC, older age, overweight or obese status, not using aspirin, and low fiber intake. In addition, arginine (21, 36, 47) and folate and folic acid (4, 40, 48) are involved in endogenous

TABLE 1Participant characteristics at baseline across quintiles of total dietary polyamine intake in the WHI Observational Study (*n* = 87,602)¹

| Participant characteristics at baseline | Total dietary polyamine intake, ² μmol/d | | | | |
|--|---|-----------------------------|-----------------------------|-----------------------------|------------------------------|
| | Quintile 1 (21.8–179.6) | Quintile 2 (179.7–243.0) | Quintile 3 (243.1–304.9) | Quintile 4 (305.0–384.7) | Quintile 5 (384.8–1293.7) |
| Participants, <i>n</i> (%) | 17,521 (20.0) ³ | 17,520 (20.0) | 17,520 (20.0) | 17,520 (20.0) | 17,520 (20.0) |
| Age, y | 63.3 ± 7.4 ⁴ | 63.5 ± 7.4 | 63.7 ± 7.3 | 63.9 ± 7.3 | 63.7 ± 7.4 |
| BMI, kg/m ² | 27.5 ± 6.0 | 27.3 ± 5.9 | 27.2 ± 5.7 | 27.1 ± 5.8 | 27.3 ± 6.0 |
| Race-ethnicity, <i>n</i> (%) | | | | | |
| Non-Hispanic white | 14,173 (81.2) | 15,004 (85.9) | 15,224 (87.1) | 15,492 (88.7) | 15,257 (87.3) |
| Black or African American | 1807 (10.4) | 1344 (7.7) | 1266 (7.2) | 1053 (6.0) | 1210 (6.9) |
| Hispanic or Latino | 884 (5.1) | 636 (3.6) | 542 (3.1) | 509 (2.9) | 587 (3.4) |
| Other | 598 (3.4) | 479 (2.7) | 453 (2.6) | 411 (2.4) | 431 (2.5) |
| Education, <i>n</i> (%) | | | | | |
| High school diploma or less | 5072 (29.2) | 3928 (22.6) | 3415 (19.6) | 2969 (17.1) | 2660 (15.3) |
| Some college | 8467 (48.7) | 8510 (49.0) | 8482 (48.8) | 8368 (48.1) | 8021 (46.2) |
| At least a college graduate | 3838 (22.1) | 4935 (28.4) | 5492 (31.6) | 6049 (34.8) | 6695 (38.5) |
| Annual household income, <i>n</i> (%) | | | | | |
| <\$10,000–\$19,999 | 3349 (20.7) | 2542 (15.7) | 2380 (14.6) | 2181 (13.4) | 2168 (13.3) |
| \$20,000–\$49,999 | 7319 (45.2) | 7107 (43.8) | 7099 (43.5) | 7107 (43.6) | 6877 (42.3) |
| \$50,000–\$99,999 | 4112 (25.4) | 4805 (29.6) | 4953 (30.4) | 5065 (31.1) | 5255 (32.3) |
| ≥\$100,000 | 1406 (8.7) | 1782 (11.0) | 1876 (11.5) | 1953 (12.0) | 1961 (12.1) |
| Previous bowel screening, ⁵ <i>n</i> (%) | 8967 (52.2) | 9263 (53.8) | 9423 (54.7) | 9727 (56.4) | 9819 (56.9) |
| Previous polyp or adenoma removed, ⁶ <i>n</i> (%) | 1705 (19.6) | 1695 (18.8) | 1673 (18.3) | 1702 (18.0) | 1728 (18.1) |
| Previous colorectal cancer, <i>n</i> (%) | 185 (1.1) | 145 (0.8) | 132 (0.8) | 172 (1.0) | 176 (1.0) |
| Family history of colorectal cancer, <i>n</i> (%) | 2624 (16.6) | 2704 (16.9) | 2592 (16.2) | 2750 (17.1) | 2751 (17.1) |
| Any hormone replacement therapy use, <i>n</i> (%) | | | | | |
| Never | 7292 (41.7) | 6791 (39.8) | 7042 (40.2) | 7004 (40.0) | 7124 (40.7) |
| Ever (past or current) | 10,211 (58.3) | 10,527 (60.2) | 10,466 (59.8) | 10,500 (60.0) | 10,380 (59.3) |
| Unopposed estrogen use, <i>n</i> (%) | | | | | |
| Never | 10,826 (61.8) | 10,937 (62.5) | 11,067 (63.2) | 10,994 (62.8) | 11,167 (63.8) |
| Ever (past or current) | 6681 (38.2) | 6597 (37.5) | 6444 (36.8) | 6513 (37.2) | 6342 (36.2) |
| Estrogen plus progesterone use, <i>n</i> (%) | | | | | |
| Never | 13,038 (74.4) | 12,476 (71.3) | 12,395 (70.8) | 12,414 (70.9) | 12,420 (70.9) |
| Ever (past or current) | 4477 (25.6) | 5034 (28.8) | 5122 (29.2) | 5103 (29.1) | 5090 (29.1) |
| Aspirin use, <i>n</i> (%) | | | | | |
| ≤1 y | 15,234 (87.0) | 15,103 (86.2) | 14,831 (84.7) | 14,771 (84.3) | 14,699 (83.9) |
| >1 y | 2287 (13.1) | 2417 (13.8) | 2690 (15.4) | 2749 (15.7) | 2820 (16.1) |
| NSAID use, ⁷ <i>n</i> (%) | | | | | |
| ≤1 y | 15,575 (88.9) | 15,476 (88.3) | 15,491 (88.4) | 15,552 (88.8) | 15,419 (88.0) |
| >1 y | 1946 (11.1) | 2044 (11.7) | 2030 (11.6) | 1968 (11.2) | 2100 (12.0) |
| Smoking, pack-years | 11.7 ± 20.3 | 10.8 ± 19.4 | 9.9 ± 18.2 | 9.3 ± 17.8 | 8.9 ± 17.7 |
| Physical activity, MET-h/wk | 10.7 ± 12.8 | 13.1 ± 14.1 | 13.8 ± 14.1 | 14.9 ± 14.5 | 16.4 ± 15.5 |

¹MET-h, metabolic equivalent task hours; NSAID, nonsteroidal anti-inflammatory drug; WHI, Women's Health Initiative.²Sum of dietary putrescine, spermidine, and spermine.³Frequency; percentage of total in parentheses (all such values for categorical variables).⁴Mean ± SD (all such values for continuous variables).⁵Any personal history of colonoscopy or sigmoidoscopy.⁶Only participants with a history of colonoscopy or sigmoidoscopy were considered for this variable.⁷Not including aspirin.

polyamine metabolism and, thus, have the potential to modify exogenous polyamine uptake and use. Further, the gut microbiome is responsive to BMI (49, 50) and fiber (51) and is believed to produce and use polyamines (17, 52, 53). Fiber, arginine, folate, and folic acid amounts were categorized into above (i.e., high intake) compared with below (i.e., low intake) median intakes to test for an effect modification. A sensitivity analysis was conducted to compare Cox-regression results with and without events that occurred within the first year of follow-up and with and without participants with a personal history of CRC. Participants were dropped from analyses when covariate data were missing (numbers of missing data by covariate were as follows: alcohol use, 29; adjusted energy intake, 8370; BMI, 1029; race-ethnicity, 242; education, 701; annual household income, 3823; previous colonoscopy or sigmoidoscopy, 1306; polyp or adenoma removed in subjects who received a colonoscopy or sigmoidoscopy, 1386; previous CRC, 7556; family history of CRC, 834; any hormone-replacement therapy use, 85; estrogen use, 64; estrogen plus progesterone use, 33; aspirin use, one; NSAID use, one; smoking, 3856; and physical activity, 992). Analyses were conducted with STATA SE 12.0 software (StataCorp LP).

RESULTS

Baseline participant and dietary characteristics

For the analyses of CRC endpoints, 87,602 participants were included with an mean \pm SD follow-up time of 12.4 ± 4.0 y.

The mean \pm SD age of participants at enrollment was 63 ± 7 y, and BMI was 27.3 ± 5.9 . Mean daily energy intake was 1574.2 ± 599.4 kcal/d, and total estimated polyamine intake (sum of putrescine, spermidine, and spermine) was 289.2 ± 127.4 μ mol/d. Mean daily intakes of specific dietary polyamines were 189.5, 64.7, and 34.9 μ mol/d for putrescine, spermidine, and spermine, respectively. Participants who were not white, with no more than a high school education, or with household income $<$ \$20,000, were more often in the lowest quintile of total dietary polyamine intake (**Table 1**). In addition, participants in the lowest quintile had the highest mean smoking pack-years and the lowest mean physical activity.

Dietary polyamine sources and correlates at baseline

Citrus fruit was the major source of total dietary polyamines. Top contributors included orange and grapefruit juice (20.6%) and oranges and grapefruit (11.0%) followed by cheddar, Swiss, and cream cheeses (8.0%); bananas and plantains (4.8%); and tomatoes and tomato products (3.3%; **Supplemental Table 1**). Because other dietary components increased along with intake of total polyamines (**Table 2**), we estimated correlations with other key dietary components (**Supplemental Table 2**). Particularly high correlations were observed between total polyamine intake and dietary fiber ($r = 0.669$, $P < 0.001$) and folate ($r = 0.820$, $P < 0.001$), which are notable because of the hypothesized roles of both fiber (35, 54) and folate (44) in CRC risk reduction.

TABLE 2

Participant dietary characteristics at baseline across quintiles of total dietary polyamine intake in the WHI Observational Study (1995-2012; $n = 87,602$)¹

| Dietary characteristics at baseline | Total dietary polyamine intake, ² μ mol/d | | | | |
|--|--|-----------------------------|-----------------------------|-----------------------------|------------------------------|
| | Quintile 1 (21.8–179.6) | Quintile 2 (179.7–243.0) | Quintile 3 (243.1–304.9) | Quintile 4 (305.0–384.7) | Quintile 5 (384.8–1293.7) |
| Total polyamine, μ mol/d ² | 136.1 \pm 30.8 ³ | 211.9 \pm 118.3 | 273.5 \pm 17.7 | 342.0 \pm 22.8 | 482.5 \pm 95.7 |
| Putrescine, μ mol/d | 74.8 \pm 24.5 | 127.9 \pm 26.3 | 176.4 \pm 30.7 | 229.2 \pm 35.5 | 339.2 \pm 90.2 |
| Spermidine, μ mol/d | 38.4 \pm 11.4 | 53.7 \pm 14.5 | 62.7 \pm 17.8 | 73.7 \pm 20.3 | 95.2 \pm 30.8 |
| Spermine, μ mol/d | 22.8 \pm 8.3 | 30.3 \pm 10.3 | 34.3 \pm 12.0 | 39.0 \pm 13.3 | 48.1 \pm 18.5 |
| Total energy, kcal/d | 1128.0 \pm 369.7 | 1388.0 \pm 436.6 | 1545.3 \pm 477.3 | 1730.9 \pm 518.1 | 2078.8 \pm 677.8 |
| Total fat, g/d | 41.9 \pm 20.5 | 49.2 \pm 25.0 | 53.3 \pm 27.3 | 58.2 \pm 29.9 | 68.2 \pm 37.0 |
| Total saturated fat, g/d | 14.0 \pm 7.5 | 16.4 \pm 9.1 | 17.7 \pm 9.8 | 19.5 \pm 10.8 | 23.0 \pm 13.4 |
| Red meat, medium servings/d | 0.5 \pm 0.4 | 0.6 \pm 0.5 | 0.6 \pm 0.6 | 0.7 \pm 0.7 | 0.8 \pm 0.7 |
| Total protein, g/d | 45.9 \pm 16.2 | 58.3 \pm 19.7 | 65.4 \pm 21.9 | 73.7 \pm 24.0 | 88.9 \pm 32.4 |
| Arginine, g/d | 2.4 \pm 0.9 | 3.0 \pm 1.1 | 3.4 \pm 1.2 | 3.8 \pm 1.3 | 4.6 \pm 1.7 |
| Methionine, g/d | 1.1 \pm 0.4 | 1.3 \pm 0.5 | 1.5 \pm 0.5 | 1.7 \pm 0.6 | 2.0 \pm 0.8 |
| Total fiber, g/d | 10.3 \pm 3.7 | 14.0 \pm 4.5 | 16.2 \pm 5.1 | 18.8 \pm 5.6 | 23.6 \pm 7.3 |
| Alcohol, servings/wk | 1.9 \pm 4.2 | 2.4 \pm 4.9 | 2.6 \pm 5.5 | 3.0 \pm 5.5 | 3.1 \pm 6.0 |
| Dietary folate, ⁴ μ g/d | 144.1 \pm 42.6 | 195.7 \pm 46.2 | 231.4 \pm 49.4 | 270.6 \pm 54.2 | 347.2 \pm 82.7 |
| Dietary folic acid, ⁵ μ g/d | 112.6 \pm 76.7 | 136.4 \pm 85.1 | 149.0 \pm 87.3 | 163.5 \pm 94.0 | 185.5 \pm 106.7 |
| Supplemental folic acid use, n (%) | 7656 (43.8) ⁶ | 8432 (48.2) | 8750 (50.0) | 9100 (52.0) | 9197 (52.6) |
| Total calcium, ⁷ mg/d | 906.6 \pm 653.4 | 1107.4 \pm 750.6 | 1217.2 \pm 806.0 | 1363.9 \pm 690.4 | 1587.3 \pm 769.0 |
| Dietary calcium, ⁴ mg/d | 559.6 \pm 323.7 | 718.1 \pm 364.7 | 819.3 \pm 388.0 | 943.8 \pm 427.9 | 1156.9 \pm 531.4 |
| Supplemental calcium use, n (%) | 9404 (53.7) | 10,406 (59.4) | 10,624 (60.6) | 11,080 (63.2) | 11,227 (64.1) |

¹WHI, Women's Health Initiative.

²Sum of dietary putrescine, spermidine, and spermine.

³Mean \pm SD (all such values for continuous variables).

⁴From food sources only.

⁵From food sources only (including fortified foods).

⁶Frequency; percentage of total in parentheses (all such values for categorical variables).

⁷Sum of dietary calcium and supplemental calcium.

Dietary polyamine intake and risk of CRC and CRC-specific death

Cumulative incidence by quintile of dietary polyamine intake is shown in **Supplemental Table 3**. In the fully adjusted model, midrange dietary polyamine intake was significantly associated with 18% and 19% decreased risk of CRC in quintiles 2 and 3, respectively (**Table 3**). Point estimates for quintiles 4 and 5 did not change appreciably across models, but significance was not consistently achieved. No evidence of a linear dose-response effect was shown; all reduced risk was achieved in the second quintile with no additional benefit with higher exposure. The addition of dietary polyamines did not significantly improve the model fit over and above other covariates as assessed by using a likelihood ratio test with versus without total polyamines ($P > 0.05$). When we excluded cancers that occurred early during the observational period (<1 y) or participants with a personal history of CRC, a similar association with decreased risk starting in the second quintile was observed (data not shown). A separate analysis of putrescine alone (diamine) and spermine plus spermidine (triamines) revealed no differential effects by amine type and no dose effect of either putrescine alone or spermidine plus spermine (data not shown).

Among participants who developed CRC during observation, participants with high polyamine intake (quintile 5) experienced 53% reduced risk of CRC-specific death when adjusted for only the stage at diagnosis (Table 3, model 1). This effect was no longer present in the fully adjusted model although data on CRC treatment was unavailable in this cohort.

Dietary polyamine intake and risk of CRC by population subgroups

Previously, we showed that, in patients with a history of colorectal adenoma (i.e., a subgroup of the population at higher risk of CRC), high polyamine intake was associated with increased odds of adenoma at follow-up (4). One explanation for the lack of a positive association between polyamines and CRC in postmenopausal women is that polyamines have different effects depending on background risk of CRC. Thus, to explore the potential heterogeneity of polyamine effect by CRC risk, we stratified analyses on established CRC risk factors at baseline. We observed a significant protective effect of dietary polyamines in women with CRC risk-lowering characteristics, specifically subjects with BMI ≤ 25 (P -interaction = 0.012) or who reported higher dietary fiber consumption (P -interaction = 0.015; **Figure 1, Table 4**). A baseline history of polyp removal (P -interaction = 0.635), family history of CRC (P -interaction = 0.687), age >65 y (P -interaction = 0.764), aspirin use >1 y (P -interaction = 0.100), and dietary arginine, folate, and folic acid (P -interaction = 0.129, 0.607, and 0.890, respectively) did not significantly modify the association between polyamines and CRC risk (Figure 1).

DISCUSSION

To our knowledge, this is the first study of the association between dietary polyamines and risk of CRC and CRC-specific mortality in a large, average-risk population of postmenopausal women. Contrary to our a priori hypothesis, we observed no significant dose-response relation between dietary polyamines and risk of CRC or CRC-specific mortality in fully adjusted

TABLE 3

Dietary polyamine intake at baseline and CRC (in the whole population) and CRC-specific death (only in subjects who had a staged local, regional, or distant CRC event on study) outcomes during the observation period¹

| Quintiles of total dietary polyamine intake, $\mu\text{mol/d}$ | CRC ² | | | | | | CRC-specific death ³ | | | | | |
|--|-------------------|----------|-------------------|----------|-------------------|----------|---------------------------------|----------|------------------------------|----------|------------------------------|----------|
| | Model 1 | | Model 2 | | Model 3 | | Model 1 + stage at diagnosis | | Model 2 + stage at diagnosis | | Model 3 + stage at diagnosis | |
| | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> | Sub-HR (95% CI) | <i>P</i> | Sub-HR (95% CI) | <i>P</i> | Sub-HR (95% CI) | <i>P</i> |
| 21.8–179.6 | 1.00 | — | 1.00 | — | 1.00 | — | 1.00 | — | 1.00 | — | 1.00 | — |
| 179.7–243.0 | 0.87 (0.73, 1.03) | 0.114 | 0.83 (0.68, 1.00) | 0.048 | 0.82 (0.68, 1.00) | 0.048 | 1.01 (0.69, 1.48) | 0.963 | 1.19 (0.76, 1.84) | 0.447 | 1.14 (0.73, 1.78) | 0.563 |
| 243.1–304.9 | 0.82 (0.69, 0.98) | 0.026 | 0.81 (0.67, 0.99) | 0.036 | 0.81 (0.66, 0.99) | 0.040 | 0.97 (0.63, 1.48) | 0.870 | 1.33 (0.81, 2.16) | 0.257 | 1.27 (0.76, 2.12) | 0.353 |
| 305.0–384.7 | 0.90 (0.76, 1.07) | 0.233 | 0.91 (0.75, 1.11) | 0.369 | 0.91 (0.74, 1.12) | 0.379 | 0.78 (0.52, 1.17) | 0.229 | 1.00 (0.62, 1.62) | 0.999 | 0.95 (0.57, 1.58) | 0.838 |
| 384.8–1293.7 | 0.83 (0.70, 0.99) | 0.035 | 0.80 (0.64, 1.00) | 0.050 | 0.80 (0.62, 1.02) | 0.073 | 0.47 (0.30, 0.74) | 0.001 | 0.64 (0.36, 1.14) | 0.130 | 0.57 (0.31, 1.06) | 0.076 |
| Overall test ⁴ | — | 0.059 | — | 0.135 | — | 0.152 | — | — | — | — | — | — |

¹Model 1 was adjusted for age only ($n = 87,602$ for CRC outcome; $n = 1084$ for CRC-specific death outcome). Model 2 was adjusted as for model 1 and for energy, income, smoking, physical activity, race-ethnicity, BMI, ever use of hormone replacement therapy, and history of bowel screening ($n = 76,066$ for CRC outcome; $n = 955$ for CRC-specific death outcome). Model 3 was adjusted as for model 2 and for dietary fat, dietary arginine, dietary fiber, dietary folate equivalents, and dietary calcium ($n = 75,924$ for CRC outcome; $n = 955$ for CRC-specific death outcome). CRC, colorectal cancer.

²Values were generated by using Cox regression with Breslow's method for ties.

³Values were generated by using competing risk regression [as outlined by Fine and Gray (25)]. The stage at diagnosis was as follows: model 1, localized CRC ($n = 479$); regional CRC ($n = 463$); and distant CRC ($n = 141$); models 2 and 3, localized CRC ($n = 425$); regional CRC ($n = 405$); and distant CRC ($n = 126$).

⁴Results of a likelihood ratio test for the comparison of Cox regression models with and without dietary polyamines as quintiles.

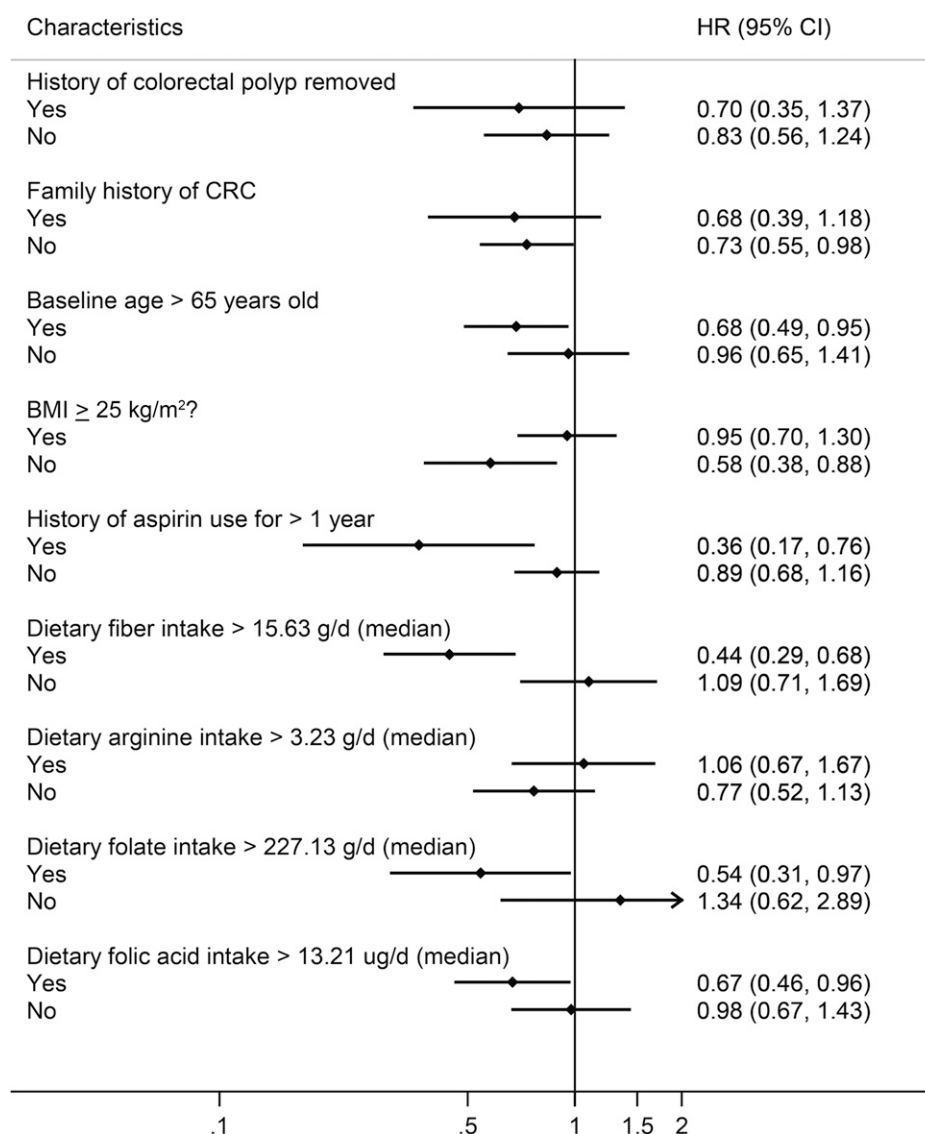


FIGURE 1 Risk of CRC associated with total dietary polyamine intake (quintile 5 compared with quintile 1) stratified by established risk factors for CRC assessed at baseline. HRs (◆), 95% CIs (line), and *P* values were generated by using Cox regression with Breslow's method for ties. The results were adjusted for all model 3 covariates (age, energy intake, income, smoking, physical activity, race-ethnicity, BMI, hormone replacement therapy, history of bowel screening, dietary fat, dietary arginine, dietary fiber, dietary folate equivalents, and dietary calcium; *n* = 76,066). CRC, colorectal cancer.

models. Before full adjustment, total polyamine intake $\geq 179.67 \mu\text{mol/d}$ (quintiles 2–5) was consistently associated with 9–20% decreased risk of CRC. After adjustment for dietary covariates, these associations were not always significant, although point estimates were consistent across all models and quintiles. These dietary covariates included dietary folate equivalents and fiber, whose food sources strongly overlapped with those of polyamines and may have resulted in overadjustment of the model. Overall, our data suggest modest, nonsignificant decreased risk of CRC in individuals with average-to-high polyamine intakes or, conversely, modest increased risk of individuals with very-low polyamine intakes.

Our findings are supported by animal and human studies that suggested effects of dietary polyamines are dependent on background risk of CRC. Similar to previous dietary polyamine studies (4, 18), we showed that citrus fruits are the largest contributor of polyamines to the diet of postmenopausal US women. In ecological

studies, the Mediterranean diet, which includes high citrus fruit intake, is associated with protection against the development of diseases including CRC (55). In experimental animal models, high-polyamine diets delayed aging (56), and putrescine was shown to mediate the effect of probiotics on longevity in mice through direct inhibitory effects on colonic cell senescence (17, 53). In contrast, low-polyamine diets were shown to delay wound healing (57) and inhibit growth (13). In humans at high risk of CRC (adenomatous polyp formers), we previously showed that dietary polyamines increased risk of adenomatous polyp recurrence (4). Similarly, mice initiated with a chemical colorectal carcinogen showed an increase in the severity of premalignant colorectal aberrant crypt foci and adenomatous polyps when exposed to a high-polyamine diet (45, 46, 56). However, these carcinogen-treated animals had fewer aberrant crypt foci in the intestinal tract when they kept receiving low-polyamine diets (13, 46), and a high-polyamine diet before carcinogen treatment decreases tumor incidence (56). Overall, our

TABLE 4Stratified models that describe the relation between BMI or dietary fiber, dietary polyamines, and colorectal cancer risk after adjustment for all model 3 covariates¹

| | <i>n</i> /total person-years | Quintile 2 | | Quintile 3 | | Quintile 4 | | Quintile 5 | |
|---------------------------------|------------------------------|-------------------|----------|-------------------|----------|-------------------|----------|-------------------|----------|
| | | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> |
| BMI, kg/m ² | | | | | | | | | |
| ≤25 | 379/393,982 | 0.56 (0.40, 0.77) | 0.001 | 0.66 (0.48, 0.92) | 0.014 | 0.73 (0.52, 1.03) | 0.077 | 0.58 (0.38, 0.88) | 0.011 |
| >25 | 715/547,985 | 1.01 (0.80, 1.29) | 0.915 | 0.91 (0.71, 1.18) | 0.486 | 1.04 (0.79, 1.35) | 0.792 | 0.95 (0.70, 1.30) | 0.754 |
| Dietary fiber, ² g/d | | | | | | | | | |
| ≤15.63 | 555/459,121 | 0.96 (0.76, 1.20) | 0.700 | 0.86 (0.66, 1.11) | 0.244 | 1.37 (1.04, 1.82) | 0.027 | 1.09 (0.71, 1.69) | 0.683 |
| >15.63 | 539/482,845 | 0.51 (0.33, 0.79) | 0.003 | 0.55 (0.36, 0.82) | 0.004 | 0.49 (0.33, 0.74) | 0.001 | 0.44 (0.29, 0.68) | <0.001 |

¹Model 3 as adjusted for age, energy intake, income, smoking, physical activity, race-ethnicity, BMI, hormone replacement therapy, history of bowel screening, dietary fat, dietary arginine, dietary fiber, dietary folate equivalents, and dietary calcium. Quintile 1 of total dietary polyamine intake was the reference group. HRs (95% CIs) and *P* values for quintiles of total dietary polyamine intake were generated by using Cox regression with Breslow's method for ties.

²Split at the median.

observations are consistent with the notion suggested by the literature that the effect of dietary polyamines is to promote health in low-risk colorectums, and polyamines may promote carcinogenesis in colorectums that experienced an initiating event.

Although the multiple subgroup analyses reported should be interpreted cautiously, our findings suggest that dietary polyamines may be particularly beneficial in lowering CRC risk in women with normal BMI and in those who consumed higher than average fiber in this population (>15.63 g/d). Fiber (58) was not protective against CRC in the WHI cohort overall, but fiber intake has been shown to reduce risk of CRC in previous studies (35). Obesity has been consistently linked with CRC risk, more so in men (33), and the effect of polyamines could be dependent on body size (49, 50). Thus, these observations also support a differential effect of dietary polyamines dependent on background CRC risk. Although the exact mechanisms by which BMI or fiber act with polyamine intake in a protective fashion are unknown, we speculate that one mechanism may be through effects on the gut microbiome. Specifically, BMI (49, 50), fiber (51), and polyamines (17, 52, 53) all interact with the gut flora, which is a major secondary source of polyamine exposure to the colonic epithelium (11, 12). This plausible mechanism of action, and others, warrants exploration in both high- and low-risk CRC model systems to better understand by what mechanism exogenous polyamines affect gut function and tumorigenesis.

In contrast with our previous findings for adenoma outcomes (4), we did not show evidence that women with a history of colorectal polyp removal had increased risk of CRC from dietary polyamine exposure. However, the WHI cohort did not differentiate between histories of colorectal polyps compared with adenomas. In addition, we failed to identify significant associations between polyamines and CRC risk in participants with some high-risk characteristics (e.g., family history of CRC or advanced age). These results may reflect differences related to past screening behaviors in these groups for which we lacked sufficient detail before enrollment in WHI. We could not discount the possibility that our findings were biased because of residual confounding because women of lower socioeconomic status are more often in the lowest quintile of dietary polyamine consumption.

The potential for misclassification of a dietary exposure, the presence of correlated nutrients, and the lack of more-precise biomarkers of polyamine intake were important limitations in this

and other similar studies of dietary factors (18). The polyamine content depends on the ripeness, bacterial activity, and age of foods (4, 18, 59), which makes estimation challenging. For example, the considerable overlap of polyamine-, fiber- and folate-rich foods may preclude a conclusive distinction of individual effects of any of these dietary exposures in the observational setting. This possibility raises the prospect that previous findings of associations between dietary folate and fiber with CRC may have been confounded by the biological effects of polyamines or that our study suffered from residual confounding. Nonetheless, the large sample size, quantity of measured variables, adjudicated CRC outcomes, and long follow-up period in this study were considerable strengths.

In conclusion and in contrast to our original hypothesis, we showed no evidence for a positive association between dietary polyamines and CRC risk. Instead, this study provides the first evidence, to our knowledge, that diets high in polyamines may decrease risk of CRC in average-risk postmenopausal women, particularly in those with other CRC risk-lowering habits such as consumption of higher fiber diets and maintenance of a normal body weight. This observation is consistent with emerging evidence of beneficial effects of polyamines on longevity and gastrointestinal health and argues for differential effects by risk status, thereby warranting additional study on the potential health benefits of polyamine-rich foods.

The short list of WHI investigators is as follows—program office: (National Heart, Lung, and Blood Institute, Bethesda, Maryland) Jacques Rossouw, Shari Ludlam, Dale Burwen, Joan McGowan, Leslie Ford, and Nancy Geller; clinical coordinating center: (Fred Hutchinson Cancer Research Center, Seattle, Washington) Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg; investigators and academic centers: (Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts) JoAnn E Manson, (MedStar Health Research Institute/Howard University, Washington, District of Columbia) Barbara V Howard, (Stanford Prevention Research Center, Stanford, California) Marcia L Stefanick, (The Ohio State University, Columbus, Ohio) Rebecca Jackson, (University of Arizona, Tucson/Phoenix, Arizona) Cynthia A Thomson, (University at Buffalo, Buffalo, New York) Jean Wactawski-Wende, (University of Florida, Gainesville/Jacksonville, Florida) Marian Limacher, (University of Iowa, Iowa City/Davenport, Iowa) Robert Wallace, (University of Pittsburgh, Pittsburgh, Pennsylvania) Lewis Kuller, (Wake Forest University School of Medicine, Winston-Salem, North Carolina) Sally Shumaker; and the WHI Memory Study: (Wake Forest University School of Medicine, Winston-Salem, North Carolina) Sally Shumaker. For a list of all investigators who contributed to WHI science, please visit <https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20List.pdf>.

The authors' responsibilities were as follows—AJV, RBW, MLN, CAT, and PAT: designed the research; AJV, ELA, and BCW: conducted the statistical research and analyses; RBW, MLN, and CAT: provided access to WHI data sets; AJV, ELA, CAT, and PAT: wrote the manuscript; and AJV: had primary responsibility for the final content of the manuscript. None of the authors reported a conflict of interest related to the study.

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