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Spermidine intake is associated with cortical thickness and hippocampal volume in older adults

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ABSTRACT

Background: The natural polyamine spermidine, known to be important for cellular function, decreases during aging. Previous research has demonstrated beneficial impact of spermidine intake on memory functions in both animal models and humans, suggesting that spermidine may be a preventive approach to delay age-related cognitive decline and possibly even Alzheimer's disease (AD). However, the association of spermidine intake with brain health in humans is still unknown. In this study, we aimed to determine the association between dietary spermidine intake and structural brain measures in older individuals with subjective cognitive decline (SCD) and healthy controls (HC).

Methods: Dietary spermidine intake and adherence to Mediterranean Diet (MeDi) were assessed by a self-reported food frequency questionnaire in 90 older adults with SCD and 47 HC. Processing of structural MRI data yielded global brain volumes, hippocampal volume, mean and regional cortical thickness, and cortical thickness in a template encompassing AD-vulnerable regions. In exploratory analyses, the association between spermidine intake and structural brain measures was assessed using adjusted and unadjusted linear regression models. Additionally, we tested for differential associations as a function of group. Mediation analyses were performed to examine whether dietary spermidine intake mediates the associations between adherence to MeDi and structural brain measures.

Results: Higher spermidine intake was associated with larger hippocampal volume (standardized $\beta = 0.262$, $p = 0.002$), greater mean cortical thickness (standardized $\beta = 0.187$, $p = 0.031$), and greater cortical thickness in AD-vulnerable brain regions (standardized $\beta = 0.176$, $p = 0.042$), the parietal (standardized $\beta = 0.202$, $p = 0.020$), and temporal lobes (standardized $\beta = 0.217$, $p = 0.012$). No significant differential effect emerged between older

Abbreviations: AD, Alzheimer's disease; ANOVA, univariate analysis of variance; APOE ϵ4, apolipoprotein E4; BMI, body mass index; CI, confidence interval; ECog-39, Everyday Cognition-39 Items Questionnaire; FFQ, food frequency questionnaire; GDS, Geriatric Depression Scale; HC, healthy controls; HPC, hippocampus; IADL, Instrumental Activities of Daily Living Scale; mCT, mean cortical thickness; MeDi, Mediterranean Diet; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; ROI, region of interest; SBP, systolic blood pressure; SCD, subjective cognitive decline; SD, standard deviation; SE, standard error; TBV, total brain volume; TGMV, total grey matter volume; TIV, total intracranial volume; TWMV, total white matter volume.

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adults with SCD and HC. Moreover, a substantial mediating effect of dietary spermidine intake on the associations between adherence to MeDi and structural brain measures was observed.
Conclusion: Higher dietary spermidine intake was positively associated with several structural brain measures, irrespective of the presence of SCD, and substantially mediated the relationship of adherence to MeDi and structural brain measures. Our data suggest that higher spermidine intake might be a promising dietary approach to preserve brain health in older adults, a hypothesis currently tested in an interventional trial.

1. Introduction

Propagation of healthy brain aging, including the maintenance of cognitive function and brain structure, is of utmost importance in our aging society (Cole et al., 2019). One promising substance to preserve brain and cognitive health is the polyamine spermidine, a natural substance in all living organisms, which is important for the maintenance of cellular homeostasis (Minois et al., 2011). The concentration of spermidine in the body declines during aging in almost all organs, including the brain (Morrison et al., 1995; Vivo et al., 2001; Liu et al., 2008). Besides cellular biosynthesis and intestinal microbial activity, endogenous spermidine concentration is maintained by nutritional supply (Madeo et al., 2019). Higher levels of spermidine were shown to be associated with greater adherence to the Mediterranean Diet (MeDi) (Binh et al., 2011), which is in turn associated with lower risk of cognitive decline and Alzheimer's disease (AD) (Scarmeas et al., 2006). Higher dietary spermidine intake and consequently higher endogenous spermidine concentration has been shown to favor several aspects of general health and the maintenance of memory performance in aging animals and humans (Gupta et al., 2013, 2016; Eisenberg et al., 2017; Wirth et al., 2018; Madeo et al., 2018). For example, a study investigating the effect of spermidine supplementation provided first evidence for an improvement in memory function in older adults with subjective cognitive decline (SCD), suggesting a potential therapeutic use of spermidine in individuals at risk for AD (Wirth et al., 2018).

Previous studies in animals and humans have shown associations of higher spermidine intake with better general health and superior memory performance (Gupta et al., 2013, 2016; Eisenberg et al., 2017; Wirth et al., 2018; Madeo et al., 2018). However, no study so far has investigated the relationship with brain structure. Thus, this cross-sectional study examined for the first time the association of dietary spermidine intake with global brain volumes, hippocampal volume, mean and regional cortical thickness, and cortical thickness in a template encompassing AD-vulnerable regions in older adults with SCD and healthy controls (HC). Furthermore, we tested for an interaction effect of group and dietary spermidine intake on brain measures and examined whether dietary spermidine intake has a differential effect on structural brain measures in the groups. In addition, we investigated whether dietary spermidine intake mediates the relationship between adherence to MeDi and structural brain measures.

2. Methods

2.1. Study design

We conducted a cross-sectional study to examine the association of self-reported dietary spermidine intake and structural brain measures. This study took place within the scope of the ongoing prospective monocentric study SmartAge (Wirth et al., 2019; ClinicalTrials.gov: NCT03094546) at the NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, analyzing the effects of 12-month spermidine supplementation (double-blinded, placebo-controlled) in individuals with SCD. Besides individuals with SCD, we recruited HC with a similar baseline protocol.

2.2. Study participants

In total, 108 older adults with SCD (SmartAge cohort) and 51 HC, aged 60–90 years, were recruited from healthcare facilities as well as through advertisements from the general German population. SCD criteria were evaluated in line with the framework of Jessen and colleagues and included I) presence of SCD for at least 6 months, II) related concerns (worries), III) previous consultation or consideration to consult a doctor due to these symptoms, IV) normal cognition, and V) no functional impairment (Jessen et al., 2014). Healthy controls were considered for inclusion if they reported no subjective cognitive worsening or if so, no related concerns (worries), showed normal cognition, and no functional impairment.

Assessment of study eligibility was undertaken during study enrollment, which included a telephone screening and an on-site screening at the Charité – Universitätsmedizin Berlin (detailed information on screening procedure are provided in the study protocol (Wirth et al., 2019)). Potential study participants in both cohorts were excluded if screening revealed a diagnosis of mild cognitive impairment or dementia. Moreover, severe or untreated medical (e.g., malignant hypertension, untreated diabetes mellitus), neurological or psychiatric disorders, malignancies, and alcohol dependency as well as drug abuse led to study exclusion. To determine the absence of objective cognitive impairment and current psychiatric disorder, the following measures were applied: Mini-Mental State Examination (MMSE) (Folstein et al., 1975) score $\geq$ 26, performance within -1.5 standard deviation (SD) of age-adjusted norms in selected neuropsychological tests, no deficits on selected items of the Instrumental Activities of Daily Living Scale (IADL) (Lawton and Brody, 1969), and the 15-item Geriatric Depression Scale (GDS) (Yesavage et al., 1982; Gauggel and Birkner, 1999) score $\leq$ 10. Further eligibility criteria for the SCD group are described in our study protocol (Wirth et al., 2019).

After successful completion of study enrollment, additional neuropsychological testing was conducted, and the participants received cerebral MRI scans and had to complete dietary questionnaires. Among the 159 participants that had provided informed consent (108 SCD, 51 HC), 8 participants (7 SCD, 1 HC) failed the on-site screening and cerebral MRI scans of 14 participants (11 SCD, 3 HC) were not available. Thus, the current analysis included 137 older participants (90 SCD, 47 HC) with MRI scans and dietary information.

2.3. Standard protocol approvals, registrations, and patient consents

The study was approved by the Ethics Committee of the Charité University Hospital Berlin, Germany, and is in accordance with the Declaration of Helsinki. Written informed consent was provided by all participants, before starting the on-site screening.

2.4. Dietary information

Spermidine intake was assessed through a self-reported 89-item food frequency questionnaire (FFQ) based on the gold standard FFQ of Willett and colleagues (Willett et al., 1985). The methodology to estimate the nutrient intake was implemented as employed in the Bruneck Study (Eisenberg et al., 2016; Kiechl et al., 2018). A common unit or serving size was specified for each item of the FFQ and participants had to provide information about their average intake of each item during the past year. Response categories ranged from "never or $<$ 1 time/month" to "$\geq$ 6 times/day" (0–8). The frequency of intake of each food was assigned a weight proportional, i.e., "1 time a day" equaled a weight of 1. Nutrition

values for each specified food size were based on the USDA Nutrient Database, release 23 (U.S. Department of Agriculture, Agricultural Research Service. 2010. USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl). Eisenberg and colleagues as well as Kiechl and colleagues assembled a special nutrient database for polyamines (Eisenberg et al., 2016; Kiechl et al., 2018). Thus, weighted sums of all contributing foods could be calculated to estimate nutrient intake. For the present analyses, we further adjusted the estimated spermidine intake (nmol/day) for total caloric intake applying a simple ratio calculation, used in log10-transformed form.

In addition to spermidine intake, the FFQ was also used to assess adherence to MeDi. Therefore, 13 food items characteristic of MeDi, in line with the validated Mediterranean Diet Adherence Screener questionnaire (except for Sofrito consumption), were scored with 1, thus comprising a score ranging from 0 to 13 (Martínez-González et al., 2012; Schroder et al., 2011).

2.5. Brain imaging

MRI measurements were performed at the Berlin Center for Advanced Neuroimaging (BCAN, Charité - Universitätsmedizin Berlin) on a 3T MRI scanner (Siemens Magnetom Trio/PrismaFit, Erlangen, Germany) employing a 12-/20-channel head coil. T1-weighted three-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE) images were acquired with the following parameters: sagittal orientation, field-of-view = 256 × 256 mm^2, matrix = 256 × 256, 192 slices, 1 mm isotropic voxels, repetition time = 1900 ms, echo time = 2.52 ms, inversion time = 900 ms, flip angle = 9°. All the T1 images were visually controlled for quality. Cortical as well as subcortical volume (mm^3) and cortical thickness (mm) were analyzed using FSL v5.0 (FMRIB Software Library, Oxford, UK) and FreeSurfer v6.0 (http://surfer.nmr.mgh.harvard.edu/). First, we performed a LAS (x-left, y-anterior, z-superior) reorientation with FSL, followed by running the automatic stream ("recon-all") of FreeSurfer. The output of FreeSurfer was subjected to visual quality control. When necessary, manual correction was performed and FreeSurfer steps were repeated.

2.5.1. Volume measures

Total intracranial volume (TIV), total brain volume (TBV), total grey matter volume (TGMV), total white matter volume (TWMV) as well as hippocampal volume were derived from FreeSurfer, summing the left and right hemisphere. Differences in head size were adjusted by using a simple ratio calculation dividing the raw volumes by TIV.

2.5.2. Cortical thickness measures

For the analyses, we used mean cortical thickness (mCT) across the whole brain of every participant from FreeSurfer, combining both hemispheres. Further, we used the individual Desikan-Killiany regions-of-interest to analyze the different brain lobes: frontal (superior frontal, rostral and caudal middle frontal, pars opercularis, triangularis and orbitalis, lateral and medial orbitofrontal and frontal pole, but excluding precentral and paracentral gyrus), parietal (superior and inferior parietal, supramarginal and precuneus gyrus excluding the postcentral one), temporal (superior, middle and inferior temporal gyrus, banks of the superior temporal sulcus, fusiform, transverse temporal and entorhinal gyrus, temporal pole and parahippocampal gyrus), and cingulate (rostral and caudal anterior frontal gyrus, posterior and isthmus parietal gyrus). Based on the template of Wirth and colleagues, we combined 5 regions (precuneus, entorhinal, inferior parietal, inferior temporal and isthmus of the cingulate gyrus), which preferably show neurodegeneration in AD (Wirth et al., 2017). To weight the different sizes of the individual gyri, weighted means were calculated using the individual surface area of each region (left and right) as provided by FreeSurfer.

2.6. Other information

Further variables were included to provide a comprehensive description of our participants including age , sex, years of education, body mass index (BMI), systolic blood pressure (SPB), current smoker, and apolipoprotein E4 (APOE ϵ4) carriers. Moreover, we used the Everyday Cognition-39 Items Questionnaire (ECog-39; self-reported) (Farias et al., 2008) to evaluate some additional parameters of subjective cognition for the characterization of the SCD group in comparison to HC.

2.7. Statistical analyses

Regarding participants' baseline characteristics, univariate analysis of variance (ANOVA) was applied to reveal potential differences between the SCD group and HC for continuous parameters. Mean, SD, and interquartile range of each measure was provided for the whole sample as well as for each diagnostic group separately, if applicable. Differences in gender, current smoker, and APOE ϵ4 carriers were analyzed between the groups using χ^2 tests.

Multiple linear regression models were conducted to analyze the association between dietary spermidine intake (independent variable) and structural brain measures (dependent variable), first unadjusted, and in a second step adjusted for potential confounders (including sex, age, years of education, adherence to MeDi, and group affiliation) across all participants. Moreover, the same method was applied to test for an interaction effect of group and dietary spermidine intake on brain measures (dependent variable). Finally, mediation analyses were performed to examine whether dietary spermidine intake (mediator; m) mediates the direct associations between adherence to MeDi (independent variable; x) and structural brain measures (dependent variable; y). In a second step, mediation analyses were adjusted for the same covariates as linear regression analyses (except adherence to MeDi). We report unstandardized regression coefficients and p-values for the direct (path a: x → m; path b: m → y; path c: x → y) and indirect pathway (path ab: x → m → y) effects. Significance of the mediation coefficients was estimated based on bias-corrected bootstrap 95% confidence interval (CI, 5000 bootstrap samples heteroscedasticity consistent standard errors (Davidson and MacKinnon, 1995)).

Statistical analyses were performed using SPSS Software Package v25 (IBM Corp. Released, 2017. IBM SPSS Statistics for Microsoft, Version 25.0. Armonk, NY: IBM Corp.) and the PROCESS macro by Hayes (v3.4.1) (Hayes, 2017) for our mediation analyses. The significance level was set at alpha = 0.05. For the analysis of cortical thickness parameters, four participants (3 SCD, 1 HC) had to be excluded because of substantial outlier characteristics. Due to the exploratory nature of the analyses, no correction for multiple comparisons was applied; therefore, all analyses have to be interpreted non-confirmatory.

3. Results

3.1. Characteristics of participants

Participants' characteristics, including demographics, general cognition and mental health, dietary information, vascular factors and genetics, brain volumes, and cortical thickness, are shown in Table 1. There were no differences between groups (SCD and HC) regarding gender distribution, years of education, general cognition, vascular factors, genetics, dietary information, brain volumes, and cortical thickness. Group differences were found for age (univariate ANOVA, $F_{(1,135)} = 5.732$, $p = 0.018$) with the SCD group being slightly younger. Moreover, group differences were observed for the ECog-39, quantifying subjective feeling of cognitive worsening (univariate ANOVA, $F_{(1,135)} = 42.199$, $p < 0.001$), as well as for higher depressive symptoms in the GDS (univariate ANOVA, $F_{(1,135)} = 18.407$, $p < 0.001$) with the SCD group expressing higher scores in both questionnaires.

Table 1
Characteristics of participants.

	All (n = 137)	HC (n = 47)	SCD (n = 90)	p-value[a]
Demographics				
Females [n] (%)	70 (51.1)	24 (51.1)	46 (51.1)	0.996
Age [years]	69 (6)	71 (6)	69 (5)	0.018
	66–74	67–75	65–71	
Education [years]	17 (3)	16 (3)	17 (3)	0.362
	14–19	14–18	14–19	
Cognition and mental health				
MMSE [score]	29.2 (0.9)	29.2 (0.9)	29.1 (0.9)	0.669
	29.0–30.0	29.0–30.0	29.0–30.0	
ECog-39 mean [score]	1.6 (0.4)	1.3 (0.3)	1.7 (0.4)	<0.001
	1.3–1.8	1.1–1.4	1.5–1.9	
GDS [score]	1.5 (1.4)	0.8 (0.8)	1.8 (1.5)	<0.001
	0–2.0	0–1.0	0.8–3.0	
Vascular factors and genetics				
SBP [mmHg]	129.2 (15.2)	131.8 (13.8)	127.8 (15.8)	0.144
	120.0–140.0	120.0–140.0	117.5–137.5	
BMI [kg/m^2]	26.2 (4.0)	26.6 (3.7)	26.0 (4.1)	0.453
	23.4–28.4	24.2–28.8	23.2–28.1	
Smoker [n] (%)	8 (5.8)	3 (6.4)	5 (5.6)	0.845
APOE ϵ4, positive [n] (%)	29 (21.2)	6 (12.8)	23 (25.6)	0.092
Dietary information				
MeDi [score]	4.1 (1.7)	3.7 (1.7)	4.3 (1.8)	0.061
	1.0–10.0	1.0–8.0	1.0–10.0	
Spermidine intake [a.u.]	1.7 (0.2)	1.6 (0.2)	1.7 (0.2)	0.073
	1.6–1.8	1.5–1.8	1.6–1.8	
Brain volumes				
TBV [%]	72.4 (3.1)	72.5 (3.3)	72.4 (3.0)	0.879
	70.2–74.2	70.2–74.7	70.1–74.1	
TGMV [%]	39.7 (2.1)	40.0 (2.1)	39.6 (2.1)	0.321
	38.4–41.4	38.5–41.7	38.3–41.0	
TWMV [%]	32.7 (2.2)	32.5 (2.5)	32.8 (2.0)	0.457
	31.2–33.9	31.0–33.4	31.4–34.1	
HPC [%]	0.5 (0)	0.5 (0.1)	0.5 (0)	0.993
	0.5–0.5	0.5–0.5	0.5–0.5	
Cortical thickness				
mCT [a.u.]	2.4 (0.1)	2.4 (0.1)	2.4 (0.1)	0.773
	2.4–2.5	2.4–2.5	2.4–2.5	
AD-ROIs [a.u.]	2.5 (0.1)	2.5 (0.1)	2.5 (0.1)	0.234
	2.4–2.6	2.4–2.6	2.4–2.6	
Frontal [a.u.]	2.5 (0.1)	2.5 (0.1)	2.5 (0.1)	0.837
	2.5–2.6	2.4–2.6	2.5–2.6	
Temporal [a.u.]	2.8 (0.1)	2.8 (0.1)	2.8 (0.1)	0.525
	2.7–2.9	2.7–2.8	2.7–2.9	
Parietal [a.u.]	2.3 (0.1)	2.3 (0.1)	2.3 (0.1)	0.392
	2.2–2.4	2.2–2.4	2.2–2.4	
Cingulate [a.u.]	2.5 (0.1)	2.6 (0.1)	2.5 (0.1)	0.277
	2.5–2.6	2.5–2.6	2.5–2.6	

Abbreviations: AD = Alzheimer's disease; APOE = apolipoprotein E; a.u. = arbitrary units; BMI = body mass index; ECog-39 = Everyday Cognition-39 Items; GDS = Geriatric Depression Scale; HC = healthy controls; HPC = hippocampus; mCT = mean cortical thickness; MeDi = Mediterranean Diet; MMSE = Mini-Mental State Examination; ROIs = regions of interest; SBP = systolic blood pressure; SCD = subjective cognitive decline; SD = standard deviation; TBV = total brain volume; TGMV = total grey matter volume; TWMV = total white matter volume. Data are given if applicable as mean (SD or %) and interquartile range. For the analysis of cortical thickness parameters, four participants (3 SCD, 1 HC) had to be excluded due to substantial outlier characteristics. Dietary spermidine intake was adjusted to total caloric intake and log10-transformed. Brain volume measures were normalized to total intracranial volume and cortical thickness measures were weighted by the individual surface area sizes.

[a] p-values estimated from χ^2 for categorical variables and univariate analysis of variance (ANOVA) for continuous variables.

3.2. Spermidine intake and brain volumes

Investigation of the relationship between spermidine intake and global brain volumes, including TBV, TGMV, and TWMV, revealed no statistically significant associations in unadjusted analyses (Table 2 and Fig. 1). However, after adjusting for covariates we found a positive relationship between spermidine intake and TGMV (standardized $\beta = 0.332$, $p = 0.001$). No statistically significant relationship of TBV and TWMV with spermidine intake could be observed after adjustment for covariates. Investigating the association between spermidine intake and hippocampal volume, we found an association of higher dietary spermidine intake with larger hippocampal volume (standardized $\beta = 0.262$, $p = 0.002$; Table 2 and Fig. 1). This association was maintained after controlling for covariates (standardized $\beta = 0.288$, $p = 0.007$).

In addition, according to the tested interaction terms (group*spermidine intake), there was no statistically significant differential effect of dietary spermidine intake between the diagnostic groups (HC vs SCD) on global brain measures, including TBV, TGMV, and TWMV (unadjusted: all p's > 0.238, adjusted: all p's > 0.442), nor on hippocampal volume (unadjusted: $p = 0.862$, adjusted: $p = 0.851$).

3.3. Spermidine intake and cortical thickness

Investigation of the associations between dietary spermidine intake and mean as well as regional cortical thickness is shown in Table 3 and Fig. 1. We found a positive association between spermidine intake and mCT, both unadjusted (standardized $\beta = 0.187$, $p = 0.031$) and after controlling for covariates (standardized $\beta = 0.222$, $p = 0.037$). More precisely, higher spermidine intake was found to be associated with greater cortical thickness within AD-vulnerable regions (standardized $\beta = 0.176$, $p = 0.042$), as well as temporal (standardized $\beta = 0.217$, $p = 0.012$), and parietal regions (standardized $\beta = 0.202$, $p = 0.020$). After adjusting for covariates, the effect remained significant for cortical thickness in temporal (standardized $\beta = 0.249$, $p = 0.016$) and parietal regions (standardized $\beta = 0.251$, $p = 0.022$), whereas the association of spermidine with AD-vulnerable regions (standardized $\beta = 0.203$, $p = 0.060$) was diminished. No statistically robust effect of group was found on the associations between dietary spermidine intake and mean as well as regional cortical thickness (unadjusted: all p's > 0.120, adjusted: all p's > 0.096).

3.4. Spermidine intake, structural brain measures, and Mediterranean Diet

Mediation analyses revealed that the relationship between MeDi and several structural brain measures, such as TGMV, hippocampal volume, mCT, and regional cortical thickness (except the cingulate cortex), was substantially mediated by dietary spermidine (unadjusted indirect effect: all 95% CI's do not include 0; Table 4). After entering the mediator into the model, adherence to MeDi was related to dietary spermidine intake (unadjusted path a: 95% CI does not include 0, $p < 0.001$) which in turn was substantially associated with TGMV, hippocampal volume, mCT as well as regional cortical thickness (unadjusted path b: all 95% CI's do not include 0, all p's ≤ 0.024). By contrast, adherence to MeDi was not directly substantially related to structural brain measures after accounting for the mediating effect of spermidine (unadjusted path c: all 95% CI's include 0, all p's ≥ 0.081), except for TGMV (unadjusted path c: 95% CI does not include 0, $p < 0.001$). Adjustment for covariates did not change these results substantially.

4. Discussion

In this cross-sectional study, we demonstrated for the first time that higher self-reported dietary intake of spermidine was associated with greater mean and regional cortical thickness, including AD-vulnerable regions, the parietal lobe, and temporal lobe, as well as with larger hippocampal volume in older individuals with SCD and HC. No differences in these associations emerged between older adults with SCD and HC. Moreover, spermidine intake was shown to substantially mediate the relationship between adherence to MeDi and several structural brain measures.

Table 2
Association between dietary spermidine intake and brain volumes.

Model	B[b]	SE	β[c]	t	95% CI	R^{2d}	adj. R^2	p-value[a]
TBV								
Unadjusted	1.004	1.308	0.066	0.768	−1.582, 3.590	0.004	−0.003	0.444
Adjusted	2.458	1.528	0.161	1.609	−0.564, 5.480	0.231	0.195	0.110
TGMV								
Unadjusted	1.569	0.899	0.149	1.746	−0.209, 3.347	0.022	0.015	0.083
Adjusted	3.508	0.989	0.332	3.547	1.551, 5.464	0.330	0.299	0.001
TWMV								
Unadjusted	−0.568	0.928	−0.053	−0.612	−2.405, 1.268	0.003	−0.005	0.541
Adjusted	−1.050	1.181	−0.097	−0.889	−3.387, 1.286	0.086	0.044	0.376
HPC								
Unadjusted	0.063	0.020	0.262	3.155	0.023, 0.102	0.069	0.062	0.002
Adjusted	0.069	0.025	0.288	2.745	0.019, 0.118	0.161	0.123	0.007

Abbreviations: adj. = adjusted; CI = confidence interval; HPC = hippocampus; SE = standard error; TBV = total brain volume; TGMV = total grey matter volume; TWMV = total white matter volume.

[a] p-values estimated from linear regressions, adjusted models include the following covariates: age, sex, years of education, diagnostic group, and Mediterranean Diet.

[b] Unstandardized regression coefficient.

[c] Standardized coefficient.

[d] Explained variance.

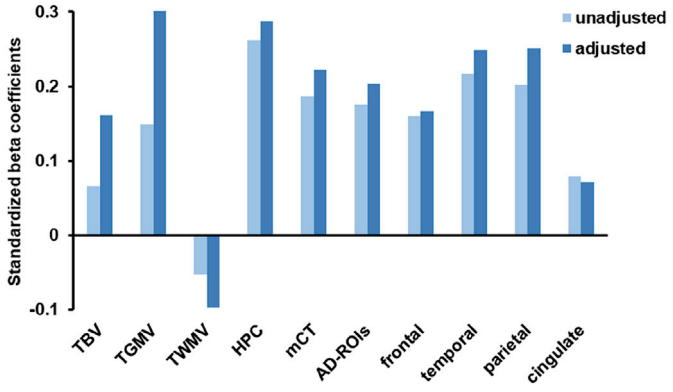

Fig. 1. Association of dietary spermidine intake with structural brain measures Association of dietary spermidine intake with structural brain measures, including global brain volumes, hippocampal volume, mean and regional cortical thickness, and cortical thickness in a template encompassing Alzheimer's disease (AD) vulnerable regions in older adults with subjective cognitive decline (SCD) and healthy controls (HC). Standardized beta coefficients of multiple linear regressions, unadjusted (light-blue columns) and adjusted for sex, age, years of education, adherence to Mediterranean Diet, and group affiliation (dark-blue columns), are reported. For the analysis of cortical thickness parameters, four participants (3 SCD, 1 HC) had to be excluded due to substantial outlier characteristics. Abbreviations: HPC = hippocampus; mCT = mean cortical thickness; ROIs = regions of interest; TBV = total brain volume; TGMV = total grey matter volume; TWMV = total white matter volume.

Higher dietary intake of spermidine, assessed by the FFQ, was associated with greater cortical thickness, especially in AD-vulnerable regions, the parietal lobe, and temporal lobe, as well as with larger hippocampal volume. The association between spermidine and memory-related brain regions, including AD-vulnerable regions, in older adults corroborates previous evidence from animal models showing that the polyamine spermidine is related to brain health and memory processes (Madeo et al., 2018). Tiboldi and colleagues demonstrated that hippocampal polyamine levels are associated with superior formation and retrieval of memory in mice (Tiboldi et al., 2012). Liu and colleagues showed that advancing age was associated with altered polyamine concentrations within memory-related brain structures in rats, possibly inducing dysfunctions in hippocampal neurogenesis and memory performance (Liu et al., 2008). Importantly, Gupta and colleagues provided first evidence for maintenance of memory function through spermidine supplementation in aging fruit flies (Gupta et al., 2013). Given that the polyamine spermidine is quickly resorbed from the intestinal lumen, higher nutritional supply might be a promising avenue to counteract age-related decrease of endogenous spermidine concentration (Madeo et al., 2019). Higher levels of spermidine may then promote hippocampal neurogenesis and preserve brain volume especially in the hippocampus. This hypothesis is supported by our findings of an association of spermidine intake with greater cortical thickness in AD-vulnerable regions, which are known to be affected early in the continuum of the disease even before the onset of first clinical symptoms. Moreover, it is in line with recent findings from our group, showing that 3-month spermidine supplementation led to an increase in a memory task sensitive to hippocampal function (Wirth et al., 2018). The biological mechanisms by which spermidine may act on brain health might encompass the induction of autophagy, driven by the inhibition of acetyltransferases (Madeo et al., 2019; Eisenberg et al., 2009). Autophagy plays a key role in the anti-aging processes of cells, including neuroprotective effects such as the proteostasis of synaptic active zones, preserving synaptic plasticity (Madeo et al., 2018). Furthermore, higher intake of spermidine may prevent neurodegeneration by mitigation of autoimmune-directed axon demyelination (Madeo et al., 2018). However, the exact mechanisms of the neuroprotective effect of spermidine are still the subject of intense research (Madeo et al., 2018).

The association between dietary spermidine intake and structural brain measures were similar for SCD and HC, providing no evidence for more pronounced brain atrophy in SCD. In contrast, previous studies showed atrophy as well as cortical thinning of the hippocampus and other memory-related brain regions in SCD versus HC (Chipi et al., 2019). However, one should note that SCD is known to be a rather heterogeneous preclinical stage of AD. Thus, our findings support the idea that the association of spermidine intake and structural brain measures may be consistent in the continuum of healthy older adults and SCD, probably irrespective of potential initiating brain pathologies. Therefore, dietary spermidine intake might be a potential dietary approach to delay or even prevent brain atrophy and cortical thinning in individuals at higher risk for AD as well as in healthy older adults.

Mediation analyses revealed that dietary spermidine intake substantially mediated the relationship between MeDi and several structural brain measures, including TGMV, hippocampal volume, mCT as well as regional cortical thickness. This finding is in line with the study of Binh and colleagues, which also showed a positive relationship between spermidine intake and adherence to MeDi, most likely attributable to the fact that most foods of the MeDi contain high concentrations of polyamines (Binh et al., 2011; Muñoz-Esparza et al., 2019). Somewhat

Table 3
Association between dietary spermidine intake and cortical thickness.

Model	B[b]	SE	β[c]	t	95% CI	R^{2d}	adj. R^2	p-value[a]
mCT								
Unadjusted	0.080	0.037	0.187	2.178	0.007, 0.153	0.035	0.028	0.031
Adjusted	0.095	0.045	0.222	2.112	0.006, 0.184	0.202	0.164	0.037
AD-ROIs								
Unadjusted	0.082	0.040	0.176	2.049	0.003, 0.162	0.031	0.024	0.042
Adjusted	0.095	0.050	0.203	1.899	−0.004, 0.193	0.173	0.134	0.060
Frontal								
Unadjusted	0.078	0.042	0.160	1.855	−0.005, 0.161	0.026	0.018	0.066
Adjusted	0.081	0.052	0.167	1.547	−0.023, 0.185	0.160	0.120	0.124
Temporal								
Unadjusted	0.115	0.045	0.217	2.541	0.025, 0.204	0.047	0.040	0.012
Adjusted	0.132	0.054	0.249	2.434	0.025, 0.239	0.242	0.206	0.016
Parietal								
Unadjusted	0.109	0.046	0.202	2.359	0.018, 0.200	0.041	0.033	0.020
Adjusted	0.135	0.058	0.251	2.312	0.020, 0.251	0.148	0.108	0.022
Cingulate								
Unadjusted	0.052	0.057	0.079	0.910	−0.061, 0.165	0.006	−0.001	0.364
Adjusted	0.047	0.073	0.072	0.652	−0.097, 0.192	0.107	0.064	0.516

Abbreviations: AD = Alzheimer's disease; adj. = adjusted; CI = confidence interval; mCT = mean cortical thickness; ROIs = regions of interest; SE = standard error.
[a] p-values estimated from linear regressions, adjusted models include the following covariates: age, sex, years of education, diagnostic group, and Mediterranean Diet. For the analyses of cortical thickness parameters, four participants (3 SCD, 1 HC) had to be excluded due to substantial outlier characteristics.
[b] Unstandardized regression coefficient.
[c] Standardized coefficient.
[d] Explained variance.

surprisingly, we did not observe a statistically significant direct association of adherence to MeDi with structural brain measures before and after entering the mediator into the model (except for TGMV), in contrast to previous studies observing positive associations between adherence to MeDi and brain volumes as well as cortical thickness (Mosconi et al., 2014; Gu et al., 2015). The fact that there were no strong associations between adherence to MeDi and several structural brain measures did not discourage us to interpret our findings of the indirect effect, as suggested by other groups (Zhao et al., 2010; Rucker et al., 2011). The findings from our mediation analyses (indirect effects) suggest that spermidine may not be a proxy of MeDi, a finding also supported by the fact that the main findings of our study remained stable after controlling for adherence to MeDi. Thus, spermidine may be one decisive ingredient of MeDi with regard to brain health.

4.1. Strengths and limitations

The use of a well-validated FFQ allowed us to investigate the relationship between dietary spermidine intake, MeDi, and a comprehensive array of structural brain measures. However, we concede that the use of a self-reported questionnaire represents only an indirect tool to determine dietary spermidine intake and increases the risk of inaccurate reports (e.g., over- or underestimation of food intake and limited assessment of food items). More direct assessments of dietary spermidine intake and consequently of endogenous spermidine concentration (e.g., markers of autophagy from peripheral blood mononuclear cells and muscle tissue) that could have supported our findings based on the FFQ were not available for the present analysis, but might be included in future studies. Moreover, the concentration of spermidine in individual foods is highly dependent on food processing, storage, and preparation. Nevertheless, the FFQ is well validated with actual food intake and reports the intake of polyamines over the last 12 months, allowing to draw conclusions of average long-term intakes (Willett et al., 1985; Eisenberg et al., 2016; Kiechl et al., 2018). In addition, our findings rely on spermidine intake from dietary sources typical for Western diets. Thus, our findings might not be applicable to cultures or ethnic groups with different eating habits and food products, an issue to be investigated in the future.

The cohort of healthy older adults as well as the cohort of individuals with SCD may not be representative of the general population, given our approach of advertising the study in the context of developing strategies to mitigate age-associated memory loss. Thereby, it might specifically target those older adults interested in brain health, which might not be representative for the general population. However, we advertised the study in very different outlets, including healthcare facilities and flyer distribution as well as through advertisements in newspapers, radio, and internet, thereby casting a wide net across different strata of the population. Note also that by recruiting healthy older adults and older individuals with SCD, we were able to include participants with a large variance in cognitive and psychological parameters in our sample, a major strength of this study. Future studies will have to validate or refute these findings in populations drawn at random from the general population.

However, note that for our exploratory analyses, we did not test for a wide array of additional potential brain-health beneficial nutrients, but included adherence to MeDi as a confounding variable (covariate) in our linear regression models, thereby controlling for dietary intake of typical Mediterranean foods and nutrients, such as omega-3 fatty acids. In addition, our results remained stable after controlling for several possible confounders such as age, sex, years of education, group (HC vs SCD), and adherence to MeDi. However, additional confounders (e.g., parameters of cardiovascular health, genetics) cannot be excluded and should be considered in future larger studies. Furthermore, the results of our study were not corrected for multiple testing given the exploratory nature of the present study, and thus need to be confirmed or refuted in another sample. Moreover, causal relationships cannot be derived from this cross-sectional sample. However, hypotheses gained from this approach can then be tested in interventional studies.

In conclusion, this study showed for the first time that higher self-reported dietary intake of spermidine is associated with greater mean cortical thickness, including AD-vulnerable regions, the parietal lobe, and temporal lobe, as well as with larger hippocampal volume. These findings suggest that higher dietary spermidine intake may have a neuroprotective effect, and thus might be a promising dietary approach to delay or even prevent brain atrophy and cortical thinning in older individuals. These beneficial structural changes could help to delay or even prevent further cognitive decline in older adults with SCD, a hypothesis

Table 4
Dietary spermidine intake as a mediator for the association of Mediterranean Diet and structural brain measures.

	Path a:		Path b:		Path c:		Path ab:	
	effect of MeDi on spermidine		*effect of spermidine on brain outcome*		*effect of MeDi on brain outcome*		*indirect effect*	
	B[b]	p-value[a]	B[b]	p-value[a]	B[b]	p-value[a]	B[b]	95% CI
Brain Volumes								
TBV								
Unadjusted	0.071	<0.001	2.538	0.121	−0.286	0.081	0.181	−0.0559, 0.4085
Adjusted	0.068	<0.001	2.458	0.152	−0.183	0.242	0.168	−0.0572, 0.3909
TGMV								
Unadjusted	0.071	<0.001	3.821	0.001	−0.420	<0.001	0.272	0.1028, 0.4710
Adjusted	0.068	<0.001	3.508	<0.001	−0.326	0.003	0.239	0.0938, 0.3988
TWMV								
Unadjusted	0.071	<0.001	−1.291	0.322	0.135	0.349	−0.092	−0.2912, 0.0717
Adjusted	0.068	<0.001	−1.050	0.451	0.144	0.310	−0.072	−0.2688, 0.0934
HPC								
Unadjusted	0.071	<0.001	0.076	<0.001	−0.003	0.325	0.005	0.0021, 0.0091
Adjusted	0.068	<0.001	0.069	0.002	−0.001	0.636	0.005	0.0016, 0.0079
Cortical thickness								
mCT								
Unadjusted	0.072	<0.001	0.121	0.008	−0.007	0.207	0.009	0.0020, 0.0156
Adjusted	0.069	<0.001	0.095	0.029	−0.005	0.278	0.007	0.0003, 0.0129
AD-ROIs								
Unadjusted	0.072	<0.001	0.128	0.010	−0.008	0.201	0.009	0.0024, 0.0165
Adjusted	0.069	<0.001	0.095	0.055	−0.007	0.226	0.007	−0.0001, 0.0135
Frontal								
Unadjusted	0.072	<0.001	0.113	0.024	−0.006	0.353	0.008	0.0011, 0.0158
Adjusted	0.069	<0.001	0.081	0.107	−0.004	0.487	0.006	−0.0013, 0.0131
Temporal								
Unadjusted	0.072	<0.001	0.158	0.006	−0.008	0.262	0.011	0.0031, 0.0202
Adjusted	0.069	<0.001	0.132	0.014	−0.006	0.341	0.009	0.0021, 0.0171
Parietal								
Unadjusted	0.072	<0.001	0.172	0.003	−0.011	0.141	0.012	0.0036, 0.0210
Adjusted	0.069	<0.001	0.135	0.027	−0.010	0.162	0.009	0.0008, 0.0178
Cingulate								
Unadjusted	0.072	<0.001	0.083	0.343	−0.006	0.501	0.006	−0.0063, 0.0185
Adjusted	0.069	<0.001	0.048	0.535	−0.003	0.743	0.003	−0.0085, 0.0149

Abbreviations: AD = Alzheimer's disease; CI = confidence interval; HPC = hippocampus; mCT = mean cortical thickness; MeDi = Mediterranean Diet; ROIs = regions of interest; TBV = total brain volume; TGMV = total grey matter volume; TWMV = total white matter volume.

[a] p-values based on CI from mediation models including adherence to MeDi as independent variable (x), structural brain measures as dependent variable (y), and dietary spermidine intake as mediator (m). Direct effects for path a (x → m), b (m → y), and c (x → y) as well as indirect effects (x → m → y) were reported. Adjusted models include the following covariates: age, sex, years of education, and diagnostic group. For the analyses of cortical thickness parameters, four participants (3 SCD, 1 HC) had to be excluded due to substantial outlier characteristics.

[b] Unstandardized regression coefficient.

to be tested in future interventional trials.

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Claudia Schwarz: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Nora Horn:** Methodology, Software, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Gloria Benson:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Isabel Wrachtrup Calzado:** Investigation, Writing - review & editing. **Katharina Wurdack:** Investigation, Writing - review & editing. **Raimund Pechlaner:** Formal analysis, Writing - review & editing. **Ulrike Grittner:** Formal analysis, Writing - review & editing. **Miranka Wirth:** Conceptualization, Methodology, Software, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Agnes Flöel:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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