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RESEARCH ARTICLE

Association of spermidine plasma levels with brain aging in a population-based study

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Abstract

Introduction: Supplementation with spermidine may support healthy aging, but elevated spermidine tissue levels were shown to be an indicator of Alzheimer's disease (AD).

Methods: Data from 659 participants (age range: 21–81 years) of the population-based Study of Health in Pomerania TREND were included. We investigated the association between spermidine plasma levels and markers of brain aging (hippocampal volume, AD score, global cortical thickness [CT], and white matter hyperintensities [WMH]).

Results: Higher spermidine levels were significantly associated with lower hippocampal volume ($\beta = -0.076$; 95% confidence interval [CI]: −0.13 to −0.02; $q = 0.026$), higher AD score ($\beta = 0.118$; 95% CI: 0.05 to 0.19; $q = 0.006$), lower global CT ($\beta = -0.104$; 95% CI: −0.17 to −0.04; $q = 0.014$), but not WMH volume. Sensitivity analysis revealed no substantial changes after excluding participants with cancer, depression, or hemolysis.

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Discussion: Elevated spermidine plasma levels are associated with advanced brain aging and might serve as potential early biomarker for AD and vascular brain pathology.

KEYWORDS
Alzheimer's disease, cortical thickness, epidemiology, hippocampal volume, spermidine, white matter hyperintensities

1 | INTRODUCTION

Polyamines are naturally occurring cell metabolites of living organisms that have gained much attention in recent years due to their function in cellular longevity and their crucial role in protein synthesis, nucleic acid synthesis, protection of cells and genes from oxidative damage, regulation of DNA methylation, and general cell development.[1,2] They can be synthesized endogenously or ingested with food.[3] The main representatives of polyamines are spermidine and spermine.

Interestingly, polyamines have been linked to positive as well as negative health effects. On the one hand, increased dietary polyamine intake has been reported to improve cognitive and general health and to slow the aging process, and has been promoted for the prevention of dementia.[4] Especially dietary spermidine was shown to be associated with benefits on different systems (i.e., cardiovascular system, brain structure and function, and immune system) in animal models.[4]

Consistent with previous research in animal models, there is also evidence that dietary spermidine intake might improve memory performance in older individuals with subjective cognitive decline (SCD) and patients with mild cognitive impairment (MCI).[5–7] In addition, a positive relationship has been shown between dietary spermidine intake and hippocampal volume, as well as cortical thickness (CT) in older adults.[8] Finally, there is evidence that increased spermidine intake might be associated with a lower mortality rate in the general population.[9] However, supplementation studies were small in sample size and therefore results may not be generalizable. In fact, a recently published larger longitudinal clinical trial failed to show a positive relationship between spermidine supplementation and memory performance in their primary analysis.[10] Moreover, it is still controversial if and to what degree spermidine is able to cross the blood–brain barrier (BBB).[11,12]

On the other hand, higher tissue polyamine levels have been reported in various diseases like dementia due to Alzheimer's disease (AD),[13–15] Parkinson's disease,[16] cancer,[17] and mental disorders like depression.[18] For instance, several *post mortem* studies showed that polyamine tissue levels (i.e., putrescine, spermine, and spermidine) were elevated in frontal and occipital lobes,[19] or in temporal cortex, white matter, and thalamus in patients with AD.[14,20] Moreover, Paik et al. demonstrated that urinary polyamine levels in AD patients were significantly elevated compared to healthy individuals.[21] Although the exact mechanisms between polyamine metabolism and the development of AD are not yet clear, it is assumed that an increase of ornithine decarboxylase as a key enzyme of polyamine synthesis, as well as a maladaptive stress response of the polyamine metabolism due to peptide-associated free radical damage, may possibly underlie its pathogenic role.[15] Polyamines are assumed to be toxic to brain cell cultures, and polyamine injections have been shown in animal models to damage the BBB.[22] Finally, using pathway enrichment analysis, Graham et al. demonstrated that patients with MCI who converted to dementia had higher spermidine and spermine production at baseline compared to MCI patients who remained stable over time.[13] Additionally, based on blood-based high-resolution mass spectrometry metabolomics, they were able to accurately predict those MCI patients converting to dementia 2 years prior to dementia diagnosis.

Although previous studies investigated the role of polyamine levels in brain tissue, urine, and blood in patients with AD and in animal models of AD, little is known about the role of polyamine blood levels during aging in the general population. In addition, there is conflicting evidence on the effect of spermidine supplementation versus spermidine tissue levels in brain health. Finally, the presumed role of spermidine as a predictive biomarker for the conversion from MCI to dementia due to AD raises the possibility that spermidine blood levels may also be a promising biomarker to predict the onset of neurodegenerative diseases in healthy cohorts. Therefore, the aim of the present work was to investigate the association between spermidine plasma levels and brain health in a population-based cross-sectional study by using neuroimaging markers for AD, to gain more insights into the role of spermidine across the lifespan.

We hypothesized that higher spermidine blood levels would be associated with less advantageous gray and white matter patterns related to advanced brain aging. In particular, we hypothesized that higher spermidine plasma levels would be associated with lower hippocampal volume, lower global CT, higher AD score (i.e., greater AD-related brain atrophy), and greater white matter hyperintensities (WMH) volume.

2 | METHODS

2.1 | Study sample

The Study of Health in Pomerania (SHIP) was designed to determine the prevalence of common risk factors and diseases in a population in northeastern Germany.[23] For this purpose, a sample was randomly drawn from local registers. Between 1997 and 2001, 4,308 individuals participated in the study. In parallel to the original SHIP-START study, a new independent sample was drawn to conduct research of similar scope (SHIP-TREND). The data used in our analyses were

RESEARCH IN CONTEXT

1. **Systematic Review**: Supplementation with the polyamine spermidine has gained attention as a potentially beneficial intervention to support healthy aging in preclinical and clinical studies. However, elevated spermidine tissue levels are also known to be an indicator of pathological processes such as Alzheimer's disease. Epidemiological studies investigating the association of spermidine blood levels and brain aging are still missing.
2. **Interpretation**: Our study provides evidence across key imaging markers of brain aging that spermidine plasma levels are associated with greater cortical and subcortical brain pathology in the general population. Thus, physiological spermidine levels might not reflect the beneficial health effects observed with spermidine supplementation in animal models and human studies.
3. **Future Directions**: Further longitudinal studies are needed to decipher the role of spermidine in neurodegenerative diseases and to determine whether spermidine blood levels are suitable biomarkers.

derived from this SHIP-TREND cohort, which was initiated 10 years after SHIP-START in the same region. A total of 4,420 individuals participated in the SHIP-TREND study. All participants gave written informed consent. The study was approved by the ethics committee of the University Medicine Greifswald (no. of ethics vote: BB39/08) and complies with the Declaration of Helsinki. Data used in our analyses originate from baseline examinations, which took place between 2008 and 2012 (SHIP-TREND-0). A detailed description of the assessment of all included variables can be found in the supporting information.

2.2 | Statistical analysis

Statistical analysis was performed using R version 3.6.1.[24] For multivariable regression analyses, we used the "lm" function for fitting ordinary least squares regression models.[24] Magnetic resonance imaging (MRI) markers of brain aging (i.e., global hippocampal volume, AD score, global CT, and WMH) were used as outcome variables. Three nested models were implemented: The first basic model examined the relationship between the exposure variable spermidine and the respective outcome variable, adjusted for total intracranial volume (ICV). A second model was additionally adjusted for age, sex, red blood cell (RBC) count, and the interaction of age and sex (spermidine confounder model), which were previously identified as possible confounder variables of spermidine. For instance, previous studies showed that spermidine levels decrease with increasing age,[25] and 90% of free circulating spermidine is associated with RBCs.[26] Moreover, age and sex are known to be associated with brain aging.[27,28] A final model was additionally adjusted for the exposure variables C-reactive protein, waist-to-hip ratio, years of education, dietary habits, alcohol consumption, smoking status, and apolipoprotein E (*APOE*) $\varepsilon 4$ (dementia risk factor model) to account for well-known dementia risk factors.[29] Total ICV was added to each model because it highly correlates with brain structure sizes.[30] Additionally, in the spermidine confounder and dementia risk factor model age and the interaction of age and sex were modeled not only linearly but also quadratically to account for non-linear age effects on the respective outcome variable. Finally, to reach normal distribution a log transformation was applied to spermidine, C-reactive protein, and WMH volume. We used false discovery rate (FDR) to control for multiple testing.[31] Results were considered statistically significant at a q-value of < 0.05. Participants with missing data on one of the variables of interest were excluded from analysis.

As sensitivity analyses, we re-ran the dementia risk factor model for each brain aging marker after excluding participants with cancer diagnosis as well as moderate and severe depression ($N = 56$, see supporting information for details on the assessment) as it is well known that individuals with cancer and depression show elevated spermidine blood level values compared to healthy individuals.[17,18]

Additionally, to rule out that hemolysis affected our results, we re-ran the dementia risk factor model for each brain aging marker after excluding participants with elevated lactate dehydrogenase or potassium values ($N = 27$, see supporting information for details on the assessment), as both blood markers are known to indicate hemolysis and 90% of free circulating spermidine is known to be associated with RBCs.[26]

3 | RESULTS

3.1 | Descriptive statistics

Descriptive characteristics of the study sample are shown in Table 1. Our final sample comprised 659 participants (mean age 50.1 years ± 13.5 years, 58% women). Participants with structural abnormalities of the brain (e.g., tumors, cysts, ventricular dilatations; $N = 44$) and low segmentation quality of WMH (e.g., over- or under-segmented WMH; $N = 115$) were excluded. Additionally, participants with spermidine plasma values exceeding ± 3 standard deviations of the mean plasma concentration were also excluded from the study sample ($N = 2$). See Figure 1 for a flowchart showing the selection of the study sample. Most participants (75.3%) indicated being physically active (i.e., including exercise during summer and winter time). The Physical Health Component Score (PCS-12) and Mental Health Component Score (MCS-12) revealed averages of 51.11 and 55.45 points, respectively, with 100 points being the maximum score corresponding to excellent physical and mental health.[32] A moderate and recommended dietary habit was present in 19.1% and 15.6% of the participants, respectively. Additionally, 2.4% of participants showed moderate or severe symptoms of depression. A total of 44% and 35.5%

TABLE 1 Descriptive characteristics of study sample

Characteristics	Median (95% CI) or *N* (%)
Sociodemographic parameters	
Age [years]	51.0 (40.5, 60.0)
Female *N* (%)	380 (58.0)
Education [years]	13 (11, 15)
Blood parameters	
Red blood cell count [Tpt/l]	4.60 (4.30, 4.90)
White blood cell count [Gpt/l]	5.46 (4.74, 6.42)
CRP [mg/l]	1.13 (0.61, 2.47)
Spermidine [μM]	0.12 (0.10, 0.14)
Brain imaging measures	
Alzheimer's disease score	−4.48 (−5.39, −3.80)
White matter [ml]	601.71 (549.86, 655.31)
Gray matter [ml]	574.60 (530.28, 618.69)
Global hippocampal volume [mm^3]	6918.34 (6514.07, 7353.55)
Global cortical thickness [mm]	4.70 (4.54, 4.84)
White matter hyperintensities volume [mm^3]	171 (69, 421)
Intracranial volume [L]	1.57 (1.46, 1.67)
Lifestyle	
Dietary habit, *N* (%)	
Poor	430 (65.3)
Moderate	126 (19.1)
Recommended	103 (15.6)
Alcohol consumption [ethanol in g/d][a]	3.92 (1.26, 9.78)
Smoking status, *N* (%)	
Non-smoker	290 (44.0)
Previous smoker	234 (35.5)
Smoker	135 (20.5)
Physical activity, *N* (%)	
Not active	163 (24.7)
Active	496 (75.3)
Waist-to-hip ratio	0.86 (0.81, 0.93)
BMI [kg/m^2]	26.79 (24.13, 29.62)
Mental health	
Depression[b], *N* (%)	16 (2.4)
Moderate	11 (1.7)
Severe	5 (0.8)
Mental health score[c]	55.45 (50.83, 57.29)
Physical health	
Physical health score[c]	51.11 (45.92, 54.83)
Cancer diagnosis, *N* (%)	36 (5.5)
Genetics	
APOE ε4, *N* (%)	152 (23.1)

Note: Values are presented as median and interquartile range or *N* and %.
Abbreviations: *APOE*, apolipoprotein E; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein.
[a]Alcohol consumption was calculated as alcohol consumption during the last 30 days.
[b]$N = 654$.
[c]$N = 648$.

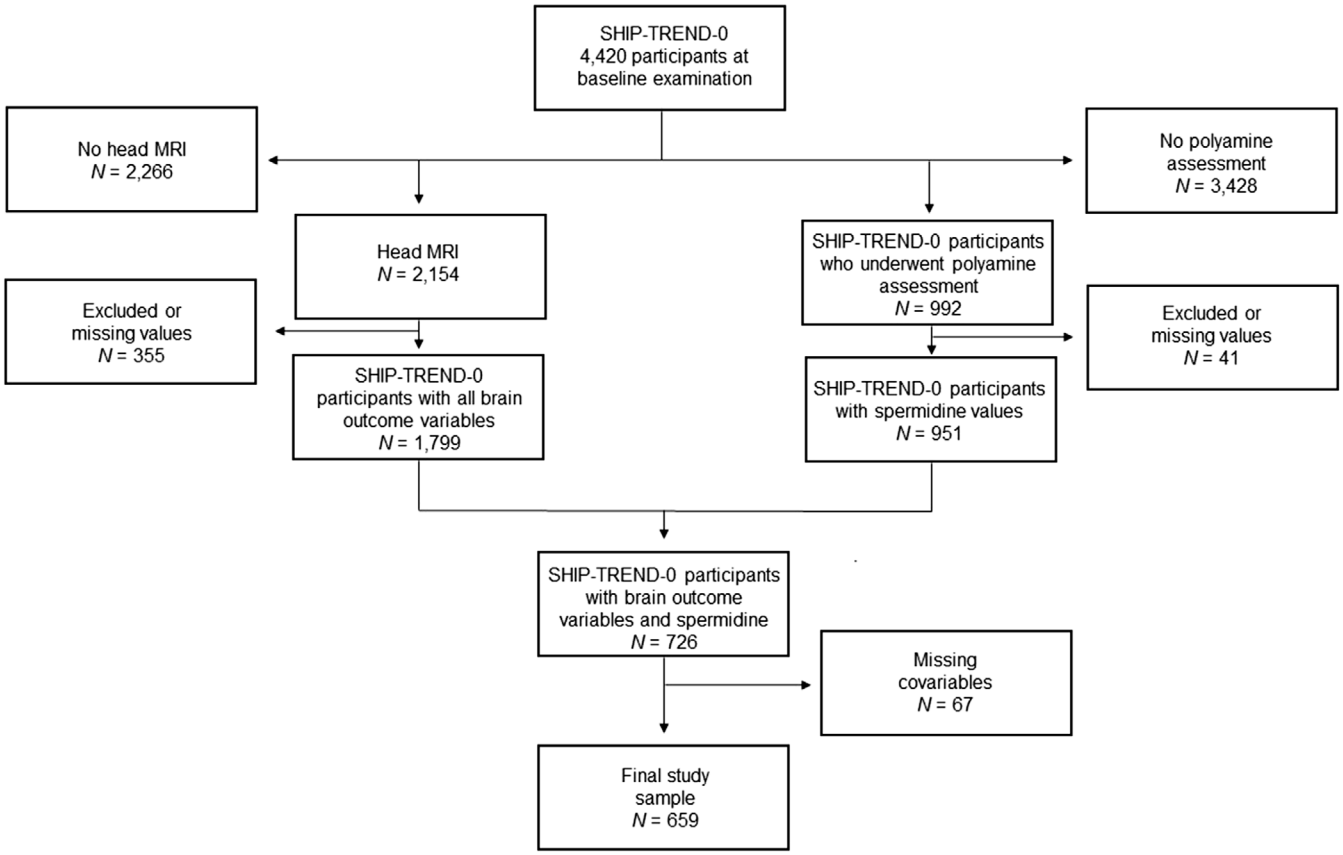

FIGURE 1 Flowchart showing the selection of the study sample. The final sample comprised 659 adults from the SHIP-TREND-0 study. MRI, magnetic resonance imaging

were never smokers and former smokers, respectively. Furthermore, 23.1% of participants were *APOE* ε4 allele carriers (i.e., either ε3/ε4 or ε4/ε4) and 5.5% had received a cancer diagnosis at least once in their lifetime.

3.2 | Association between spermidine and markers of brain aging

Table 2 shows the association between spermidine and the four different markers of brain aging considered in this study. Results for all regression models including all covariates can be found in the supporting information (Table S1–S4).

3.2.1 | Association between spermidine and global hippocampal volume

To evaluate the relationship between spermidine blood values and global hippocampal volume in our sample we ran a first regression model (basic model), which revealed an inverse association between both variables (ß = −0.063, *q* = 0.047). We also ran a second partially adjusted multivariable regression model (spermidine confounder model). This regression model explained 54% of the variance (R^2 = 0.54, *adj.* R^2 = 0.53, *F*[8, 650] = 93.74) and revealed an inverse association between spermidine and hippocampal volume (ß = −0.076, *q* = 0.013). The final fully adjusted regression model (dementia risk factor model) explained 54% of the variance (R^2 = 0.54, *adj.* R^2 = 0.53, *F*[17, 641] = 44.99) and revealed again an inverse association between spermidine and hippocampal volume (ß = −0.076, *q* = 0.026). Thus, all three regression models consistently indicate that higher spermidine blood levels are associated with lower hippocampal volume. Detailed results are shown in Table S1.

3.2.2 | Association between spermidine and AD score

The basic model revealed a positive association between spermidine and AD score (ß = 0.103, *q* = 0.026). To further investigate this relationship, we ran the spermidine confounder model. This regression model explained 21% of the variance (R^2 = 0.21, *adj.* R^2 = 0.20, *F*[8, 650] = 22.05) and showed a positive association between spermidine and AD score (ß = 0.117, *q* = 0.005). The fully adjusted dementia risk factor model explained 25% of the variance (R^2 = 0.25, *adj.* R^2 = 0.23, *F*[17, 641] = 12.27) and again revealed that spermidine was positively associated with AD score (ß = 0.118, *q* = 0.006). All three regression models consistently indicate that higher spermidine blood levels

TABLE 2 Association between spermidine and markers of brain aging

	Spermidine	
	ß (95% CI)	*q*-value
Global hippocampal volume		
Basic model	−0.063 (−0.12, −0.01)	0.047
Spermidine confounder model	−0.076 (−0.13, −0.02)	0.013
Dementia risk factor model	−0.076 (−0.13, −0.02)	0.026
Alzheimer's disease score		
Basic model	0.103 (0.03, 0.18)	0.026
Spermidine confounder model	0.117 (0.04, 0.19)	0.005
Dementia risk factor model	0.118 (0.05, 0.19)	0.006
Global cortical thickness		
Basic model	−0.125 (−0.20, −0.05)	0.004
Spermidine confounder model	−0.105 (−0.17, −0.04)	0.004
Dementia risk factor model	−0.104 (−0.17, −0.04)	0.014
White matter hyperintensities volume		
Basic model	0.073 (0.00, 0.15)	0.093
Spermidine confounder model	0.082 (0.02, 0.15)	0.045
Dementia risk factor model	0.081 (0.03, 0.16)	0.103

Note: *ß*: standardized regression coefficient; 95% CI with lower and upper bound; *q*: *q*-value. A log transformation was applied to spermidine, C-reactive protein, and white matter hyperintensities volume. Basic model: adjusted for ICV. Spermidine confounder model: adjusted for basic model variables and age, age^2, sex, RBC count, the interaction age x sex and age^2 x sex. Dementia risk factor model: adjusted for spermidine confounder model variables and C-reactive protein, waist-to-hip ratio, years of education, dietary habits, alcohol consumption, smoking status, and *APOE ε*4. All *P*-values are FDR corrected for multiple testing (e.g., *q*-value). Detailed results for all regression models including all variables can be found in the supporting information (Table S1–S4).
Abbreviations: *APOE*, apolipoprotein E; CI, confidence interval; FDR, false discovery rate; ICV, intracranial volume; RBC, red blood cell.

are associated with higher AD scores. Detailed results are shown in Table S2.

3.2.3 | Association between spermidine and global CT

The basic model revealed an inverse association between spermidine and global CT ($ß = -0.125$, $q = 0.004$). The spermidine confounder model explained 38% of the variance ($R^2 = 0.38$, *adj.* $R^2 = 0.37$, $F[8, 650] = 49.45$). Spermidine blood levels were inversely associated with global CT ($ß = -0.105$, $q = 0.004$). Finally, the dementia risk factor model explained 40% of the variance ($R^2 = 0.40$, *adj.* $R^2 = 0.38$, $F[17, 641] = 24.69$) and confirmed again the already found inverse association between spermidine and global CT ($ß = -0.104$, $q = 0.014$). All three regression models consistently indicate that higher spermidine blood levels are associated with lower CT. Detailed results are shown in Table S3.

3.2.4 | Association between spermidine and WMH volume

Finally, the basic model revealed no significant association between spermidine and WMH volume ($ß = 0.073$, $q = 0.093$). The spermidine confounder model explained 34% of the variance ($R^2 = 0.34$, *adj.* $R^2 = 0.33$, $F[8, 650] = 41.53$). Spermidine plasma levels were positively associated with WMH volume ($ß = 0.082$, $q = 0.045$). The fully adjusted dementia risk factor model explained 35% of the variance ($R^2 = 0.35$, *adj.* $R^2 = 0.33$, $F[17, 641] = 19.89$). After FDR correction for multiple testing, spermidine was not significantly associated with WMH volume ($ß = 0.081$, $q = 0.103$, $P = 0.017$). Taken together, the spermidine confounder model indicates that higher spermidine blood levels are associated with greater WMH volume. Although the association between spermidine and WMH volume in the dementia risk factor model was not significant after FDR correction, the standardized regression coefficient for this model supports the relation between spermidine and WMH volume found in the spermidine confounder model. Detailed results are given in Table S4 in the supporting information.

3.3 | Sensitivity analyses

Because elevated spermidine levels are also more common in individuals with cancer and depression compared to healthy individuals, we performed a sensitivity analysis excluding participants with cancer diagnosis and moderate and severe depression. This analysis did not substantially change results related to the association between spermidine and brain imaging markers. Detailed results

including all variables are given in Table S5 in the supporting information.

Furthermore, as 90% of free circulating spermidine is associated with RBCs,[26] the presence of hemolysis could have affected spermidine plasma levels. Therefore, in a second sensibility analysis we excluded participants with indication of possible hemolysis. Again, this analysis did not substantially change results related to the association between spermidine and brain imaging markers. Detailed results including all variables are given in Table S6 in the supporting information.

4 | DISCUSSION

The aim of the present study was to investigate the association of spermidine blood levels and brain aging across the lifespan in a community-based cross-sectional sample. In particular, we were interested in the association of spermidine with four different imaging-based biomarkers of brain aging and AD, that is, global hippocampal volume,[33] global CT,[34] WMH volume,[35] and AD score.[36]

We found that increased spermidine levels were associated with advanced brain aging independent of dementia risk factors (i.e., age, C-reactive protein, waist-to-hip ratio, dietary habits, alcohol consumption, smoking, and *APOE* ε4 allele carrier status) and potential confounders of spermidine levels in peripheral blood (i.e., age, sex, and RBC count). More specifically, elevated spermidine plasma levels were associated with lower hippocampal volume, higher AD score, and lower CT volume in the dementia risk factor model. Additionally, spermidine plasma levels were associated with greater WMH volume in the spermidine confounder model. Although the association between spermidine and WMH volume failed to reach significance after FDR correction for multiple testing in the dementia risk factor model, the standardized β-estimate revealed the same inverse tendency.

Our results are substantiated by previous cross-sectional evidence on the role of spermidine tissue and blood levels in patients with MCI and with dementia due to AD. Evidence on the association of elevated polyamine, in particular spermidine tissue levels, and AD is mainly based on *post mortem* studies.[14,19,37] It has been shown that spermidine tissue levels were elevated in the temporal cortex, white matter, and thalamus in patients with AD compared to spermidine tissue levels of participants without neurodegenerative disease.[14,20] This increase of spermidine in brain tissue from deceased patients with dementia due to AD indicates that an alteration of the polyamine metabolism might be involved in AD pathology.

However, evidence on the role of spermidine blood levels in AD is limited and partially contradicting.[13,38,39] In line with our findings, the study of Graham et al. showed that polyamine metabolism was altered in MCI patients who later transitioned to dementia leading to elevated spermidine (and spermine) production.[13] Similarly, Sternberg et al. showed that polyamine plasma levels were increased in patients with MCI compared to healthy controls.[39]

In contrast, a 4-year longitudinal study including patients with MCI, dementia due to AD, and healthy controls observed that spermidine plasma levels were lower in patients with MCI and dementia compared to healthy controls, whereas spermine plasma levels were increased in patients with MCI.[38] Moreover, our results seem to contradict previous findings on the effects of higher dietary spermidine intake and of spermidine supplementation on brain health, including brain structure and memory performance.[5–8]

However, one study showed that higher dietary spermidine intake was associated with smaller CT and smaller left hippocampal volume,[40] and a larger supplementation study in humans could not demonstrate an improvement in memory performance.[10] In fact, there is still controversy as to whether spermidine can cross the BBB in non-diseased states. Some reports have pointed out that spermidine only crosses the BBB in animal models suffering from fatal stress,[41] but there is also some evidence that spermidine can cross the BBB in healthy aged mice.[12] Moreover, supplementation studies have not consistently shown an increase in spermidine blood levels after dietary spermidine supplementation.[6,7,42–44] One possible explanation could be that polyamine metabolism is highly regulated and can thus preserve individual polyamine concentrations in blood over a wide range of dietary intake.[4] Therefore, short-term increases of polyamine intake will not change polyamine blood levels;[45] rather, an increase in dietary polyamine intake over at least 26 weeks might be necessary to detect significant changes in blood levels, as shown in mice.[42]

Mechanisms underlying the detrimental association of spermidine blood levels with brain health still remain to be fully elucidated. Interestingly, disruption of BBB has been reported in animal models after polyamine injections.[22] Accordingly, it may be possible that elevated polyamine blood levels could disrupt the BBB under certain circumstances. This in turn may facilitate the penetration of free radicals, viruses, and bacteria into the central nervous system, a theory also suggested by Zheng et al.[46] In addition, mechanistic explanations for the beneficial effects of polyamines on brain and overall health have been questioned, such as their putative ability to directly induce autophagy by inhibiting lysine acetyltransferase EP300.[47] Other studies have shown that in the presence of ruminant serum, exogenously added polyamines do not play a direct role in autophagy induction. Rather, autophagy may have been induced artificially by toxic metabolites such as aldehydes, peroxides, and ammonia, byproducts resulting from the oxidation of spermidine and spermine by bovine serum amine oxidase.[48] To ensure that our results were not driven by other possible pathological processes that may lead to elevated spermidine plasma levels, we performed two sensitivity analyses excluding participants with cancer diagnosis and moderate and severe depression or hemolysis, showing almost the same pattern as in our main analyses. Therefore, our findings indicate the robustness of the association of spermidine plasma levels and brain aging beyond pathologies in which polyamines play an already well-described decisive role.

Taken together, our findings consistently show an association between higher blood-derived spermidine levels and lower brain health, as indicated by several imaging biomarkers for brain aging and AD. To the best of our knowledge, this is the first study that investigated the role of spermidine blood levels across the lifespan in a community-based cross-sectional sample. While previous studies

have focused on the role of elevated spermidine levels in populations with preexisting neurodegenerative diseases like MCI or dementia due to AD and Parkinson's disease, our study is the first to show that these inverse associations already exist in individuals without previously diagnosed neurodegenerative disease or cognitive impairment. Moreover, we show for the first time that elevated spermidine blood levels are associated with white matter pathology, besides the already known association with gray matter atrophy. Our findings support the hypothesis that spermidine could not only be an indicator of incipient pathological states but may also serve as a potential biomarker for preclinical AD, and a predictor for further clinical deterioration.[13] Given the currently ongoing intensive search for blood-based biomarkers for AD that entail lower cost and less invasive procedures compared to biomarkers found in cerebrospinal fluid,[49] and still unsatisfactory sensitivity and specificity of amyloid beta, phosphorylated tau, neurofilament light chain, or glial fibrillary acidic protein in peripheral blood,[50] spermidine might be an interesting addition or possibly even alternative to these parameters.

4.1 | Limitations

Several limitations should be considered when interpreting our results. First, this study is cross-sectional; therefore, it is not possible to draw causal conclusions. Nevertheless, our study, which includes a large data set drawn from the general population, provides important insights on how elevated spermidine plasma levels are associated with markers of brain aging. Moreover, we currently have no information if participants included in this study developed neurodegenerative diseases at a later time point, an issue to be addressed in future studies.

4.2 | Conclusion

Our results show for the first time that higher spermidine levels are associated with more advanced cortical and subcortical brain aging in the general population. Thus, physiological spermidine blood levels do not reflect the beneficial health effects observed with supplementation in animal models. Rather, elevated spermidine blood levels are associated with more advanced brain aging and might even play a role as a potential biomarker for AD and vascular brain pathology. Future longitudinal research is needed to show to which extent elevated spermidine blood levels are specific and prognostic for neurodegenerative diseases.

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CONFLICTS OF INTEREST

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REFERENCES

1. Pegg AE. Functions of polyamines in mammals. *J Biol Chem*. 2016;291(29):14904-14912.
2. Fukui T, Soda K, Takao K, Rikiyama T. Extracellular spermine activates DNA methyltransferase 3A and 3B. *Int J Mol Sci*. 2019;20(5):1254.
3. Heby O, Holm I, Frostesjö L, et al. Polyamines: regulators of mammalian cell growth and differentiation. In: polyamines in the Gastrointestinal Tract. *Falk Symposium*. 1992;62:19-28.
4. Madeo F, Eisenberg T, Pietrocola F, Kroemer G. Spermidine in health and disease. *Science*. 2018;359(6374):eaan2788.
5. Pekar T, Wendzel A, Flak W, et al. Spermidine in dementia. *Wien Klin Wochenschr*. 2020;132(1):42-46.
6. Pekar T, Bruckner K, Pauschenwein-Frantsich S, et al. The positive effect of spermidine in older adults suffering from dementia. *Wien Klin Wochenschr*. 2021;133(9):484-491.
7. Wirth M, Benson G, Schwarz C, et al. The effect of spermidine on memory performance in older adults at risk for dementia: a randomized controlled trial. *Cortex*. 2018;109:181-188.
8. Schwarz C, Horn N, Benson G, et al. Spermidine intake is associated with cortical thickness and hippocampal volume in older adults. *Neuroimage*. 2020;221:117132.
9. Kiechl S, Pechlaner R, Willeit P, et al. Higher spermidine intake is linked to lower mortality: a prospective population-based study. *Am J Clin Nutr*. 2018;108(2):371-380.
10. Schwarz C, Benson GS, Horn N, et al. Effects of spermidine supplementation on cognition and biomarkers in older adults with subjective cognitive decline: a randomized clinical trial. *JAMA Netw Open*. 2022;5(5):e2213875-e2213875.
11. Shin W, Fong W, Pang S, Wong PC. Limited blood-brain barrier transport of polyamines. *J Neurochem*. 1985;44(4):1056-1059.
12. Schroeder S, Hofer SJ, Zimmermann A, et al. Dietary spermidine improves cognitive function. *Cell Rep*. 2021;35(2):108985.
13. Graham SF, Chevallier OP, Elliott CT, et al. Untargeted metabolomic analysis of human plasma indicates differentially affected polyamine and L-arginine metabolism in mild cognitive impairment subjects converting to Alzheimer's disease. *PLoS One*. 2015;10(3):e0119452.

14. Morrison LD, Kish SJ. Brain polyamine levels are altered in Alzheimer's disease. *Neurosci Lett*. 1995;197(1):5-8.
15. Polis B, Karasik D, Samson AO. Alzheimer's disease as a chronic maladaptive polyamine stress response. *Aging (Albany NY)*. 2021;13(7):10770.
16. Lewandowski NM, Ju S, Verbitsky M, et al. Polyamine pathway contributes to the pathogenesis of Parkinson disease. *Proc Natl Acad Sci*. 2010;107(39):16970-16975.
17. Casero RA, Stewart TM, Pegg AE. Polyamine metabolism and cancer: treatments, challenges and opportunities. *Nat Rev Cancer*. 2018;18(11):681-695.
18. Limon A, Mamdani F, Hjelm BE, Vawter MP, Sequeira A. Targets of polyamine dysregulation in major depression and suicide: activity-dependent feedback, excitability, and neurotransmission. *Neurosci Biobehav Rev*. 2016;66:80-91.
19. Inoue K, Tsutsui H, Akatsu H, et al. Metabolic profiling of Alzheimer's disease brains. *Sci Rep*. 2013;3(1):1-9.
20. Morrison LD, Becker L, Ang LC, Kish SJ. Polyamines in human brain: regional distribution and influence of aging. *J Neurochem*. 1995;65(2):636-642.
21. Paik M-J, Lee S, Cho K-H, Kim K-R. Urinary polyamines and N-acetylated polyamines in four patients with Alzheimer's disease as their N-ethoxycarbonyl-N-pentafluoropropionyl derivatives by gas chromatography – mass spectrometry in selected ion monitoring mode. *Anal Chim Acta*. 2006;576(1):55-60.
22. Glantz L, Nates JL, Trembovler V, Bass R, Shohami E. Polyamines induce blood-brain barrier disruption and edema formation in the rat. *J Basic Clin Physiol Pharmacol*. 1996;7(1):1-10.
23. John U, Hensel E, Lüdemann J, et al. Study of Health In Pomerania (SHIP): a health examination survey in an east German region: objectives and design. *Sozial-und Präventivmedizin*. 2001;46(3):186-194.
24. Team RC. R: A language and environment for statistical computing (Version 3.6. 1)[Software package]. 2019.
25. Pucciarelli S, Moreschini B, Micozzi D, et al. Spermidine and spermine are enriched in whole blood of nona/centenarians. *Rejuvenation Res*. 2012;15(6):590-595.
26. Minois N, Carmona-Gutierrez D, Madeo F. Polyamines in aging and disease. *Aging (Albany NY)*. 2011;3(8):716.
27. Coffey CE, Lucke JF, Saxton JA, et al. Sex differences in brain aging: a quantitative magnetic resonance imaging study. *Arch Neurol*. 1998;55(2):169-179.
28. Salat DH, Buckner RL, Snyder AZ, et al. Thinning of the cerebral cortex in aging. *Cereb cortex*. 2004;14(7):721-730.
29. Chen J-H, Lin K-P, Chen Y-C. Risk factors for dementia. *J Formos Med Assoc*. 2009;108(10):754-764.
30. Barnes J, Ridgway GR, Bartlett J, et al. Head size, age and gender adjustment in MRI studies: a necessary nuisance? *Neuroimage*. 2010;53(4):1244-1255.
31. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B*. 1995;57(1):289-300.
32. Ware Jr JE, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Med Care*. 1996;34(3):220-233.
33. Gosche KM, Mortimer JA, Smith CD, Markesbery WR, Snowdon DA. Hippocampal volume as an index of Alzheimer neuropathology: findings from the Nun Study. *Neurology*. 2002;58(10):1476-1482.
34. Dickerson BC, Bakkour A, Salat DH, et al. The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. *Cereb cortex*. 2009;19(3):497-510.
35. Habes M, Erus G, Toledo JB, et al. White matter hyperintensities and imaging patterns of brain ageing in the general population. *Brain*. 2016;139(4):1164-1179.
36. Frenzel S, Wittfeld K, Habes M, et al. A biomarker for Alzheimer's disease based on patterns of regional brain atrophy. *Front psychiatry*. 2020;10:953.
37. Morrison LD, Cao X, Kish SJ. Ornithine decarboxylase in human brain: influence of aging, regional distribution, and Alzheimer's disease. *J Neurochem*. 1998;71(1):288-294.
38. Joaquim HPG, Costa AC, Forlenza OV, Gattaz WF, Talib LL. Decreased plasmatic spermidine and increased spermine in mild cognitive impairment and Alzheimer's disease patients. *Arch Clin Psychiatry (São Paulo)*. 2019;46:120-124.
39. Sternberg Z, Podolsky R, Nir A, et al. Elevated spermidine serum levels in mild cognitive impairment, a potential biomarker of progression to Alzheimer dementia, a pilot study. *J Clin Neurosci*. 2022;100:169-174.
40. Tavares JF, Landstra EN, Brunner J, et al. Associations between dietary spermidine intake, cognition and brain volumes: prevention (nonpharmacological)/Nutrition. *Alzheimers Dement*. 2020;16:e045750.
41. Trout JJ, Koenig H, Goldstone AD, Lu CY. Blood-brain barrier breakdown by cold injury. Polyamine signals mediate acute stimulation of endocytosis, vesicular transport, and microvillus formation in rat cerebral capillaries. *Lab Invest*. 1986;55(6):622-631.
42. Soda K, Uemura T, Sanayama H, Igarashi K, Fukui T. Polyamine-rich diet elevates blood spermine levels and inhibits pro-inflammatory status: an interventional study. *Med Sci*. 2021;9(2):22.
43. Schwarz C, Stekovic S, Wirth M, et al. Safety and tolerability of spermidine supplementation in mice and older adults with subjective cognitive decline. *Aging (Albany NY)*. 2018;10(1):19.
44. Brodal BP, Eliassen KA, Rönning H, Osmundsen H. Effects of dietary polyamines and clofibrate on metabolism of polyamines in the rat. *J Nutr Biochem*. 1999;10(12):700-708.
45. Soda K. Overview of polyamines as nutrients for human healthy long life and effect of increased polyamine intake on DNA methylation. *Cells*. 2022;11(1):164.
46. Zheng L, Xie Y, Sun Z, et al. Serum Spermidine in relation to risk of stroke: a multilevel study. *Front Nutr*. 2022;9:496.
47. Pietrocola F, Lachkar S, Enot DP, et al. Spermidine induces autophagy by inhibiting the acetyltransferase EP300. *Cell Death Differ*. 2015;22(3):509-516.
48. Holbert CE, Dunworth M, Foley JR, Dunston TT, Stewart TM, Casero RA. Autophagy induction by exogenous polyamines is an artifact of bovine serum amine oxidase activity in culture serum. *J Biol Chem*. 2020;295(27):9061-9068.
49. Amin M, Tang S, Shalamanova L, et al. Polyamine biomarkers as indicators of human disease. *Biomarkers*. 2021;26(2):77-94.
50. Teunissen CE, Verberk IMW, Thijssen EH, et al. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. *Lancet Neurol*. 2022;21(1):66-77.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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