The effect of spermidine on memory performance in older adults at risk for dementia: A randomized controlled trial

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Abstract

Introduction

Nutritional intervention with the natural polyamine spermidine, an autophagy-enhancing agent, can prevent memory loss in aging model organisms. This is the first human study to evaluate the impact of spermidine supplementation on memory performance in older adults at risk for the development of Alzheimer's disease.

Methods

Cognitively intact participants with subjective cognitive decline ($n = 30$, 60–80 years of age) were included in this three-months, randomized, placebo-controlled, double-blind Phase IIa pilot trial with a spermidine-rich plant extract supplement. Effects of intervention were assessed using the behavioral mnemonic similarity task, measured at baseline and post-intervention visits. Data analysis was focused on reporting and interpreting effectiveness based on effect sizes.

Results

Memory performance was moderately enhanced in the spermidine group compared with placebo at the end of intervention [contrast mean = 0.17, 95% confidence interval (CI): -0.01, 0.35, Cohen's $d = 0.77$, 95% CI: 0, 1.53]. Mnemonic discrimination ability improved in the spermidine-treated group with a medium effect size (mean difference = -0.11, 95% CI: -0.19, -0.03, Cohen's $d = 0.79$, 95% CI: 0.01, 1.55). A similar effect was not found in the placebo-treated group (mean difference = 0.07, 95% CI: -0.13, 0.27, Cohen's $d = -0.20$, 95% CI: -0.94, 0.54).

Discussion

In this pilot trial, nutritional spermidine was associated with a positive impact on memory performance in older adults with subject cognitive decline. The beneficial effect might be mediated by stimulation of neuromodulatory actions in the memory system. A follow-up Phase IIb randomized controlled trial will help validate the therapeutic potential of spermidine supplementation and delineate possible neurophysiological mechanisms of action.

Trial registration

Registered in ClinicalTrials.gov with the Identifier NCT02755246.

Keywords: Diet supplementation; Polyamine; Cognition; Subjective cognitive decline; Prevention; SmartAge

1 Introduction

Episodic memory is a highly vulnerable cognitive domain affected in aging and neurodegenerative disease (Jagust, 2013), calling for effective treatment strategies. A well-characterized behavioral marker of age- and disease-related memory loss is the inability to discriminate among similar items held in memory (Stark, Stevenson, Wu, Rutledge, & Stark, 2015; Stark, Yassa, Lacy, & Stark, 2013), here referred to as mnemonic discrimination deficit. This behavioral phenotype of brain aging tracks well with neural dysfunction in the memory network (Leal & Yassa, 2018; Marks, Lockhart, Baker, & Jagust, 2017; Yassa et al., 2011). Moreover, this marker has been identified as a possible outcome measure for interventions that target hippocampal functions in older individuals with mild cognitive impairment (Bakker et al., 2012).

Age-related decline of cognitive abilities may be prevented through healthy lifestyle behaviors (Mattson, 2015; Wirth, Haase, Villeneuve, Vogel, & Jagust, 2014), including caloric restriction (Witte, Fobker, Gellner, Knecht, & Floel, 2009). A novel candidate substance proposed to mimic beneficial effects of dietary restriction is the natural polyamine spermidine (Madeo, Eisenberg, Pietrocola, & Kroemer, 2018). Previous findings in aging model systems have highlighted several health benefits of oral spermidine intake (Eisenberg et al., 2009, 2016), including restoration of memory performance in fruit flies (Gupta et al., 2013). In light of these findings, spermidine supplementation is proposed to open a new avenue for prevention of memory loss in older individuals at risk for dementia. However, to this date there are no systematic evaluations of spermidine-induced benefits on cognitive health in suitable target populations.

An alerting sign of accelerated (and potentially pathological) aging appears to be the presence of subjective cognitive decline (SCD). Expressed by cognitively intact older adults, SCD was previously associated with higher risk of objective cognitive decline (Peter et al., 2014) and clinical progression to Alzheimer's disease (AD) (Jessen, Wolfsgrubber, Wiese, Bickel, Mosch, & Kaduszkiewicz, 2014b; Mitchell, Beaumont, Ferguson, Yadegarfar, & Stubbs, 2014). Moreover, studies have demonstrated functional and structural alterations in brain regions typically affected by AD (Cherbuin, Sargent-Cox, Easteal, Sachdev, & Anstey, 2015; Erk et al., 2011; Jessen et al., 2006; van der Flier et al., 2004; Wirth et al., 2017) and (albeit less frequent) brain beta-amyloid burden (Amariglio et al., 2012) in older individuals with SCD. Based on this evidence, it has been recognized that this at-risk group will benefit from early intervention strategies (Jessen, Amariglio, van Boxtel, Breteler, Ceccaldi, & Chetelat, 2014; Smart et al., 2017) that aim to restore brain function and help maintain cognitive abilities.

We conducted the first randomized, placebo-controlled, double-blind Phase IIa pilot trial to evaluate impacts of three-months spermidine supplementation on cognitive performance in cognitively intact older individuals with SCD ($n = 30$). We recently documented compliance, safety, and tolerability of a spermidine-rich plant extract supplement in this group (Schwarz et al., 2018). Given the available literature on aging model systems (Gupta et al., 2013), we anticipated a positive impact of spermidine intake on memory. Therefore, we evaluated mnemonic discrimination performance as the primary outcome, measured using the mnemonic similarity task (MST) (Stark et al., 2013). This behavioral memory task was selected, due to its robustness and sensitivity to isolate memory deficits in the elderly (Marks et al., 2017; Stark et al., 2015). In addition, we

explored effects of intervention using conventional neuropsychological tests on memory and executive functions.

2 Materials and methods

2.1 Participants

In total thirty older individuals (60 – 80 years of age, fluent German speakers) participated in this study. Participants were recruited through health care facilities and through advertisement in the general population. Presence of SCD was diagnosed using established guidelines (Jessen, Amariglio, et al., 2014) that included (1) subjective cognitive complaints for at least 6 months and self-reported associated concerns (worries), (2) no deficits in activities of daily living, and (3) normal cognitive performance. The later was measured using the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) (score ≥ 26), the Logical Memory subscale total delayed recall (Wechsler, 1987), and the Trail Making Test A (Reitan, 1955) [both scores > -1.5 standard deviation (SD) of age-adjusted norms]. Exclusion criteria comprised current major neurological, internal or psychiatric diseases, renal dysfunction, malignancies (current or on medical history), known allergy to wheat germs, gluten or histamine intolerance, and pre-trial polyamine intake, as summarized by Schwarz et al. (2018).

Written informed consent was obtained from each participant in accordance with the ethics Committee of the Charité – Universitätsmedizin, Berlin, Germany (EA1/233/15) and the declaration of Helsinki. The study was registered at ClinicalTrials.gov (identifier: NCT02755246). Out of the 30 participants, 28 completed the trial. Two participants, one in each group, dropped out due to loss of motivation.

2.2 Study design and intervention

The design of our study was reported elsewhere (Schwarz et al., 2018). In brief, this monocentric, randomized, double-blind, placebo-controlled Phase IIa trial was carried out at the NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin. The trial involved a screening visit, a baseline visit, an intervention period, and a post-intervention visit. On-site screening for eligibility criteria and baseline assessments of neuropsychology and behavior were carried out on the same day. Participants were then randomly assigned to spermidine or placebo intervention. After the three-month intervention period, assessment procedures from baseline were repeated, here referred to as post-intervention assessment. The primary outcome of the present trial was the effect of spermidine intervention on memory performance.

The spermidine group received a daily dose of 750 mg of spermidine-rich plant extract (with a daily spermidine dose of 1.2 mg) and 510 mg cellulose, dispersed into three capsules. Capsules in the placebo group, three per day, contained in total 750 mg potato starch and 510 mg cellulose. All study capsules (spermidine and placebo) were identical in all aspects (shape, color, taste, and smell) and provided by TLL The Longevity Labs GmbH (Graz, Austria).

2.3 Behavioral assessment

Memory performance (primary outcome) was measured using the behavioral non-verbal MST (version 0.8, Figure 1A) (Stark et al., 2015), available for public download including task description (<http://faculty.sites.uci.edu/starklab/mnemonic-similarity-task-mst/>). Briefly, during incidental encoding, participants viewed images of everyday objects

and decided on each trial (64 items total, self-paced via button press), whether the object was typically found “outdoor” or “indoor”. During immediate probed recognition, 32 images were repeated (repetitions), randomly inter-mixed with 32 novel images (foils), and 32 images that were similar to those pictures seen during encoding (lures). Participants indicated for each trial (96 items total, self-paced via button press), whether the image was “old”, “new” or “similar”. Matched sets with different images were presented at baseline and post-intervention assessments, respectively.

Fig. 1 Mnemonic similarity task (MST) and the effect of intervention. A. MST: Participants encoded pictures of objects during an indoor/outdoor judgment task. Recognition was examined with old/new/similar judgments on repetitions, foils, and similar lure items. Schematic task representation was adapted with

permission (Stark et al., 2013). B. Between-group effect: Error bar plots show mnemonic discrimination (MD) performance in the spermidine group compared with placebo at post-intervention assessment. C. Within-group effect: Error bar plots show MD performance in the spermidine group and the placebo group at baseline and post-intervention assessments. Black error bars indicate mean with 95% confidence interval (CI). Dots specify individual data points.

Two behavioral measures were evaluated from the MST at baseline and post-intervention assessments. Our primary outcome was the effect of intervention on mnemonic discrimination performance. This index was calculated comparable to Marks et al. (2017), as percentage of lure items endorsed as similar [$P(\text{“similar”}|\text{lure})$] plus percentage of lure items classified as new [$P(\text{“new”}|\text{lure})$] minus percentage of foil items endorsed as similar [$P(\text{“similar”}|\text{foil})$], to correct for a response bias. Low values in this performance index capture the inability to discriminate lures from previously seen items that are perceptually similar. In addition, we evaluated recognition memory performance as a measure of task compliance, calculated as percentage of repetitions identified as old [$P(\text{“old”}|\text{repetition})$] minus percentage of foils identified as old [$P(\text{“old”}|\text{foil})$] to correct for response bias (Stark et al., 2013).

As additional outcome measures, we explored conventional neuropsychological tests. Measures for memory and executive functions (one for each domain) were selected from a standard neuropsychological test battery. Selection was based on conceptual relevance (sensitivity to age-related cognitive deficits) and suitable test characteristics, as done previously (Wirth et al., 2013). The German version of the Rey Auditory Verbal Learning Test (AVLT), total immediate recall (Lezak, Howieson, Loring, Hannay, &

Fischer et al., 2004) was included as a measure of verbal episodic memory and the digit symbol substitution test (Wechsler, 1981) as a measure of executive functions.

2.4 Statistical analysis

Statistical analyses were carried out with the SPSS 24.0 statistical package (PASW, SPSS; IBM, Armonk, NY). Given that this is a pilot study with a small sample size and the study objective was to investigate, if there is any effect of intervention on memory performance, we chose to evaluate effect sizes, rather than relying on p-values.

For the a-priori sample size calculation, we used data from Soda et al. (2009). This earlier study demonstrated a positive effect of polyamine-rich diet on whole-blood polyamine levels in humans, but did not include cognitive outcome measures. The resulting effect size was used for the present power analysis (estimated effect size Cohen's d for change in spermine concentration = 1.1). With 15 participants in the spermidine group and 15 participants in the placebo group, a two-tailed t-test for independent samples (i.e., the respective differences between baseline and post-intervention assessments for each group in a cognitive outcome measure) may show a difference with 80% power and a two-sided significance level: $\alpha = 0.049$. This sample size calculation was done conservatively for an independent t-test based on the difference values (post-intervention – baseline) of polyamine concentrations in both intervention groups (Soda et al., 2009). However, the main analysis procedure was conducted using analysis of covariance (ANCOVA) models to adjust for the baseline value and using the post-intervention measure as dependent variable. No drop-out rate was incorporated in the sample size estimation for this pilot trial.

Descriptive sample characteristics were evaluated as follows: The total sample was characterized with regard to available SCD plus criteria (Jessen, Amariglio, et al., 2014), known to increase the likelihood of preclinical AD in cognitively intact older individuals with SCD. In particular, we evaluated self-reported subjective decline in memory (yes/no), self-reported onset of SCD (recommended within the past 5 years and age of 60 years or older), and self-reported feeling of memory performance in comparison to others of the same age group [1 (better than most), 2 (about the same), 3 (a bit worse), and 4 (much worse)]. In addition, self-reported family history of dementia of first-degree relatives was assessed as an important risk factor for AD (Farrer, O'Sullivan, Cupples, Growdon, & Myers, 1989). Subsequently, intervention groups were characterized descriptively at baseline on demographic and cognitive measures using mean, SD, and interquartile range (IQR).

The effect of intervention on memory performance (primary outcome) was assessed using the effect size measure Cohen's *d*. To examine the effect of intervention between groups, Cohen's *d* was converted from eta-squared measures that were estimated using ANCOVA models. Conversions were conducted using the formula by Cohen (1988). Respective ANCOVA models included each outcome measure obtained at post-intervention assessment as dependent variable, intervention group (spermidine and placebo) as independent variable, and behavioral performance at baseline as a covariate. Cohen's *d* for the intervention effect within each group was estimated, using the change in behavioral performance from baseline to post-intervention assessment. Behavioral data of the MST was incomplete for one participant at baseline assessment due to premature termination. Statistical analysis was thus conducted first including and then excluding this

participant's data. In addition, exploratory evaluations of between-group effects were carried out, using selected neuropsychological tests.

All between- and within-group comparisons were evaluated descriptively using means and 95% confidence intervals (CIs). The effect size measure Cohen's *d* was classified as small (0.2–0.5), medium (0.5–0.8) or large (0.8 and larger) effects (Cohen, 1988). Ninety-five percent confidence intervals (95% CI) for Cohen's *d* effect sizes were calculated using the *r* package MBESS (Kelley, 2018).

3 Results

3.1 Participant characteristics

The total sample was characterized as follows: The majority of participants indicated SCD in the memory domain [yes: $n = 27$ (96%), no: $n = 1$ (4%)]. The onset of SCD was on average within the past five years (mean = 4.5, SD = 4.4) and above 60 years of age (mean = 65.6, SD = 6.2). Memory performance was reported in tendency to be a bit worse in comparison to others of the same age group (mean = 2.5, SD = 0.7). A positive family history of dementia was indicated by 13 [46%] out of the 28 participants. Stratification by intervention (spermidine: $n = 14$, placebo: $n = 14$) showed comparable demographics as well as cognitive and affective characteristics between the two groups at baseline (Table 1).

Table 1 – Characterization of participants.

	Spermidine group	Placebo group
n (women)	14 (9)	14 (9)
Age (years)	70.4 (5.2), 67.0 – 74.0	69.4 (5.6), 65.0 – 74.0
Education (years)	16.0 (3.7), 13.0 – 18.0	15.3 (1.7), 14.0 – 16.0
GDS (score)	1.7 (1.7), 0.0 – 3.0	2.0 (1.5), 1.0 – 3.0
MMSE (score)	29.1 (1.1), 29.0 – 30.0	29.2 (0.9), 28.0 – 30.0
LMS delayed recall (score)	25.0 (6.9), 18.0 – 29.0	24.7 (5.6), 21.0 – 29.0
TMT A (sec)	47.5 (17.8), 34.0 – 64.0	43.4 (14.5), 30.0 – 50.5

Sample description data are given if applicable as mean, standard deviation (SD), and interquartile range (IQR). Abbreviations: GDS, Geriatric Depression Scale; MMSE, Mini-Mental State Examination; LMS, Logical Memory subscale; TMT, Trail Making Test.

3.2 Behavioral results

Performance in the behavioral memory task (MST, Table 2) at baseline was similar for both intervention groups and comparable to previous reports in older adults. Mnemonic discrimination performance was low in both groups, while recognition memory performance was reasonably high.

Table 2 – Effect of intervention in the mnemonic similarity task (MST).

	Spermidine group		Placebo group		Contrast Mean [95% CI]	Cohen's d [95% CI]
	Baseline	Post-intervention	Baseline	Post-intervention		
Mnemonic discrimination	0.32 (0.11)	0.43 (0.15)	0.33 (0.17)	0.26 (0.29)	0.17 [-0.01, 0.35]	0.77 [0, 1.53]
Recognition memory	0.85 (0.14)	0.71 (0.34)	0.83 (0.11)	0.78 (0.12)	-0.09 [-0.28, 0.11]	-0.37 [-1.11, 0.38]

Performance data are given as mean and standard deviation (SD). Contrast values [mean and 95% confidence interval (CI)] and effect sizes (Cohen's d and 95% CI) for the effect of group (spermidine, placebo) were estimated using ANCOVA models with the outcome measure (post-intervention) as dependent variable.

Effects of intervention in the MST were as follows: Mnemonic discrimination performance (primary outcome) was enhanced in the spermidine group compared with placebo at the end of intervention with a medium effect size (Cohen's $d = 0.77$, 95% CI: 0, 1.53; Table 2, Fig. 1B). Within-group comparisons corroborated that mnemonic discrimination moderately improved in the spermidine group [mean difference (baseline – post-intervention) = -0.11, 95% CI: -0.19, -0.03, Cohen's $d = 0.79$, 95% CI: 0.01, 1.55], while this effect was not observed in the placebo group [mean difference (baseline – post-intervention) = 0.07, 95% CI: -0.13, 0.27, Cohen's $d = -0.20$, 95% CI: -0.94, 0.54] (Table 2, Fig. 1C). There was no analogous group-dependent effect of intervention for recognition memory performance (Cohen's $d = -0.37$, 95% CI: -1.11, 0.38; Table 2, data not shown). After excluding one participant with an incomplete data set, results remained essentially unchanged, that is, memory performance was moderately enhanced in the spermidine group compared with placebo (data not shown).

In exploratory evaluations, we assessed group-dependent effects of spermidine intervention on selected neuropsychological tests. Cohen's d values were below 0.5, indicative of small effects. In the spermidine group, test scores were slightly higher for the AVLT (contrast mean = 2.49, 95% CI: -2.70, 7.68, Cohen's $d = 0.41$, 95% CI: -0.34, 1.16) and slightly lower for the digit symbol substitution test (contrast mean = -0.61, 95% CI: -5.28, 4.06, Cohen's $d = -0.11$, 95% CI: -0.85, 0.63) in comparison with placebo at the end of intervention.

4 Discussion

In this Phase IIa trial, spermidine supplementation was associated with improved memory performance in older adults at risk for AD. Specifically, we found a moderate improvement of mnemonic discrimination ability after three months of spermidine intake compared with placebo. The finding suggests that nutritional spermidine may be a potential intervention strategy to delay memory loss in older individuals with SCD, a recognized at-risk population (Jessen, Amariglio, et al., 2014).

Memory performance, as assessed by the MST, was evaluated as the primary outcome of this pilot trial. The ability to differentiate among similar items held in memory is robustly decreased in older individuals, while recognition memory appears to be intact (Stark et al., 2013). A similar behavioral pattern was seen in our SCD participants at baseline. After spermidine administration, specifically mnemonic discrimination performance was improved, as indicated by a medium effect size. This intervention effect may reflect a therapeutic benefit of the natural compound in the preservation of memory functions. The finding is of importance, because memory improvement through safe and well-tolerated spermidine supplementation (Schwarz et al., 2018) may offer a potential intervention strategy in persons at risk for AD.

We did not observe comparable positive effects of intervention using neuropsychological tests on memory and executive functions. Results may reflect a lack of sensitivity to detect subtle cognitive changes in cognitively intact older adults using such tests (Jessen, Amariglio, et al., 2014). The MST appears to be suitable for capturing even subtle effects, since a number of shorter-term intervention studies have employed this task

in samples of young and older individuals (Bakker et al., 2012; Borota et al., 2014; Hanert, Weber, Pedersen, Born, & Bartsch, 2017). For example, Bakker et al. (2012) demonstrated improved mnemonic discrimination performance in patients with mild cognitive impairment after two weeks of low-dose antiepileptic drug administration, which was not detected by standard neuropsychological tests. Moreover, the MST is computerized and available online including multilingual instructions, which may be an advantage for potential multi-site trials.

The exact mechanisms of action through which spermidine might influence memory performance in older adults are unknown. However, data from aging model organisms propose several pathways, involving cellular and systemic levels of the memory system (Korte & Schmitz, 2016). In aging fruit flies, for example, memory-promoting effects of spermidine appear to be mediated via recuperation of autophagy (Gupta et al., 2013, 2016). This cellular mechanism is believed to eliminate pathogenic protein aggregates (Schaeffer et al., 2012) and, as a result, protect higher-order brain functions. Such neuroprotective effects of spermidine may likely necessitate longer intervention periods in humans. More immediate effects of spermidine administration might include proposed neuromodulatory actions (Madeo et al., 2018). Spermidine has been shown to regulate synaptic activity (Gupta et al., 2016), which could counteract subtle neural dysfunction in the memory system and thereby target pathological aging at an early stage. To understand brain correlates underlying cognitive benefits of nutritional spermidine intake, neuroimaging assessments of neural structure and function will be crucial.

One might hypothesize positive relationships between memory performance and whole-blood polyamine concentrations. As reported previously, polyamine levels were

evaluated for our participants using baseline and post-intervention blood samples, with no concentration changes in response to spermidine supplementation (Schwarz et al., 2018). Correlation coefficients (examined post-hoc) between spermidine/spermine levels and mnemonic discrimination performance were small at baseline and post-intervention for the whole sample and within the spermidine group (all r 's: $-0.3 < r < 0.3$). There was one moderate positive correlation between spermine and memory performance measured after polyamine treatment ($r = 0.41$). The overall lack of such relationships could be explained by the small sample size, measurement errors, and limited relations between whole-blood polyamine levels and tissue cell concentrations (Teixeira, Santaolalia, Meneu, & Alonso, 2002). Further investigation of possible relations between polyamines, their metabolites, and cognitive functioning is warranted.

In general, nutrition interventions that may delay cognitive decline in the elderly are an important area of research. In the present Phase IIa pilot trial, short-term spermidine supplementation was associated with a positive impact on memory performance in older adults with SCD. Outcomes of this study serve as a rationale to conduct a Phase IIb randomized controlled trial (ClinicalTrials.gov identifier: NCT03094546) with a longer intervention period, sufficient sample size, as well as clinical and biomarker-based assessments to validate the therapeutic potential of nutritional spermidine intake. In addition, this study includes functional and structural neuroimaging to delineate possible neurophysiological mechanisms of action associated with spermidine intervention.

Limitations of our study include the small sample size and limited intervention period, which are common features of pilot trials (Shader, 2015). Data analysis was focused on reporting and interpreting effectiveness based on effect sizes and does not allow

generalization to population level. Based on results of this Phase IIa trial, larger intervention studies with appropriate sample sizes will be possible. In the attempt to optimize power, a global discrimination index was assessed that included the percentage of lure items endorsed as “similar” and “new”, comparable to a previous approach (Marks et al., 2017). Also, short-term repeats of behavioral assessments may encourage practice effects, although not observed previously using the MST (Stark et al., 2015). Future studies should incorporate assessments on the efficacy of blinding from the participants' perspective. Finally, we acknowledge that SCD is a non-specific condition, which likely includes individuals, who will progress to dementia, as well as individuals, who will remain cognitively stable for many years. Nevertheless, this diagnostic group is considered eligible to early intervention strategies, given the higher risk of clinical progression in previous studies (Jessen, Amariglio, et al., 2014; Smart et al., 2017).

5 Conclusions

This Phase IIa pilot trial suggests that nutritional spermidine may protect memory performance in cognitively intact older adults with SCD, presumably through regulating neural mechanisms in the memory system. The therapeutic potential of spermidine supplementation in persons at risk for dementia warrants further investigation and identification of possible mechanisms of action.

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7 Disclosures

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