

Original Article

Effects of Spermidine Supplementation on Vascular Brain Health in Older Individuals with Subjective Cognitive Decline – An Exploratory MRI Analysis

Claudia Schwarz^{1,2,3#,*}, Katharina Wurdack^{2,3#}, Stefan Hetzer⁴, Andrea Dell’Orco^{2,3,5}, Silke M. Wortha^{1,6}, Daria Antonenko¹, Gloria S. Benson^{2,3,7}, Nora Horn^{2,3}, Ulrike Grittner^{8,9}, Sebastian J. Hofer^{10,11}, Tobias Eisenberg^{10,12,13}, Stephan J. Sigrist^{3,14}, Dietmar Schmitz³, Miranka Wirth^{2,3,15}, Frank Madeo^{10,12,13}, Agnes Flöel^{1,16}

¹Department of Neurology, University Medicine Greifswald, Greifswald, Germany; ²Department of Neurology, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ³NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ⁴Berlin Center for Advanced Neuroimaging (BCAN), Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ⁵Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Neuroradiology, Berlin, Germany; ⁶Centre for Mathematical Cognition, Loughborough University, Loughborough, United Kingdom; ⁷Department of Geriatric Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; ⁸Institute of Biometry and Clinical Epidemiology, Charité – Universitätsmedizin Berlin, Berlin, Germany; ⁹Berlin Institute of Health (BIH), Berlin, Germany; ¹⁰Institute of Molecular Biosciences, NAWI Graz, University of Graz, Graz, Austria; ¹¹Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany; ¹²BioTechMed Graz, Graz, Austria; ¹³Field of Excellence BioHealth – University of Graz, Austria; ¹⁴Institute for Biology/Genetics, Freie Universität Berlin, Berlin, Germany; ¹⁵German Center for Neurodegenerative Diseases (DZNE), Dresden, Germany; ¹⁶German Center for Neurodegenerative Diseases (DZNE), Standort Rostock/Greifswald, Greifswald, Germany.

[Received May 7, 2026; Revised August 13, 2026; Accepted August 14, 2026]

ABSTRACT: Spermidine supplementation benefits brain health and function in animal models, with emerging evidence suggesting cognitive benefits in humans. However, effects on human brain health remain unclear. In this randomized, double-blind, placebo-controlled trial (SmartAge), we investigated 12-month spermidine supplementation (0.9 mg spermidine/day) in individuals with subjective cognitive decline aged 60-90 years. This exploratory secondary MRI analysis included 75 participants and assessed changes in vascular brain health measures, including cerebral blood flow (CBF), white matter microstructure, and white matter hyperintensities. Brain volume and cortical thickness were also assessed. Regression analyses were adjusted for age, sex, and baseline measures. Between-group differences were observed in regional CBF and white matter microstructure. CBF increased in the precuneus from baseline to post-intervention in the spermidine group versus placebo (between-group difference, 4.9 ml/100 g/min; 95%CI, 1.0–8.9; $\eta^2_p=.11$ [90%CI, .012–.250]; $P=.015$), with a similar trend in the posterior cingulate gyrus (3.7 ml/100 g/min; 95%CI, -.5–7.8; $\eta^2_p=.06$ [90%CI, 0–.182]; $P=.08$). Fractional anisotropy in the cingulate gyrus white matter bundle remained stable with spermidine but decreased with placebo (.016; 95%CI, .002–.031; $\eta^2_p=.06$ [90%CI, .004–.208]; $P=.029$). CBF and fractional anisotropy changes were not substantially associated. No substantial between-group differences were found in

*Correspondence should be addressed to: Dr. Claudia Schwarz, University Medicine Greifswald, Department of Neurology, Ferdinand-Sauerbruch-Straße, D-17475 Greifswald, Germany. Email: claudia.schwarz@med.uni-greifswald.de. #These authors contributed equally to this work.

Copyright: © 2026 Schwarz C. et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

global or regional brain volume, cortical thickness, or white matter hyperintensities, and regional MRI changes were not associated with changes in memory performance, subjective cognitive complaints, or plasma soluble intercellular adhesion molecule-1 levels. This exploratory analysis identified selected regional MRI differences in cerebral perfusion and white matter microstructure among older adults at risk for cognitive decline. These findings are preliminary and require independent confirmation.

Keywords: Alzheimer's Disease, Polyamines, Cerebral Blood Flow, Arterial Spin Labeling, Diffusion Tensor Imaging, White Matter Microstructure

Introduction

Promoting healthy brain aging and developing strategies to counteract geriatric decline in cognitive function is of utmost importance in aging societies. The persistent perception of subjectively deteriorating cognitive capacities without objectively measurable cognitive decline in older adults has been characterized as subjective cognitive decline (SCD) [1-3]. This condition is suggested to reflect an early pre-clinical stage of Alzheimer's Disease (AD), as the presence of SCD has been linked to an increased risk of future cognitive deterioration and dementia [4, 5]. Furthermore, pathological brain changes typical of AD have been identified in SCD, including gray matter (GM) atrophy and white matter (WM) changes in AD-vulnerable regions [6-9] as well as detrimental changes in functional connectivity and cerebral perfusion [10-12]. Among these brain alterations, impaired cerebral vascular function may accelerate cognitive decline and promote progression from preclinical AD stages to manifest AD [13, 14]. Consequently, individuals with SCD may represent an ideal population to investigate early intervention strategies targeting vascular brain health to prevent cognitive decline and development of dementia.

Spermidine, a naturally occurring polyamine, is crucial for several intracellular processes, notably regulation of autophagy and maintenance of cellular homeostasis [15-17]. Studies in animal models suggest that spermidine crosses the blood-brain barrier [18, 19] and its dietary supplementation may counteract age-related cognitive decline by preserving autophagic and mitophagic capacity [18, 20-23]. Moreover, spermidine demonstrated positive effects on vascular health in animal models [24, 25], and improved endothelial function and arterial aging in mice [26, 27] and humans [28]. Approximately one third of the endogenous spermidine pool originates from dietary sources, with daily consumption levels estimated to be around 10 mg in humans, with high geographic variability [29]. Epidemiological studies in humans showed that increased dietary spermidine intake was linked to decreased mortality [30-32]. This was mostly attributed to

potentially cardioprotective properties and improved vascular health, which is also supported by preclinical data [17]. Moreover, diets rich in spermidine appear to protect against cognitive decline [18, 33] and are associated with greater hippocampal volume and cortical thickness in older adults [34]. Overall, a growing body of literature suggests that elevated dietary spermidine intake may protect from age-related decline of cardiovascular and brain function.

In older adults with SCD, a 3-month spermidine supplementation study showed first evidence for memory improvement [35]. A subsequent 12-month spermidine supplementation (SmartAge study) demonstrated no beneficial effects of the intervention on cognitive, behavioral, and physiological parameters across the whole cohort [36]. However, in exploratory analyses in participants who had successfully completed the 12-month intervention period with compliance rates above 90%, a positive intervention effect on the soluble intercellular adhesion molecule-1 (sICAM-1; i.e., reduced plasma levels), a suggested marker of blood vessel injury, endothelial dysfunction, cardiovascular disease (CVD) progression, and vascular inflammation [37-39], was observed. In addition, exploratory analyses suggested beneficial effects on memory performance in specific subgroups of participants, including individuals aged 70 years and older, in men, and individuals with more severe subjective cognitive complaints.

Based on previous research on the importance of preserving vascular brain health in preclinical AD, the possible beneficial effects of spermidine on cardiovascular health from animal and epidemiological studies, and evidence for beneficial effects of spermidine supplementation on a vascular pro-inflammatory marker, we conducted a comprehensive evaluation of the effect of increased spermidine intake on vascular brain health. In this exploratory secondary analysis of the SmartAge trial, we used multimodal magnetic resonance imaging (MRI) data to examine the effect of 12-month spermidine supplementation on cerebral perfusion, WM microstructure, and WM hyperintensities (WMH) in older individuals with SCD. In addition, the impact of the

intervention on the macrostructural measures of brain volumetry and cortical thickness was evaluated.

MATERIALS AND METHODS

Study design and participants

The SmartAge study was a monocenter, randomized, double-masked, placebo-controlled phase IIb trial investigating the effect of 12-month dietary spermidine supplementation on memory performance and biomarkers in older individuals with SCD (ClinicalTrials.gov identifier: NCT03094546; [40]). Between January 2017 and April 2019, 100 healthy older adults with SCD were randomly assigned (1:1 ratio; blockwise randomization sequence) to receive either a spermidine-rich wheat germ extract (750mg per day [corresponding to approximately 0.9 mg spermidine, 0.5 mg spermine, 0.2 mg putrescine], equivalent to a 10% increased daily supply; target intervention) or placebo (750mg microcrystalline cellulose; control intervention) during an intervention period of 12 months (spermidine supplement was obtained from TLL The Longevity Labs). The study protocol [40] was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Charité Universitätsmedizin Berlin, Germany. All participants gave written informed consent before being included in the study. Older individuals with SCD, aged 60 to 90 years, were recruited through health care facilities and advertisements in the general population (eMethods 1 in Supplement 1). A telephone screening and an on-site screening were conducted before baseline assessment to ensure individual study eligibility. During on-site screening, the following measures were applied to determine the absence of objective cognitive impairment and current psychiatric disorders: Mini-Mental State Examination [41] score ≥ 26 , performance within -1.5 standard deviation (SD) of age-adjusted norms in appropriate neuropsychological tests, no deficits on selected items of the Instrumental Activities of Daily Living Scale [42], and 15-item Geriatric Depression Scale [43] score ≤ 10 (detailed information on screening procedure are provided in the trial protocol [40]). Inclusion criteria encompassed a diagnosis of SCD based on established guidelines [1], comprising I) expression of subjective cognitive complaints for at least six months, II) associated concerns (worries), III) affirmation to consult and/or previous consultation of a doctor due to these symptoms, IV) normal cognitive performance, and V) no restrictions on activities of daily living. Potential study participants were excluded if screening revealed a diagnosis of mild cognitive impairment or dementia. Further exclusion criteria comprised severe or untreated medical (e.g., malignant hypertension, untreated diabetes mellitus), neurological or psychiatric disorders,

malignancies (current or on medical history), known allergies to wheat germs, gluten or histamine intolerance, pretrial polyamine intake, and alcohol dependency as well as drug abuse. During baseline assessment, participants underwent neuropsychological testing including questionnaires, a standardized medical examination, and MRI scanning. These assessments were repeated after 12 months of intervention (post-intervention visit). In total, 89 participants successfully completed the 12-month study intervention. The primary outcome (memory performance) and various secondary outcomes have been published previously [36].

Brain imaging

MRI acquisition was performed at the Berlin Center for Advanced Neuroimaging (BCAN, Charité - Universitätsmedizin Berlin) on a 3T MRI scanner (Siemens Magnetom Trio, Erlangen, Germany). MRI data for baseline and 12-month post-intervention visits were available for 75 participants. Global and regional regions of interest (ROIs) for each MRI modality were selected *a priori* and blinded to treatment outcomes (eMethods 2 in Supplement 1).

Arterial Spin Labeling (ASL). Perfusion maps, representing cerebral blood flow (CBF) values, were quantified from the control-label ASL image pairs using ASLtbx [44] – a toolbox based on image processing routines of the Statistical Parametric Mapping (SPM) package (Wellcome Trust Centre for Neuroimaging, London, UK, version SPM12, www.fil.ion.ucl.ac.uk/spm) using MATLAB (The MathWorks, Natick, MA, USA, version R2013b). We focused on whole brain GM CBF and regional CBF values, comprising selected anatomical ROIs that previously showed cerebral perfusion alterations in predementia stages, i.e., inferior temporal gyrus, precuneus, posterior cingulate gyrus, supramarginal gyrus, angular gyrus, and hippocampus [45-47]. Furthermore, ROIs of both hemispheres were combined into a binary mask of AD-vulnerable regions (in the following referred to as AD signature), to extract regional CBF values. Normalized CBF maps underwent quality control by combining standardized visual inspection as well as automated outlier detection (eMethods 3 in Supplement 1) [48].

Diffusion Tensor Imaging (DTI). To assess WM microstructure, DTI was conducted to obtain fractional anisotropy (FA) and mean diffusivity (MD) measures. Generally, higher FA and lower MD values are considered markers for a higher degree of spatial organization and coherence in WM tracts. However, it is important to consider that DTI parameters – especially FA – are susceptible to complex fiber architecture (e.g., branching or merging fibers), warranting caution in the

interpretation of DTI results [49]. After visual inspection of the raw data, four participants were excluded from the analysis due to poor data quality. A diffusion tensor model was fitted using FSL's dtifit [50, 51]. Voxelwise statistical analysis of the DTI data was conducted with FSL's Tract-Based Spatial Statistics (TBSS) [52] and the longitudinal FreeSurfer streamline TRActs Constrained by UnderLying Anatomy (TRACULA) [53] was applied for automatic reconstruction of four major WM tracts belonging to the limbic system, which is vulnerable to AD pathology [6, 8, 9, 54]: anterior thalamic radiation, angular bundle of the cingulum, cingulate gyrus WM bundle, and uncinate fasciculus. To increase robustness of the diffusion metrics, the bihemispheric mean value was calculated for each of the tracts. If a given tract was successfully reconstructed in only one of the two hemispheres, the respective subject was not included in further analyses (eMethods 4 in Supplement 1).

White Matter Hyperintensities (WMH). Segmentation of WMH was performed using WMHPypes [55, 56] on the Fluid-Attenuated Inversion Recovery (FLAIR) images. For each participant, WMH volume and WMH number were calculated on both a global and regional level, yielding periventricular WMH and deep WMH. Note that higher WMH values indicate worse vascular health (eMethods 5 in Supplement 1).

Volumetry and cortical thickness. For reliable global and regional volumetric as well as cortical thickness estimates, T1-weighted images were automatically processed using the longitudinal stream [57] in FreeSurfer v6.0 (FMRIB Software Library, Oxford, UK; <http://surfer.nmr.mgh.harvard.edu/>) (eMethods 6 in Supplement 1).

Table 1. Participants' characteristics.

	Spermidine group (N=36)	Placebo group (N=39)
Women, N (%)	18 (50)	21 (54)
Age (years)	68 (5), 60-79	69 (5), 60-78
Education (years)	17 (3), 11-24	16 (3), 12-23
CVD risk (%)	17 (12), 2-42	17(12), 3-49
Family history of		
Dementia, N (%)	9 (25.0)	11 (28.2)
Alzheimer's Disease, N (%)	6 (16.7)	9 (23.1)
MMSE (score)	29.2 (.9), 26-30	29.1 (.9), 26-30
APOE ε4 carrier, N (%)	5 (14)	12 (31)
GDS (score)	1.8 (1.3), 0-4	1.9 (1.6), 0-6
FFQ, Spermidine	1.7 (.2), 1.2-2.2	1.7 (.2), 1.4-2.1

Data are given as mean, standard deviation (SD), and range for the spermidine and placebo group, respectively. APOE: apolipoprotein E, CVD: cardiovascular disease, FFQ: Food Frequency Questionnaire (see eMethods 1 in Supplement 1 for details on calculation). GDS: Geriatric Depression Scale, MMSE: Mini Mental State Examination.

Statistical analysis

Statistical analyses were conducted using R v4.4.1 [58]. Characteristics of study participants were reported descriptively using mean, standard deviation (SD), and range for each intervention group. Linear regression models (Analysis of Covariance, ANCOVA) with change in the outcome MRI measure (CBF, FA/MD from tractography, WMH number/volume, and structural measures) from baseline to 12-month post-intervention visit as dependent variable, intervention group as factor, and the respective baseline measure as well as age and sex as covariates were conducted. We performed one model per ROI or tract (N = 38) to analyze intervention effects on global and regional CBF (n = 7), WM microstructure

in pre-selected tracts (n = 8), WMH measures (n = 6), and volumetry and cortical thickness (n = 17). For statistical analysis of the DTI data processed with TBSS, differential FA and MD maps (i.e., difference between baseline and post-intervention values) were investigated for between-group differences, corrected for age and sex [59-61]. Additional inclusion of CVD risk (calculated with the Framingham risk score for CVD [62]) as a covariate was performed as sensitivity analysis and did not lead to relevant changes in results (eMethods 7, eResults 1, and eTables 6-9 in Supplement 1). In addition, effects of the intervention on different MRI parameters were correlated with each other using Pearson correlation of the respective differences between baseline and post-intervention values. Lastly, linear regression models were performed

to investigate possible associations between intervention-related changes in MRI measures and changes in memory performance (operationalized using the mnemonic discrimination index of the Mnemonic Similarity Task [35, 63, 64]), subjective cognitive complaints (operationalized using the 39-item Everyday Cognition Scales, ECog-39 [65]), and plasma sICAM-1 levels, adjusted for age, sex, and the respective baseline measure. A 2-sided significance level of $\alpha = .05$ was used for all analyses. Given the exploratory nature of these secondary

analyses, no correction of multiple testing was applied. Rather, we focused on effect sizes, particularly partial eta squared (η^2_p indicating small $\geq .01$, medium $\geq .06$ or large $\geq .14$ effects), than on p-values when interpreting our analyses. We calculated 90% confidence intervals for η^2_p , thus matching the two-sided significance level of $\alpha = .05$. An independent statistician oversaw the analyses and was not involved in the study (eMethods 7 in Supplementary 1).

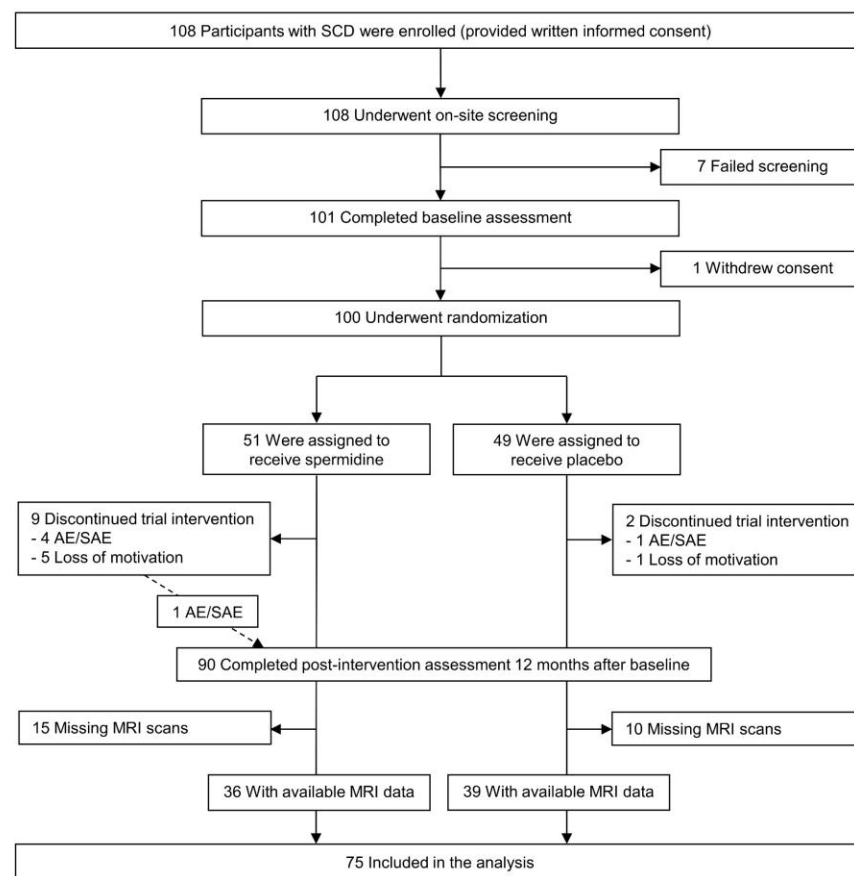


Figure 1. Study flowchart. This figure is adapted and modified from Schwarz and colleagues [36]. AE: adverse event, MRI: magnetic resonance imaging, SAE: serious adverse event, SCD: subjective cognitive decline.

RESULTS

Characteristics of study participants

In total, 89 participants completed the 12-month study intervention, and 75 participants had MRI data for both time points available (Fig. 1).

Participants in both groups showed comparable characteristics in terms of sex distribution, age, CVD risk, global cognitive performance, apolipoprotein $\epsilon 4$ carrier status, family history of dementia/AD, and depressive

symptoms (Table 1). In addition, high compliance rates, as measured by the number of remaining capsules at post-intervention visit, were observed in both intervention groups (spermidine group, mean [SD]: 96.1% [5.3]; placebo group, mean [SD]: 96.9% [4.3]).

Moreover, we observed similar baseline characteristics between participants with successful preprocessing and quality control of DTI, WMH, and volumetric data and those who were excluded (eTable 1 in Supplement 1). However, for ASL, participants with

excluded datasets were older and had higher CVD risk scores compared to those with analyzable CBF data.

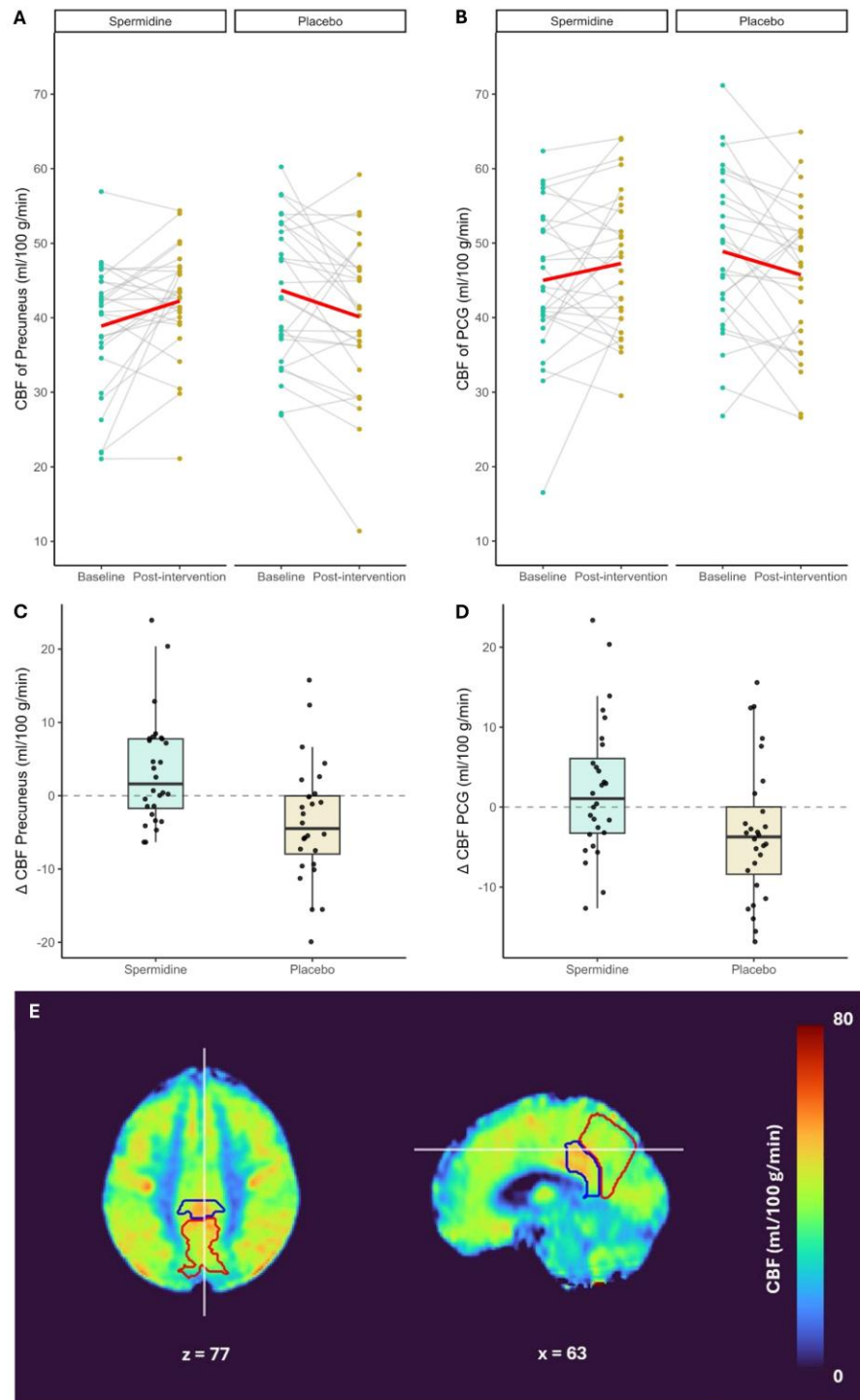


Figure 2. Effect of spermidine supplementation on regional cerebral blood flow. Spaghetti plots depict individual longitudinal changes in cerebral blood flow (CBF) in A) the precuneus and B) the posterior cingulate gyrus from baseline to post-intervention (total N=56; spermidine group: n=28, placebo group: n=28). The group mean is indicated by the red line. Individual change scores (Δ CBF = post-intervention – baseline) are presented for C) the precuneus and D) the posterior cingulate gyrus. Each dot represents one participant; boxes indicate the median and interquartile range. E) Color-coded mean CBF maps illustrate the regional effects of the intervention. Regions of interest are outlines in red (precuneus) and blue (posterior cingulate gyrus).

Table 2. Effect of spermidine supplementation on cerebral perfusion.

	B (ml/100 g/min)	95% CI	η^2_p	90% CI	<i>P</i> value
Global CBF					
Whole brain GM	.8	-2.3 to 4.0	<.01	0 to .079	.60
Regional CBF					
AD signature	1.2	-2.7 to 5.0	<.01	0 to .086	.55
Precuneus	4.9	1.0 to 8.9	.11	.012 to .250	.015
Posterior cingulate	3.7	-.5 to 7.8	.06	0 to .182	.08
Inferior temporal	-2.6	-7.6 to 2.4	.02	0 to .120	.31
Inferior parietal	1.2	-4.2 to 6.6	<.01	0 to .071	.67
Hippocampus	1.4	-2.7 to 5.6	<.01	0 to .093	.49

Linear regression models were adjusted for the corresponding baseline measure, age, and sex. In total, 56 participants (spermidine group: N=28; placebo group: N=28) were included in the analysis. The unstandardized regression coefficient (B) indicates the between-group differences in the respective CBF measures. AD: Alzheimer's disease, CBF: cerebral blood flow, CI: confidence interval, GM: gray matter, T: *t*-value, η^2_p : partial eta squared.

Cerebral blood flow

ASL data of sufficient quality was available for 56 participants (spermidine group: N=28; placebo group: N=28). We did not observe substantial between-group differences in global GM CBF or CBF in the AD signature (Table 2). We observed a medium effect on regional CBF in the precuneus (between-group difference, 4.9 ml/100 g/min; 95% CI, 1.0 to 8.9; η^2_p =.11 [90% CI, .012 to .250]; P =.015; Table 2 and Figure 2), with an adjusted CBF increase of 2.3 ml/100 g/min in the spermidine group and an adjusted CBF decrease of -2.6 ml/100 g/min in the placebo group. In addition, we found a medium effect on CBF in the posterior cingulate gyrus (between-group difference, 3.7 ml/100 g/min; 95% CI, -.5 to 7.8; η^2_p =.06 [90% CI, 0 to .182]; P =.08), with an adjusted CBF increase of 1.3 ml/100 g/min in the spermidine group and an adjusted CBF decrease of -2.4 ml/100 g/min in the placebo group.

White matter microstructure

Completion of the TBSS pipeline was successful for 71 participants (spermidine group: N=33; placebo group: N=38). TBSS-based analyses did not identify areas where changes in central WM FA or MD between baseline and follow-up were different between the placebo and verum group. Tractography analyses demonstrated a medium effect on FA of the WM bundle underlying the cingulate gyrus (between-group difference, .016; 95% CI, .002 to .031; η^2_p =.06 [90% CI, .004 to .208]; P =.029; Figure 3). Participants who received spermidine supplementation showed a slight increase in FA over the intervention period, while we observed a decrease in FA in the placebo group. For all other tracts, there were no substantial group-differences regarding FA or MD values (eTable 2 in Supplement 1).

No correlations were found between FA changes of the cingulate gyrus WM bundle and CBF changes in the precuneus ($r(44)$ =.004; P =.98) or in the posterior cingulate gyrus ($r(44)$ =-.042; P =.78) after the intervention period. In addition, no significant associations were observed between intervention-related changes in regional MRI measures and changes in memory performance, subjective cognitive complaints, or plasma sICAM-1 levels (all η^2_p ≤.06; eTable 10 in Supplement 1).

White matter hyperintensities

WMH analyses were conducted on 63 participants (spermidine group: N=28; placebo group: N=35), revealing no substantial differences between intervention groups, neither for global as well as regional WMH number and WMH volume (all η^2_p <.01; eTable 3 in Supplement 1).

Volumetry and cortical thickness

Completion of the FreeSurfer pipeline was successful for 71 participants (spermidine group: N=33; placebo group: N=38). No substantial differences between intervention groups were observed for global as well as regional volume (all η^2_p ≤.05; eTable 4 in Supplement 1) and cortical thickness (all η^2_p ≤.03; eTable 5 in Supplement 1).

DISCUSSION

This secondary neuroimaging analysis investigated the effect of 12-month dietary spermidine supplementation on vascular brain health (operationalized through CBF, WM microstructure, and WMH) as well as on volume and cortical thickness in older individuals with SCD. Between-group differences were observed in regional cerebral perfusion and regional WM microstructure, whereas no substantial effects on WMH, volume, or

cortical thickness were identified. Given the exploratory nature of this secondary analysis and the absence of correction for multiple comparisons, all regional MRI

findings should be interpreted as preliminary and require independent confirmation.

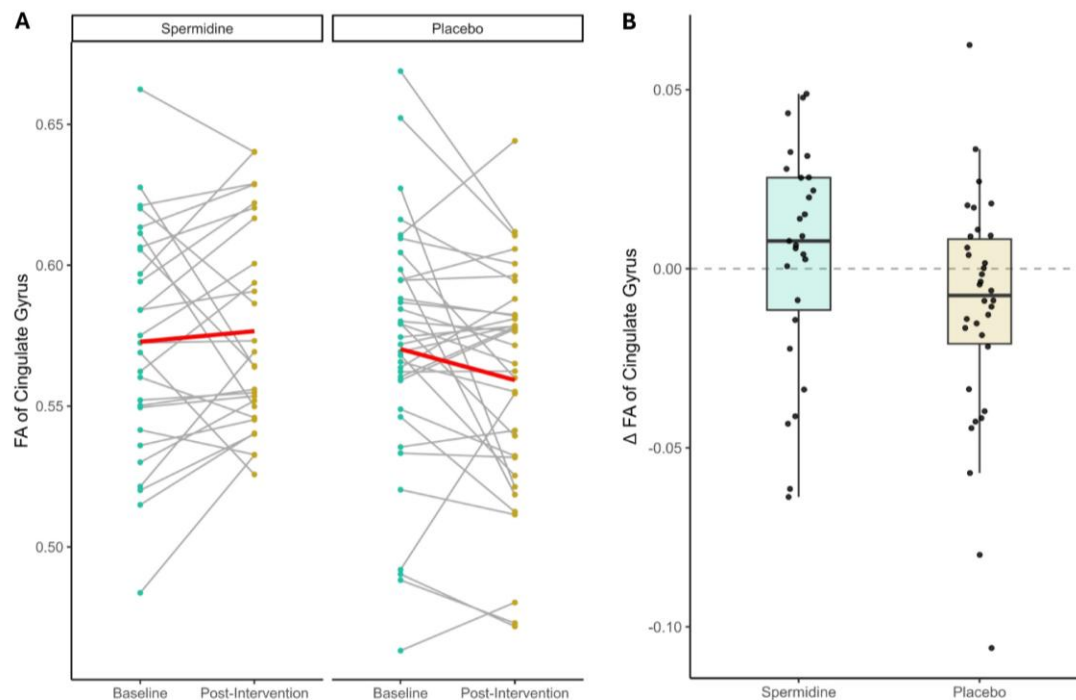


Figure 3. Effect of spermidine supplementation on white matter microstructure. A) Spaghetti plots depict individual longitudinal changes in fractional anisotropy (FA) of the cingulate gyrus bundle from baseline to post-intervention (total N=61; spermidine group: n=27, placebo group: n=34). The group mean is indicated by the red line. B) Individual change scores (Δ FA = post-intervention – baseline) are presented for the cingulate gyrus. Each dot represents one participant; boxes indicate the median and interquartile range.

Maintaining CBF is essential for proper neuronal functioning, ensuring a constant supply of blood flow to the brain [66]. CBF decreases with age and even more so in individuals with AD, where it is considered one of the earliest preclinical pathological events [67, 68]. Decreased CBF can, in turn, lead to impaired vascular clearance, resulting in the accumulation of amyloid beta proteins [69]. Our analyses identified between-group differences of medium effect size in the precuneus and posterior cingulate gyrus, with CBF increasing over time in the spermidine group and decreasing in the placebo group. These regions are known to play a pivotal role as central hubs of the default mode network and have been linked to various higher cognitive processes, supporting their relevance in SCD [69, 70]. The substantial decline in regional CBF in the precuneus and posterior cingulate gyrus in the placebo group is in line with previous studies showing CBF reductions in those regions in early stages of the AD continuum [69, 71]. This reduction could indicate pre-existing hypoperfusion in the precuneus and posterior cingulate gyrus in individuals with SCD, which

may have been restored by spermidine supplementation. Interestingly, no between-group difference was observed in global GM CBF, suggesting that any potential associations with spermidine supplementation may be region-specific and most apparent in AD-vulnerable brain regions. We further observed a medium effect on higher FA of the WM bundle underlying the cingulate gyrus in the spermidine group. Simplified, FA indicates the degree to which free water diffusion is directionally restricted through surrounding cellular structures. In highly organized WM bundles, higher FA is considered to reflect higher fiber density and uniform spatial organization, though interpretation of FA values in areas of branching or crossing fibers requires caution [51, 72]. Previous studies have shown altered microstructure in the cingulate gyrus in SCD and mild cognitive impairment and described associations between reduced cingulate WM bundle FA and worse cognitive function in the early AD continuum [73, 74]. These findings suggest that lower cingulate WM FA may reflect incipient neurodegeneration in the context of SCD and mild

cognitive impairment. Thus, our study might indicate a modulation of cingulate WM microstructure through spermidine supplementation, possibly mitigating the negative effects of AD pathology on WM bundles maintaining cognitive function. Similar to CBF, we did not find between-group differences in global central WM microstructure. It is important to note that TBSS does not represent true tractography and is subject to statistical corrections for multiple testing that may limit the detection of subtle, tract-specific differences [75]. The discrepancy between TBSS and tractography results is thus most likely explained by methodological differences and the restriction of microstructural changes to the cingulate bundle in this study sample. The observed effects on regional CBF and WM microstructure have to be interpreted cautiously in this exploratory study and should be confirmed in larger studies. Future longer-lasting intervention studies should also evaluate whether spermidine supplementation can beneficially influence global CBF and WM microstructure beyond the cingulate gyrus and precuneus.

Taken together, spermidine supplementation was associated with regional differences in CBF and WM microstructure in AD-vulnerable brain regions. Whether these findings are relevant for cognitive function or AD-related disease progression remains to be determined. Notably, we observed parallel effects on CBF in the precuneus as well as posterior cingulate gyrus and FA of the cingulate gyrus WM bundle. No correlation was detected between changes in regional CBF and FA, likely because the cingulate gyrus WM bundle is anatomically distinct from the precuneus, and because CBF changes only occurred in the posterior cingulate gyrus. Previous studies have demonstrated regionally specific associations of CBF and microstructure [76, 77], indicating a physiological link between cortical CBF and subcortical WM microstructure. On this basis, we suggest that improved vascular brain health may account for the observed effect as a common underlying mechanism [76, 77]. In sum, our findings provide convergent evidence that 12-month spermidine supplementation was associated with early vascular brain alterations on a microstructural and perfusion level in individuals with SCD.

These findings are in line with various spermidine supplementation studies in animal models that demonstrate delayed arterial aging and improved proangiogenic as well as endothelial function [24-27]. Further, human studies have linked increased spermidine levels with favorable effects on endothelial function, blood pressure, and lower mortality due to improved cardiovascular health [28, 30-32]. Our study results also align with earlier exploratory subgroup findings from the SmartAge trial [36], where we observed a possible

positive intervention effect on sICAM-1, a suggested marker of vascular pathology that is elevated in aging and dementia, in participants who had successfully completed the 12-month intervention period with compliance rates above 90% [37-39, 78]. However, changes in regional MRI measures observed between groups were not associated with concurrent changes in memory performance, subjective cognitive complaints, or plasma sICAM-1 levels. Therefore, the clinical relevance of these findings remains uncertain and requires further investigation in future intervention studies.

We did not observe between-group differences in WMH volume or number, nor on GM volume or CT. WMH arise from both cerebral small vessel disease and amyloid-related pathology [79] and are already present in SCD, reflecting WM macrostructural alterations in the early AD continuum [13]. However, there is accumulating evidence that alterations in WM microstructure [79, 80] and CBF [81] precede WMH manifestation. Similarly, reductions in GM volume and cortical thickness occur secondary to CBF changes [82] and WM microstructural alterations [8, 83] in the course of AD. The macrostructural manifestations of AD pathology are likely secondary to a continuous process of microstructural degeneration and cerebral hypoperfusion over time. Thus, we hypothesize that the intervention period in this study may have been too short to show significant changes in WMH and GM volume as well as cortical thickness. Future trials should thus include longer intervention periods and higher spermidine concentrations to improve our understanding of the potential influence of spermidine on brain health.

Limitations

The interpretation of our results is also subject to some limitations. First, quantification of CBF in ROIs close to large blood vessels was not possible due to problems during post-processing of the images. However, these are common issues with ASL imaging as this subtractive MRI technique is very sensitive to motion, which does not diminish the great advantages of ASL over other perfusion imaging methods [84]. In addition, a relatively high proportion of ASL datasets had to be excluded during preprocessing and quality control. Participants excluded from the ASL analyses were older and had higher CVD risk scores than those with analyzable ASL data. This finding is in line with previous literature showing that increased motion artifacts and more pronounced vascular pathology (e.g., cerebral small vessel disease) in older individuals may limit the feasibility and quality of ASL-based CBF quantification [84]. Although exclusions were based on predefined data quality criteria, this selection may have resulted in underrepresentation of individuals

with higher vascular risk profiles. Consequently, the observed regional CBF findings may not be fully generalizable to older individuals with greater vascular risk, and the direction and magnitude of any potential bias cannot be determined. Nevertheless, the final sample size was comparable to that of previous ASL studies in older adults [69, 85]. Furthermore, the use of predefined ROIs with relatively large volumes and high baseline perfusion, together with a detailed semi-automated quality control including expert review, likely improved the signal-to-noise ratio and supported the robustness of the reported findings. In line, previous studies have demonstrated excellent reproducibility of pseudo-continuous ASL-derived CBF measurements in the precuneus and posterior cingulate gyrus, with test-retest variability typically below 10% [86, 87]. Second, FA and MD are indirect measures of microstructural integrity and thus should be interpreted with caution, especially in regions with many branching or crossing fibers [53, 56, 88]. Third, we did not collect AD blood biomarkers from study participants, precluding identification of AD-related pathology. However, inclusion criteria comprised currently accepted guidelines for SCD [1] and our intervention did not specifically target amyloid beta or tau. Fourth, although adherence to the intervention was high, circulating spermidine concentrations did not differ between groups [36], and no tissue-level or downstream biomarkers of spermidine activity were available. Therefore, the extent of biological exposure and target engagement following supplementation could not be determined, which represents an important limitation when interpreting the observed MRI findings.

Conclusion

One year of spermidine supplementation, corresponding to a 10% increase in daily spermidine intake, was associated with regional changes in MRI measures related to cerebral perfusion and white matter microstructure in brain regions vulnerable to early AD pathology. These exploratory regional MRI findings were not accompanied by global changes in brain imaging measures or associated with changes in memory performance, subjective cognitive complaints, or plasma sICAM-1 levels. As these findings are hypothesis-generating, further intervention studies with longer follow-up periods and higher spermidine supplementation doses in at-risk populations for AD are warranted to confirm their reproducibility and clarify their clinical relevance.

Acknowledgments

We offer our sincerest thanks to our volunteers for participating in the study and all members of the

SmartAge Study Team for help and advice in planning, organization, and/or conduction of the trial.

Conflict of Interest Disclosure

The Longevity Labs, TLL, provided the study supplement free of charge but had no role in study design, data collection, data analysis, data interpretation, manuscript preparation, or the decision to publish the results. FM and TE served as advisors for TLL and held equity interests in the company during the conduct of the study. FM and TE were not involved in data collection, statistical analyses, or outcome assessment. FM has equity interest in Samsara Therapeutics. TE reports personal fees from Infectopharm (Germany) and work-related lectures. AF reports personal fees from Lilly, Roche, Biogen, and Eisai, outside the submitted work. No other conflicts were reported.

Authors' contributions

CS, KW, and AF had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Concept and design:* CS, KW, SJS, MW, FM, AF. *Acquisition, analysis, or interpretation of data:* CS, KW, SH, AD, SMW, DA, GSB, NH, UG, SJH, TE, MW, FM, AF. *Drafting of the manuscript:* CS, KW, SH, AD, SJH, TE, FM, AF. *Critical revision of the manuscript for important intellectual content:* CS, KW, SH, AD, SMW, DA, GSB, NH, UG, SJH, TE, SJS, DS, MW, FM, AF. *Statistical analysis:* CS, KW, UG. *Obtained funding:* SJS, DS, MW, FM, AF. *Supervision:* CS, KW, AF.

Funding

Funding for this trial was provided by the Bundesministerium für Bildung und Forschung (SMARTAGE, grant No. FKZ 01GQ1420B) and the Hans Gerhard Creutzfeldt scholarship (Nos. FKZ CSB II, 01EO1301 TP T2). The NeuroCure Clinical Research Center was supported by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy (Nos. EXC-2049-390688087 and EXC-257 NeuroCure) as well as by the Berlin Institute of Health at Charité (BIH). The Longevity Labs GmbH provided funds for supporting the development of spermidine-rich wheat germ extract during the conduct of the study. This research was also funded by the German Research Foundation (project grants to AF: FL 379/26-1; CRC INST 276/741-2 and 292/155-1; Research Unit 5429/1 (467143400), FL 379/34-1, FL 379/35-1, and project grant 539593253, AN 1103/6-1 to DA). SMW was supported by UKRI Economic and Social Research Council (grant number

ES/W002914/1). FM and TE are grateful to the Austrian Science Fund (FWF) for excellence cluster 10.55776/COE14. For open access purposes, the author has applied for a CC BY public copyright license to any author-accepted manuscript version arising from this submission. We thank the University of Graz, the City of Graz and the Provincial Government of Styria for financial support to excellence cluster 10.55776/COE14. FM is grateful to FWF for DOC-50, F3012, W1226, P29203, P29262, P27893 and P31727 and the Austrian Federal Ministry of Education, Science and Research, as well as from the University of Graz for grants 'Unkonventionelle Forschung-InterFast' and 'Fast4Health' as well as 'flysleap' (BMFWF-80.109/0001-WF/V/3b/2015). The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2026.0559.

References

- [1] Jessen F, Amariglio RE, van Boxtel M, Breteler M, Ceccaldi M, Ch  telat G, et al. (2014). A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimers Dement*, 10:844-852.
- [2] Molinuevo JL, Rabin LA, Amariglio R, Buckley R, Dubois B, Ellis KA, et al. (2017). Implementation of subjective cognitive decline criteria in research studies. *Alzheimers Dement*, 13:296-311.
- [3] Jessen F, Amariglio RE, Buckley RF, van der Flier WM, Han Y, Molinuevo JL, et al. (2020). The characterisation of subjective cognitive decline. *Lancet Neurol*, 19:271-278.
- [4] Jessen F, Wolfgruber S, Wiese B, Bickel H, M  sch E, Kaduszkiewicz H, et al. (2014). AD dementia risk in late MCI, in early MCI, and in subjective memory impairment. *Alzheimers Dement*, 10:76-83.
- [5] van Harten AC, Mielke MM, Swenson-Dravis DM, Hagen CE, Edwards KK, Roberts RO, et al. (2018). Subjective cognitive decline and risk of MCI: The Mayo Clinic Study of Aging. *Neurology*, 91:e300-e312.
- [6] Meiberth D, Scheef L, Wolfgruber S, Boecker H, Block W, Tr  ber F, et al. (2015). Cortical thinning in individuals with subjective memory impairment. *J Alzheimers Dis*, 45:139-146.
- [7] Luo C, Li M, Qin R, Chen H, Yang D, Huang L, et al. (2019). White Matter Microstructural Damage as an Early Sign of Subjective Cognitive Decline. *Front Aging Neurosci*, 11:378.
- [8] Ryu SY, Lim EY, Na S, Shim YS, Cho JH, Yoon B, et al. (2017). Hippocampal and entorhinal structures in subjective memory impairment: a combined MRI volumetric and DTI study. *Int Psychogeriatr*, 29:785-792.
- [9] Zhao W, Luo Y, Zhao L, Mok V, Su L, Yin C, et al. (2019). Automated Brain MRI Volumetry Differentiates Early Stages of Alzheimer's Disease From Normal Aging. *J Geriatr Psychiatry Neurol*, 32:354-364.
- [10] Hays CC, Zlatar ZZ, Campbell L, Meloy MJ, Wierenga CE (2018). Subjective Cognitive Decline Modifies the Relationship Between Cerebral Blood Flow and Memory Function in Cognitively Normal Older Adults. *J Int Neuropsychol Soc*, 24:213-223.
- [11] Yang Z, Rong Y, Cao Z, Wu Y, Zhao X, Xie Q, et al. (2021). Microstructural and Cerebral Blood Flow Abnormalities in Subjective Cognitive Decline Plus: Diffusional Kurtosis Imaging and Three-Dimensional Arterial Spin Labeling Study. *Front Aging Neurosci*, 13:625843.
- [12] Zhang Y, Du W, Yin Y, Li H, Liu Z, Yang Y, et al. (2021). Impaired cerebral vascular and metabolic responses to parametric N-back tasks in subjective cognitive decline. *J Cereb Blood Flow Metab*, 41:2743-2755.
- [13] Helda P, Patricia D-G, Jos   B, Atef B, Jonas KO, Eric W, et al. (2022). Cerebrovascular damage in subjective cognitive decline: A systematic review and meta-analysis. *Ageing Research Reviews*, 82:101757.
- [14] Hays CC, Zlatar ZZ, Wierenga CE (2016). The Utility of Cerebral Blood Flow as a Biomarker of Preclinical Alzheimer's Disease. *Cell Mol Neurobiol*, 36:167-179.
- [15] Eisenberg T, Knauer H, Schauer A, B  ttner S, Ruckenstein C, Carmona-Gutierrez D, et al. (2009). Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol*, 11:1305-1314.
- [16] Madeo F, Eisenberg T, Pietropaolo F, Kroemer G (2018). Spermidine in health and disease. *Science*, 359.
- [17] Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med*, 22:1428-1438.
- [18] Schroeder S, Hofer SJ, Zimmermann A, Pechlaner R, Dammbrueck C, Pendl T, et al. (2021). Dietary spermidine improves cognitive function. *Cell Rep*, 35:108985.
- [19] Shin WW, Fong WF, Pang SF, Wong PC (1985). Limited blood-brain barrier transport of polyamines. *J Neurochem*, 44:1056-1059.
- [20] Gupta VK, Scheunemann L, Eisenberg T, Mertel S, Bhukel A, Koemans TS, et al. (2013). Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner. *Nat Neurosci*, 16:1453-1460.
- [21] Hofer SJ, Liang Y, Zimmermann A, Schroeder S, Dengjel J, Kroemer G, et al. (2021). Spermidine-induced hypusination preserves mitochondrial and cognitive function during aging. *Autophagy*, 17:2037-2039.
- [22] Maglione M, Kochlamazashvili G, Eisenberg T, R  cz B, Michael E, Toppe D, et al. (2019). Spermidine protects

- from age-related synaptic alterations at hippocampal mossy fiber-CA3 synapses. *Sci Rep*, 9:19616.
- [23] Liang Y, Piao C, Beuschel CB, Toppe D, Kollipara L, Bogdanow B, et al. (2021). eIF5A hypusination, boosted by dietary spermidine, protects from premature brain aging and mitochondrial dysfunction. *Cell Rep*, 35:108941.
- [24] Ueno D, Ikeda K, Yamazaki E, Katayama A, Urata R, Matoba S (2023). Spermidine improves angiogenic capacity of senescent endothelial cells, and enhances ischemia-induced neovascularization in aged mice. *Scientific Reports*, 13:8338.
- [25] Liu X, Chen A, Liang Q, Yang X, Dong Q, Fu M, et al. (2021). Spermidine inhibits vascular calcification in chronic kidney disease through modulation of SIRT1 signaling pathway. *Aging Cell*, 20:e13377.
- [26] Kim H, Massett MP (2024). Effect of Spermidine on Endothelial Function in Systemic Lupus Erythematosus Mice. *Int J Mol Sci*, 25.
- [27] LaRocca TJ, Gioscia-Ryan RA, Heaton CM, Jr., Seals DR (2013). The autophagy enhancer spermidine reverses arterial aging. *Mech Ageing Dev*, 134:314-320.
- [28] Matsumoto M, Kitada Y, Naito Y (2019). Endothelial Function is improved by Inducing Microbial Polyamine Production in the Gut: A Randomized Placebo-Controlled Trial. *Nutrients*, 11:1188.
- [29] Muñoz-Esparza NC, Latorre-Moratalla ML, Comas-Basté O, Toro-Funes N, Veciana-Nogués MT, Vidal-Carou MC (2019). Polyamines in Food. *Front Nutr*, 6:108.
- [30] Kiechl S, Pechlaner R, Willeit P, Notdurfter M, Paulweber B, Willeit K, et al. (2018). Higher spermidine intake is linked to lower mortality: a prospective population-based study. *Am J Clin Nutr*, 108:371-380.
- [31] Han S, Qian M, Zhang N, Zhang R, Liu M, Wang J, et al. (2024). The Association of Dietary Polyamines with Mortality and the Risk of Cardiovascular Disease: A Prospective Study in UK Biobank. *Nutrients*, 16.
- [32] Wu H, Wang J, Jiang H, Liu X, Sun X, Chen Y, et al. (2022). The association of dietary spermidine with all-cause mortality and CVD mortality: The U.S. National Health and Nutrition Examination Survey, 2003 to 2014. *Front Public Health*, 10:949170.
- [33] Ma Y, Qi L, Zhu F, Xiao M, Li M, Zhang X, et al. (2025). Association between dietary spermidine intake and cognitive performance in older adults: The U.S. National Health and Nutrition Examination Survey, 2011-2014. *J Affect Disord*, 381:174-182.
- [34] Schwarz C, Horn N, Benson G, Wrachtrup Calzado I, Wurdack K, Pechlaner R, et al. (2020). Spermidine intake is associated with cortical thickness and hippocampal volume in older adults. *Neuroimage*, 221:117132.
- [35] Wirth M, Benson G, Schwarz C, Köbe T, Grittner U, Schmitz D, et al. (2018). The effect of spermidine on memory performance in older adults at risk for dementia: A randomized controlled trial. *Cortex*, 109:181-188.
- [36] Schwarz C, Benson GS, Horn N, Wurdack K, Grittner U, Schilling R, et al. (2022). Effects of Spermidine Supplementation on Cognition and Biomarkers in Older Adults With Subjective Cognitive Decline: A Randomized Clinical Trial. *JAMA Netw Open*, 5:e2213875.
- [37] Jenny NS, Arnold AM, Kuller LH, Sharrett AR, Fried LP, Psaty BM, et al. (2006). Soluble intracellular adhesion molecule-1 is associated with cardiovascular disease risk and mortality in older adults. *J Thromb Haemost*, 4:107-113.
- [38] Haydinger CD, Ashander LM, Tan ACR, Smith JR (2023). Intercellular Adhesion Molecule 1: More than a Leukocyte Adhesion Molecule. *Biology*, 12:743.
- [39] Witkowska AM (2005). Soluble ICAM-1: a marker of vascular inflammation and lifestyle. *Cytokine*, 31:127-134.
- [40] Wirth M, Schwarz C, Benson G, Horn N, Buchert R, Lange C, et al. (2019). Effects of spermidine supplementation on cognition and biomarkers in older adults with subjective cognitive decline (SmartAge)-study protocol for a randomized controlled trial. *Alzheimers Res Ther*, 11:36.
- [41] Folstein MF, Folstein SE, McHugh PR (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*, 12:189-198.
- [42] Lawton MP, Brody EM (1969). Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist*, 9:179-186.
- [43] Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. (1982). Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res*, 17:37-49.
- [44] Wang Z, Aguirre GK, Rao H, Wang J, Fernández-Seara MA, Childress AR, et al. (2008). Empirical optimization of ASL data analysis using an ASL data processing toolbox: ASLtbx. *Magn Reson Imaging*, 26:261-269.
- [45] Wirth M, Pichet Binette A, Brunecker P, Köbe T, Witte AV, Flöel A (2017). Divergent regional patterns of cerebral hypoperfusion and gray matter atrophy in mild cognitive impairment patients. *J Cereb Blood Flow Metab*, 37:814-824.
- [46] Li W, Jiang J, Zou X, Zhang Y, Sun M, Jia Z, et al. (2022). The characteristics of arterial spin labeling cerebral blood flow in patients with subjective cognitive decline: The Chinese imaging, biomarkers, and lifestyle study. *Frontiers in Neuroscience*, Volume 16 - 2022.
- [47] Lacalle-Aurioles M, Mateos-Pérez JM, Guzmán-De-Villoria JA, Olazarán J, Cruz-Orduña I, Alemán-Gómez Y, et al. (2014). Cerebral blood flow is an earlier indicator of perfusion abnormalities than cerebral blood volume in Alzheimer's disease. *J Cereb Blood Flow Metab*, 34:654-659.
- [48] Proisy M, Corouge I, Legouhy A, Nicolas A, Charon V, Mazille N, et al. (2019). Changes in brain perfusion in successive arterial spin labeling MRI scans in neonates with hypoxic-ischemic encephalopathy. *Neuroimage Clin*, 24:101939.
- [49] Jbabdi S, Johansen-Berg H (2011). Tractography: where do we go from here? *Brain Connect*, 1:169-183.
- [50] Behrens TE, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, et al. (2003).

- Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magn Reson Med*, 50:1077-1088.
- [51] Behrens TE, Berg HJ, Jbabdi S, Rushworth MF, Woolrich MW (2007). Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? *Neuroimage*, 34:144-155.
- [52] Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. (2006). Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage*, 31:1487-1505.
- [53] Yendiki A, Panneck P, Srinivasan P, Stevens A, Zöllei L, Augustinack J, et al. (2011). Automated probabilistic reconstruction of white-matter pathways in health and disease using an atlas of the underlying anatomy. *Front Neuroinform*, 5:23.
- [54] Selnes P, Fjell AM, Gjerstad L, Bjørnerud A, Wallin A, Due-Tønnessen P, et al. (2012). White matter imaging changes in subjective and mild cognitive impairment. *Alzheimer's & Dementia*, 8:S112-S121.
- [55] Gaubert M, Dell'Orco A, Lange C, Garnier-Crussard A, Zimmermann I, Dyrba M, et al. (2022). Performance evaluation of automated white matter hyperintensity segmentation algorithms in a multicenter cohort on cognitive impairment and dementia. *Front Psychiatry*, 13:1010273.
- [56] Li H, Jiang G, Zhang J, Wang R, Wang Z, Zheng WS, et al. (2018). Fully convolutional network ensembles for white matter hyperintensities segmentation in MR images. *Neuroimage*, 183:650-665.
- [57] Reuter M, Schmansky NJ, Rosas HD, Fischl B (2012). Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage*, 61:1402-1418.
- [58] Team RC (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- [59] Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE (2014). Permutation inference for the general linear model. *Neuroimage*, 92:381-397.
- [60] Nichols TE, Holmes AP (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp*, 15:1-25.
- [61] Smith SM, Nichols TE (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*, 44:83-98.
- [62] D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. (2008). General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*, 117:743-753.
- [63] Stark SM, Yassa MA, Lacy JW, Stark CE (2013). A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia*, 51:2442-2449.
- [64] Marks SM, Lockhart SN, Baker SL, Jagust WJ (2017). Tau and β -Amyloid Are Associated with Medial Temporal Lobe Structure, Function, and Memory Encoding in Normal Aging. *J Neurosci*, 37:3192-3201.
- [65] Farias ST, Mungas D, Reed BR, Cahn-Weiner D, Jagust W, Baynes K, et al. (2008). The measurement of everyday cognition (ECog): scale development and psychometric properties. *Neuropsychology*, 22:531-544.
- [66] Nagata K, Yamazaki T, Takano D, Maeda T, Fujimaki Y, Nakase T, et al. (2016). Cerebral circulation in aging. *Ageing Res Rev*, 30:49-60.
- [67] Swinford CG, Risacher SL, Wu YC, Apostolova LG, Gao S, Bice PJ, et al. (2023). Altered cerebral blood flow in older adults with Alzheimer's disease: a systematic review. *Brain Imaging Behav*, 17:223-256.
- [68] Mokhber N, Shariatzadeh A, Avan A, Saber H, Babaei GS, Chaimowitz G, et al. (2021). Cerebral blood flow changes during aging process and in cognitive disorders: A review. *The Neuroradiology Journal*, 34:300-307.
- [69] Ghaderi S, Mohammadi S, Fatehi F (2025). Cerebral blood flow alterations in the precuneus of Alzheimer's disease using arterial spin labeling: A systematic review and meta-analysis. *Brain Research Bulletin*, 229:111444.
- [70] Foster BL, Koslov SR (2025). Functions of the posterior cingulate cortex and default network. *Current Opinion in Behavioral Sciences*, 65:101560.
- [71] Graff BJ, Harrison SL, Payne SJ, El-Bouri WK (2023). Regional Cerebral Blood Flow Changes in Healthy Ageing and Alzheimer's Disease: A Narrative Review. *Cerebrovascular Diseases*, 52:11-20.
- [72] Concha L (2014). A macroscopic view of microstructure: using diffusion-weighted images to infer damage, repair, and plasticity of white matter. *Neuroscience*, 276:14-28.
- [73] Gozdas E, Fingerhut H, Chromik LC, O'Hara R, Reiss AL, Hosseini SMH (2020). Focal white matter disruptions along the cingulum tract explain cognitive decline in amnesic mild cognitive impairment (aMCI). *Scientific Reports*, 10:10213.
- [74] Brueggen K, Dyrba M, Cardenas-Blanco A, Schneider A, Fliessbach K, Buerger K, et al. (2019). Structural integrity in subjective cognitive decline, mild cognitive impairment and Alzheimer's disease based on multicenter diffusion tensor imaging. *Journal of Neurology*, 266:2465-2474.
- [75] Bach M, Laun FB, Leemans A, Tax CM, Biessels GJ, Stieltjes B, et al. (2014). Methodological considerations on tract-based spatial statistics (TBSS). *Neuroimage*, 100:358-369.
- [76] Bouhrara M, Triebswetter C, Kiely M, Bilgel M, Dolui S, Erus G, et al. (2022). Association of Cerebral Blood Flow With Longitudinal Changes in Cerebral Microstructural Integrity in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *JAMA Netw Open*, 5:e2231189.
- [77] Chen JJ, Rosas HD, Salat DH (2013). The relationship between cortical blood flow and sub-cortical white-matter health across the adult age span. *PLoS One*, 8:e56733.
- [78] Rentzos M, Michalopoulou M, Nikolaou C, Cambouri C, Rombos A, Dimitrakopoulos A, et al. (2004). Serum Levels of Soluble Intercellular Adhesion Molecule-1 and Soluble Endothelial Leukocyte Adhesion Molecule-1 in

- Alzheimer's Disease. *Journal of Geriatric Psychiatry and Neurology*, 17:225-231.
- [79] Prins ND, Scheltens P (2015). White matter hyperintensities, cognitive impairment and dementia: an update. *Nat Rev Neurol*, 11:157-165.
- [80] Sun P, He Z, Chu E, Fan X, Cai Y, Lan G, et al. (2025). White matter fractional anisotropy decreases precede hyperintensities in Alzheimer's disease. *Cell Reports Medicine*, 6:102138.
- [81] Bernbaum M, Menon BK, Fick G, Smith EE, Goyal M, Frayne R, et al. (2015). Reduced blood flow in normal white matter predicts development of leukoaraiosis. *J Cereb Blood Flow Metab*, 35:1610-1615.
- [82] Zhang N, Gordon ML, Goldberg TE (2017). Cerebral blood flow measured by arterial spin labeling MRI at resting state in normal aging and Alzheimer's disease. *Neurosci Biobehav Rev*, 72:168-175.
- [83] Agosta F, Pievani M, Sala S, Geroldi C, Galluzzi S, Frisoni GB, et al. (2011). White matter damage in Alzheimer disease and its relationship to gray matter atrophy. *Radiology*, 258:853-863.
- [84] Alsop DC, Detre JA, Golay X, Günther M, Hendrikse J, Hernandez-Garcia L, et al. (2015). Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson Med*, 73:102-116.
- [85] Bernetti C, D'Andrea V, Buoso A, Barbalace I, Greco F, Pilato F, et al. (2025). Arterial Spin Labeling MRI in Alzheimer's Disease: A Systematic Review of Cerebral Perfusion Biomarkers. *J Neuroimaging*, 35:e70035.
- [86] Almeida JRC, Greenberg T, Lu H, Chase HW, Fournier JC, Cooper CM, et al. (2018). Test-retest reliability of cerebral blood flow in healthy individuals using arterial spin labeling: Findings from the EMBARC study. *Magn Reson Imaging*, 45:26-33.
- [87] Chen Y, Wang DJ, Detre JA (2011). Test-retest reliability of arterial spin labeling with common labeling strategies. *J Magn Reson Imaging*, 33:940-949.
- [88] Figley CR, Uddin MN, Wong K, Kornelsen J, Puig J, Figley TD (2022). Potential Pitfalls of Using Fractional Anisotropy, Axial Diffusivity, and Radial Diffusivity as Biomarkers of Cerebral White Matter Microstructure. *Frontiers in Neuroscience*, Volume 15 - 2021.