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der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Dietary polyamine intake and its association with brain structure, cognition, and cardiovascular risk in older adults

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Nora Horn

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PREFACE

Note on preliminary publications of partial results

Partial results of the presented work have been published in *Alzheimer's & Dementia* in 2019 (as abstract) and presented at the *Alzheimer's Association International Conference* in Los Angeles (AAIC 2019, poster presentation):
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Partial results of the presented work have been submitted at *Neuroimage* in April 2020:
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* These authors contributed equally to the manuscript
(Contribution: Major role in the acquisition of data, recruitment of study participants, medical examination, MRI-acquisition and -analyses, data entry, data analyses, statistical analyses, interpretation of data. Drafting of the manuscript.)

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LIST OF ABBREVIATIONS

Aβ	Beta-Amyloid
AD	Alzheimer's Disease
ADL	Activities of daily life
Aparc	Automated cortical parcellation
Aseg	Automatic subcortical segmentation
AvgRisk	Average Risk
AVLT	Auditory Verbal Learning Test
BET	Brain Extraction Tool
BMI	Body Mass Index
CA	Cornu Ammonis
CI	Confidence Interval
Cig	Cigarettes, current smoker
CVD	Cardiovascular Disease
DG	Dentate Gyrus
DICOM	Digital Imaging and Communications in Medicine
DM	Diabetes Mellitus
e.g.	exempli gratia
etc.	et cetera
eTIV	estimated Total Intracranial Volume
FFL	Food Frequency List
FFQ	Food Frequency Questionnaire
FSL	FMRIB Software Library
GC	Granule Cell layer
GDS	Geriatric Depression Scale
GLM	General Linear Model
HATA	Hippocampal-Amygdaloid Transition region
HC	Healthy Controls
HDL	High-Density Lipoprotein
HPC	Hippocampus
i.a.	inter alia
IADL	Instrumental Activities of Daily Living scale
LAS	Left Anterior Superior
LMS	Logical Memory IIa Subtest
LPS	Left Posterior Superior
MCI	Mild Cognitive Impairment

MEDAS	Mediterranean Diet Adherence Screener
mmHg	Millimeter of mercury
MMSE	Mini-Mental State Examination
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo
MRI	Magnetic Resonance Imaging
NFTs	Neurofibrillary Tangles
NIFTI	Neuroimaging Informatics Technology Initiative
NMDA	N-Methyl-D-Aspartate
PACC	Preclinical Alzheimer Cognitive Composite
PAO	Polyamine Oxidase
ROI	Region-of-interest
SCD	Subjective Cognitive Decline
SD	Standard Deviation
SE	Standard Error
SSAT	Spermidine/spermine-N1-acetyltransferase
SysBP	Systolic Blood Pressure
TMT	Trail Making Test
TotalChol	Total Cholesterol

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ABSTRACT (German)

Hintergrund: Endogene Polyamine sind wichtige Produkte des Zellstoffwechsels, die an wesentlichen Zellprozessen wie der Autophagie beteiligt sind und deren Konzentration mit zunehmendem Alter abnimmt. Polyamine sind in gesundem sowie auch pathologischem Altern, z.B. im Rahmen der Alzheimer Krankheit (AD), involviert. Studien haben gezeigt, dass polyaminreiche Nahrung helfen kann, die kognitive und kardiovaskuläre Gesundheit zu erhalten und das Erreichen eines hohen Lebensalters zu fördern, was bisher hauptsächlich in Tiermodellen beobachtet wurde. Die Rolle von Autophagie-anregenden Polyaminen in der Gesundheit von Gehirn, Kognition und Herz-Kreislauf-System beim Menschen muss hingegen noch genauer untersucht werden. Diese Studie ermittelt, ob ein Zusammenhang zwischen der Polyaminaufnahme über die Ernährung und Hippocampusvolumen, kortikaler Dicke und Volumen, Kognition, kardiovaskulärem Risiko und anderen bekannten gesundheitsfördernden Ernährungsgewohnheiten bei älteren Menschen besteht.

Methoden: Insgesamt wurden 101 kognitiv uneingeschränkte Probanden*innen (Alter 70 ± 6 Jahre) medizinisch und neuropsychologisch untersucht. Die Polyaminzufuhr (Spermidin und Spermin) sowie weitere Diätgewohnheiten wurden anhand von Fragebögen zur Häufigkeit von Nahrungsmitteleinnahmen erfasst. Gesamtwerte einzelner neuropsychologischer Tests wurden berechnet, um Leistungen verschiedener kognitiver Domänen zu quantifizieren. Zur Einschätzung des kardiovaskulären Risikos wurde ein Prozentwert berechnet. Eine strukturelle Magnetresonanztomographie wurde durchgeführt um Hippocampusvolumen, hippocampale Subfeldvolumina, kortikale Dicke und Volumen von unter anderem AD-vulnerablen Regionen abzuleiten. In einem Querschnittsdesign wurde das Verhältnis der Polyaminzufuhr zu diesen abhängigen Variablen mit Hilfe von allgemeinen linearen Modellen analysiert, welche für potenzielle Störfaktoren korrigiert wurden.

Ergebnisse: Die Aufnahme von Spermidin über die Nahrung war mit einer mediterranen Ernährungsweise assoziiert. Eine höhere Aufnahme von Spermidin war mit einem größeren Hippocampus sowie gesamtkortikalen Volumen assoziiert, selbst nach Anpassung an Störfaktoren. Spermidin war mit dem Volumen einiger hippocampaler Unterregionen (Cornu Ammonis 1, Subiculum, Hippocampusschweif) verbunden. Eine höhere Spermidinzufuhr war zudem mit einer ausgeprägteren mittleren sowie AD-vulnerablen, frontalen, temporalen und parietalen kortikalen Dicke verbunden. Anpassungen an die potenziellen Störfaktoren haben die Ergebnisse nicht sonderlich verändert, mit Ausnahme der frontalen kortikalen Dicke und der hippocampalen Subfeldvolumina. Für die Nahrungsaufnahme von Spermin wurden keine Korrelationen mit hirnstrukturellen Parametern beobachtet. Zudem zeigten sich keine

Zusammenhänge zwischen diätischer Polyaminaufnahme und regionalen Kortikalvolumina, Kognition oder kardiovaskulärem Risiko.

Schlussfolgerung: Diese Studie ergab, dass eine spermidinreiche Ernährung mit einem größeren Hippocampus- und gesamtkortikalen Volumen sowie einer größeren kortikalen Dicke unter anderem in AD-vulnerablen Regionen bei älteren Menschen zusammenhängt. Diese Ergebnisse deuten darauf hin, dass eine höhere Spermidinzufuhr durch eine spezifische Ernährung oder Nahrungsergänzung dazu beitragen kann, die Gehirngesundheit während des menschlichen Alterns zu erhalten. Diese Hypothese sollte in zukünftigen Interventionsstudien eingehender untersucht werden.

ABSTRACT (English)

Background: Endogenous polyamines are important products of cell metabolism, involved in essential cell processes like autophagy, whose concentration decreases with age. Polyamines are involved in healthy as well as pathological aging, e.g. in the context of Alzheimer's disease (AD). Previous studies have shown that polyamine-rich food can help maintain cognitive and cardiovascular health as well as promote longevity, most thoroughly described in animal models. However, in humans the role of autophagy-enhancing polyamines on brain, cognitive and cardiovascular health needs to be better understood. This study aimed to determine whether dietary polyamine intake is related to hippocampal volume, cortical thickness and volume, cognition, and cardiovascular risk as well as to other well-known health promoting dietary habits in older adults.

Methods: In total, 101 cognitively unimpaired individuals (age 70 ± 6) underwent medical examination and neuropsychological testing. Polyamine intake (spermidine and spermine) as well as further dietary patterns were assessed through food frequency questionnaires. Composite scores of individual neuropsychological tests were calculated to quantify the performance of different cognitive domains. A score was calculated to assess cardiovascular risk. Cerebral magnetic resonance imaging was conducted to derive hippocampal and hippocampal subfield volumes, cortical thickness and volume among others in AD-vulnerable regions. The relationship of polyamine intake on these dependent variables was analysed using general linear models, adjusting for potential confounding factors.

Results: Spermidine intake was related to Mediterranean Diet adherence. Higher dietary intake of spermidine was associated with larger hippocampal and total grey matter volume, even after adjusting for confounders. Dietary spermidine was related with several hippocampal subfield volumes (cornu ammonis 1, subiculum, tail). Higher spermidine intake was also associated with greater mean cortical thickness as well as cortical thickness in AD-vulnerable, frontal, temporal and parietal regions. Adjustments for confounding factors did not substantially attenuate the relationships, except for frontal cortical thickness and hippocampal subfield volumes. No correlations with brain-structural parameters were observed for dietary intake of spermine. There were no associations between dietary polyamine intake and regional cortical volume, cognition or cardiovascular risk.

Conclusions: This study provided first evidence, that a spermidine-rich diet is related to larger hippocampal and total grey matter volume as well as greater cortical thickness among others in AD-vulnerable regions in older adults. These findings suggest that higher spermidine intake,

through specific diet or supplementation, may help preserve brain health during human aging, a hypothesis to be further evaluated in future interventional studies.

1 INTRODUCTION

Given the worldwide constant growth of the elderly population, and the life span currently rising faster than the health span, age-related diseases are increasing. In Europe, the average life expectancy at birth was 78 years in 2016, whereas the healthy life expectancy at birth was only 68 (World Health Organization). By 2030, more than 25 per cent of the European population will be over 60 years old, mainly due to increased longevity and reduced fertility rates (United Nations, 2015). This implicates that health care will be confronted with a higher number of diseases of older people, in particular non-communicable, chronic diseases such as cardiovascular or neurodegenerative diseases, the prevalence of which is associated with old age (United Nations, 2015). However, it is not only of great socio-economic and medical interest, but also of personal interest in quality of life to ensure healthy aging by finding preventive measures during the healthy phase of life that counteract degenerative processes.

1.1 Healthy brain aging in humans

Healthy, normal aging is generally understood as age-related changes with no significant disorder, meaning disease-free aging. The underlying mechanisms of aging are diverse and implicate molecular and cellular alterations, including telomere shortening, genomic instability, epigenetic modifications, loss of proteostasis, dysregulation of nutrient sensing, cellular senescence, mitochondrial dysfunction, exhaustion of stem cells, intercellular communication alterations, and chronic inflammation (López-Otín et al., 2013, Franceschi et al., 2000). To extend the health span these alterations must be attenuated or retarded as they drive the process of aging and the induction of age-related pathologies (López-Otín et al., 2016). Another mechanism involved in the aging process is the decrease in cellular autophagic activity. Autophagy is an intracellular digestion system, allowing for the degradation of damaged and potentially harmful aggregates that accumulate during aging through autophagosome sequestration and lysosomal digestion (Rubinsztein et al., 2011). Consequently, autophagy alterations are implicated in the development and exacerbation of age-related diseases (Cuervo, 2008, Choi et al., 2013).

Aging is unavoidable and affects all organs, including the brain. Age-related brain changes during healthy aging are part of a very complex multifactorial process including neurochemical as well as structural changes (Hof and Mobbs, 2010), strongly varying among elderly people. Autophagy changes also occur during normal aging of the human brain, where autophagy processes are transcriptionally down-regulated, as related proteins decrease with age (Lipinski et al., 2010). Even during a "normal aging process" the brain experiences various structural changes (Raz et al., 2004, Salat et al., 2004). In healthy elderly people, the brain volume loss of cortical and

subcortical brain structures and the expansion of the ventricular system can be perceived in almost all brain areas over only one year and changes in cortical thickness are accelerated with increasing age (Fjell et al., 2009). Brain shrinkage is not random or uniform, but rather selective and differential (Raz and Rodrigue, 2006). There are two time windows of brain aging. One is exemplified by a steady cortical volume decline in different regions with varying decline rates throughout the whole adult life span. The second one is characterized by an accelerated decline in specific regions such as the hippocampus and is limited to late adulthood (Raz et al., 2004). Cortical thickness in most areas also linearly declines with age (Fjell et al., 2012). Finally, these alterations are paralleled by a deterioration of cognitive performance (Fjell and Walhovd, 2010).

1.2 Pathological brain aging in humans

Severe mental decline is not a normal part of healthy aging. Dementia is one of the most common chronic diseases among older people contributing to physical and mental need for care and loss of healthy life years (World Health Organization, 2015). It is not a specific disease, but a generic term to sum up acquired brain progressive syndromes characterized by the decline of cognition e.g. memory, thinking, language, behaviour, and emotion abilities, severe enough to impair the ability of performing activities of daily living (World Health Organization, 2015). The most common subtype is Alzheimer's Disease (AD) accounting for 60 to 80% of all cases (Alzheimer's Association, 2019). Worldwide, 50 million people were affected by dementia in 2018 and according to estimations, this number will triple to 152 million by 2050 (Patterson, 2018). To date, there is no cure for most types of dementia (Alzheimer's Association, 2019) and the diagnosis itself as well as its stigma are very difficult for patients and their relatives to process and live with.

Between normal aging of the brain and dementia, there is a prodromal stage, called mild cognitive impairment (MCI), which has a higher risk of developing AD (Roberts et al., 2014). MCI is characterized by a cognitive decline (e.g. in the domains of memory) that differs significantly from the normal aging process, which can be objectified by neuropsychological tests (outside the age- and education-adapted normal range) and related concerns (Albert et al., 2011). The difference between MCI and AD is that mild cognitive deterioration in MCI patients does not yet compromise independent daily living (Albert et al., 2011) (Figure 1).

A pre-clinical condition referred to as subjective cognitive decline (SCD) has also received great scientific attention lately. Individuals with subjectively experienced cognitive decline but no impairment of measurable cognitive performance have an increased risk of objective cognitive decline and progression to MCI and AD dementia (Jessen et al., 2014b, Mitchell et al., 2014, van

Harten et al., 2018). If, in addition, they express associated worries about their perceived memory deterioration, the risk of AD dementia doubles once again (Jessen et al., 2010, Jessen et al., 2014b). Thus, SCD could be regarded as a predictor of pathological cognitive deterioration during aging (Figure 1) and persons affected by it could be used as a suitable target population for prevention and intervention strategies years before the onset of clinical symptoms of dementia (Jessen et al., 2014a, Smart et al., 2017).

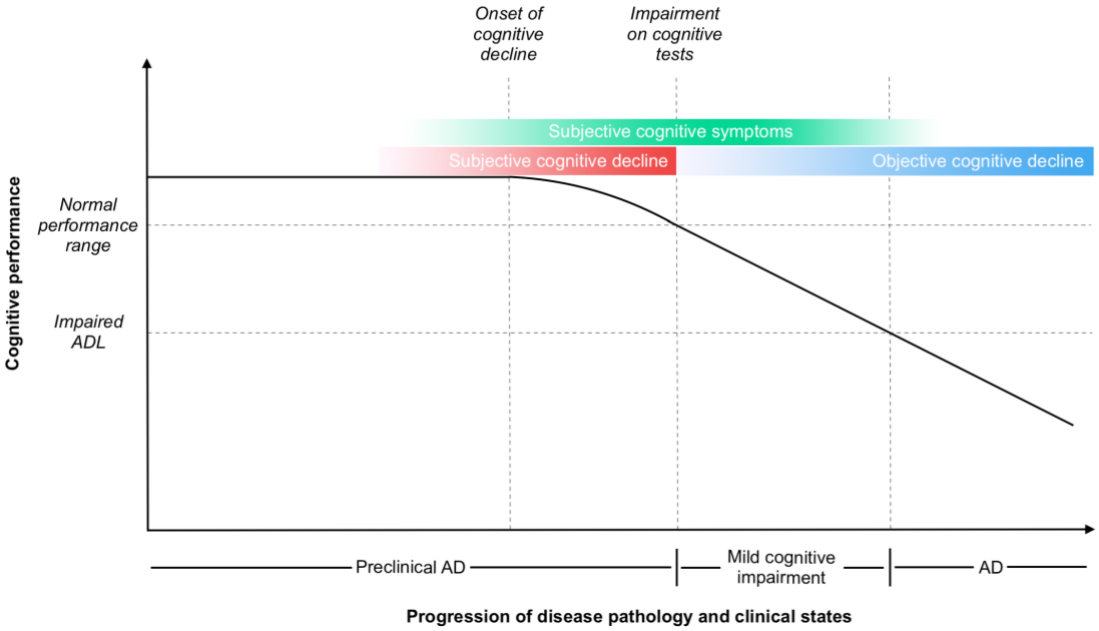

Figure 1: Cognitive decline in relation to progressive disease pathology
This figure illustrates the initial stable phase of cognitive performance, followed by subtle cognitive decline due to increasing pathology, but still within the normal age-, sex-, and education-adjusted cognitive performance range. In a late stage of preclinical Alzheimer's Disease (AD), self-experienced subjective cognitive decline (SCD) occurs without impairment on standardized cognitive tests, due to compensatory cognitive efforts. When the threshold of adjusted normal cognitive performance is crossed (impairment on cognitive tests) mild cognitive impairment (MCI) is reached, but activities of daily life (ADL) are still unimpaired. With further progressing cognitive decline, ADL become increasingly restricted and the stage of AD dementia is reached. As objective impairment progresses into dementia, the experience of subjective decline diminishes. Figure and legend based on Jessen et al. (2014a) and Rabin et al. (2017).

Multiple pathological pathways are involved in the development of AD, acting on the microscopic as well as the macroscopic scale. In addition, it is suggested that the neuropathological mechanisms underlying the cognitive impairments begin years to decades before the onset of clinical symptoms (Sperling et al., 2011, Jack Jr et al., 2013). There are two main neuropathological hallmarks characteristic for AD: increased extracellular beta-amyloid (Aβ) plaque deposition and intra-neuronal accumulation of Aβ peptide and neurofibrillary tangles (NFTs) consisting of tau proteins (Serrano-Pozo et al., 2011, Jack Jr et al., 2018, Blennow et al., 2006). The NFT and Aβ aggregates are strongly influenced by cellular autophagy capacities, which are early impaired in AD affected neurons (Nilsson et al., 2013, Schaeffer et al., 2012).

Other pathological mechanisms damaging cellular structures and accompanying AD include inflammation, oxidative stress, neurovascular dysfunction, hypometabolism and hypoperfusion, cell cycle abnormalities, mitochondrial dysfunction, astrocytosis and microgliosis (Blennow et al., 2006, Serrano-Pozo et al., 2011).

These microscopic/molecular changes are followed by macroscopic changes, which in turn are associated with cognitive decline. Structural alterations include grey matter atrophy, reflected in decreased cortical thickness and volume loss in susceptible regions, i.a. the temporal lobe and the hippocampus (Lerch et al., 2004, Scahill et al., 2002). Cortical thinning in AD signature regions can be related to symptom severity even in early stages of clinical symptoms (Dickerson et al., 2008). Moreover, the severity of atrophy in the hippocampus and entorhinal cortex can predict the progression from healthy to MCI and MCI to AD dementia (Jack et al., 2000, Tapiola et al., 2008, Henneman et al., 2009). The hippocampus is affected very early in AD and allows differing between healthy controls and AD patients (Jack et al., 1997). Most of the imaging literature focuses on the hippocampus as unitary entity, but taking into account that the hippocampus consists of subregions exerting different functions and connecting to other brain regions, it is also obvious that these regions are affected differently in aging and AD (Aggleton, 2012, Maruszak and Thuret, 2014, Small et al., 2011). Specifically in SCD, hippocampal subfields exhibit a differentiated pattern of grey matter atrophy that mimics the pattern of AD (Perrotin et al., 2015).

1.2.1 Boundary between healthy and pathological brain aging

Normal brain aging aspects often appear to be accelerated in pathological aging processes like AD, such as hippocampal atrophy and cortical thinning, especially during early stages of the disease (Sabuncu et al., 2011). However, accelerated cortical thickness decline in areas vulnerable to AD does not necessarily mean that it is due to neurodegenerative disease (Fjell et al., 2012). In addition, the hippocampus, which is already severely damaged at the time of AD diagnosis, is also affected during healthy aging where it is one of the structures that show a sharp decrease in volume over only one year (Fjell et al., 2009). In general, most brain alterations in early AD are also common in healthy aging, but on a smaller scale (Fjell et al., 2009). Equally, Aβ and NFTs are not only present in brains affected by AD, but also to a lesser extent in healthy aging as well as adults with MCI and SCD (Arriagada et al., 1992, Bennett et al., 2005, Wirth et al., 2018a). These changes can still have an impact on memory function, even though they are unrelated to AD (Fjell et al., 2012). Furthermore, brain changes i.a. in temporal and parietal cortices occur many years before diagnosis during preclinical AD (Bernard et al., 2014, Smith et al., 2012, Smith et al., 2007, Saykin et al., 2006). Thus, the boundary between healthy and pathological aging is not clearly definable (Jagust, 2013), and given the life-long ability of the brain

to undergo morphological change or stagnation through cognitive stimulation (Engvig et al., 2010), the boundaries become even more blurred.

1.3 Risk factors and preventive research

The search for new prevention strategies to maintain higher brain function throughout life is an imperative public health goal. Already in healthy aging and early stages of dementia, brain changes are notable but concealed many years before the onset of first cognitive symptoms which empowers the focus on preventive research. As AD is known to be a multifactorial disease, numerous independent but also interrelated risk factors have been identified, some of which are possible starting points for prevention. Among others, these risk factors comprise age, cardiovascular factors (i.a. hypertension, hypercholesterolemia, obesity, diabetes), lifestyle related aspects (dietary patterns, smoking and alcohol, social interactions, physical and cognitive activity, education), genetic factors (i.a. Apolipoprotein E ε4 allele), and other influences such as traumatic brain injuries, sleep and depression (Imtiaz et al., 2014, Baumgart et al., 2015).

While some risk factors such as age and genetics cannot be changed, several studies suggest that modifying unhealthy lifestyle behaviours could have potential positive effects on brain aging and cognitive function (Wirth et al., 2014, Floel et al., 2008, Mattson, 2015, Arenaza-Urquijo et al., 2015, Schwarz et al., 2018b). Lifestyle results from consciously choosing a behaviour which can have a strong influence on body and brain health (Vaynman and Gomez-Pinilla, 2006), which is why changing lifestyle behaviours is a good approach for prevention. In this context, nutrition has increasingly gained attention as an easily modifiable lifestyle factor to protect against age- and disease-related brain changes and maintain cognitive function (Otaegui-Arrazola et al., 2014, Swaminathan and Jicha, 2014).

1.4 Dietary factors

Dietary patterns have been shown to affect cognitive and brain health in both positive and negative ways. On the one hand, previous studies provided evidence that mid-life obesity (through high energy intake) accelerated age-related cognitive decline and increased the risk for dementia in later life (Cournot et al., 2006, Whitmer et al., 2005, Fitzpatrick et al., 2009). Brain structures most susceptible to aging were negatively affected by obesity (Bischof and Park, 2015). On the other hand, dietary or caloric restriction showed beneficial effects. For instance, Witte et al. (2009) found that after a 3-month period of caloric restriction, memory performance in older adults improved. In addition, as a result of caloric restriction, grey matter volume in brain areas

vulnerable to aging increased, hippocampal resting-state functional connectivity to parietal areas was augmented and recognition memory improved (Prehn et al., 2016). Moderate life-long caloric restriction also contributed to longevity and healthy aging (Willcox et al., 2007). However, the benefit of caloric restriction can be limited by the degree and duration of the restriction required (Ingram et al., 2006) and by the risk of malnutrition with several adverse health effects (de Cabo et al., 2014) which is therefore not ideal for elderly and diseased people.

Described as one of the healthiest diets, adherence to a Mediterranean Diet is associated with reduced risk of AD (Scarmeas et al., 2006, Singh et al., 2014) as well as enhanced longevity and delayed onset of age-associated health decline in Mediterranean and non-Mediterranean countries (Kouris-Blazos et al., 1999, Haveman-Nies et al., 2003). Moreover, a lower adherence to a Mediterranean Diet is predictive of greater brain atrophy over a 3-year interval (Luciano et al., 2017). Mediterranean Diet is defined by the abundant intake of plant food such as vegetables, fruits, legumes, nuts, and cereals. Further, it comprises olive oil as the main source of fat, daily low to moderate consumption of dairy products (especially yoghurt and cheese), low to moderate intake of fish, poultry and eggs, low to moderate wine consumption (accompanying meals), and a low amount of red meat (Willett et al., 1995).

There were some compounds identified, which share beneficial traits with the aforementioned caloric restriction, called “caloric restriction mimetics”, such as polyamines. The positive aspect is that they mimic the calorie-reducing effects without the actual reduction of caloric intake and thus represent an easier and safer approach to everyday life. Moreover, high amounts of polyamines are contained in most foods in the Mediterranean Diet, such as vegetables and fruits (Binh et al., 2011).

Polyamines as novel candidates combining beneficial effects of caloric restriction and Mediterranean Diet have gained attention lately.

1.5 Polyamines

Natural polyamines, present in all living organisms, are important agents in cell growth, proliferation and survival (Pegg, 2016, Bardocz et al., 1995, Igarashi and Kashiwagi, 2010). Polyamines are organic polycations, positively charged aliphatic amines, interacting with negatively charged molecules like deoxyribonucleic acid, ribonucleic acid, proteins and phospholipids thus stabilising their structure (Carter, 1994). Further, polyamines have antioxidative and enzyme modulating functions, and exert diverse roles in protein synthesis, cell reprogramming, and autophagy regulation (Pegg, 2016, Bardocz et al., 1995, Igarashi and

Kashiwagi, 2010) and have also been described to be involved in learning and memory (Guerra et al., 2016). Thus, the variety of polyamines' essential cellular interactions and functions suggest a complex and significant role in controlling life and death in cells (Minois et al., 2011). The diamine putrescine and the polyamines spermine and spermidine are involved in the very strongly regulated polyamine metabolic pathway (Figure 2), physiological concentrations being required for optimal cell growth and function (Wallace et al., 2003). Exogenous food intake, endogenous/cellular biosynthesis, and microbial synthesis in the intestines are the three main sources for sustaining polyamine levels in organisms, in combination with its catabolism and urinary excretion, the polyamines' concentration is determined (Madeo et al., 2019).

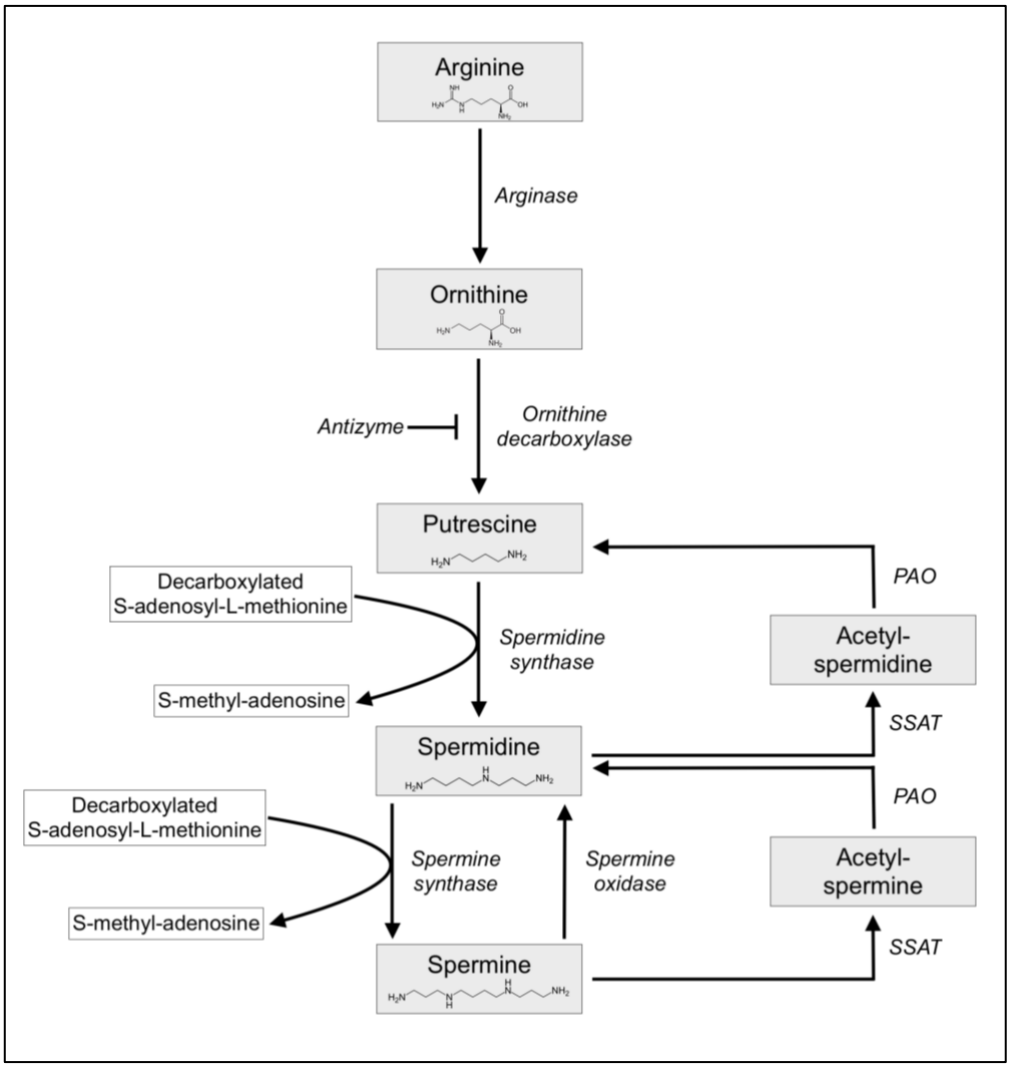

Figure 2: The polyamine pathway
The figure shows the de novo polyamine synthesis and catabolism. By the mitochondrial arginase, arginine is synthesized to ornithine. Ornithine decarboxylase decarboxylates ornithine to putrescine. Decarboxylated S-adenosyl-L-methionine is the aminopropyl group donor to putrescine for the synthesis of spermidine by spermidine synthase and/or to spermidine for the synthesis of spermine by spermine synthase. Spermidine and spermine can be reconverted to spermidine and putrescine, respectively, by being converted to acetylated spermidine/spermine by spermidine/spermine-N1-acetyltransferase (SSAT) then oxidized by polyamine oxidase (PAO) to spermidine/putrescine. Another mechanism of spermine's back-conversion to spermidine is by spermine-oxidase. Figure and legend based on Minois et al. (2011).

Polyamines are present in most foods but concentrations differ considerably (Bardócz et al., 1993, Cipolla et al., 2007). Especially fruit, vegetables, legumes, cereal products, and meat are the main sources for polyamines (Atiya Ali et al., 2011, Zoumas-Morse et al., 2007). Cheese and other products obtained by microbial fermentation such as sauerkraut and soya products (e.g. natto) contain high amounts of polyamines, as do i.a. wheat germ, green pepper, cauliflower, broccoli, and mushrooms (Bauza et al., 2007, Nishimura et al., 2006, Kiechl et al., 2018). In European countries, the average estimated polyamine intakes were about 211.910 nmol/day putrescine, 86.959 nmol/day spermidine, and 54.704 nmol/day spermine (Ralph et al., 1999, Buyukuslu et al., 2014). The amount of polyamine intake is strongly influenced by dietary pattern differences between cultural, educational and socio-economic backgrounds. Interestingly, it has been shown that maintaining a spermidine-rich diet is associated with increased health and life span (Kiechl et al., 2018).

However, intracellular polyamine levels of several organs, including serum, were shown to decrease in many aging model organisms and humans (Scalabrino and Ferioli, 1984, Gupta et al., 2013, Das and Kanungo, 1982, Pucciarelli et al., 2012, Vivo et al., 2001, Eisenberg et al., 2009), one reason being that the polyamine-producing enzymes' biosynthetic activities decline with age (Das and Kanungo, 1982). The idea of externally supplementing these essential polyamines is therefore an obvious approach to counteract their decline.

The external supply of polyamines through food or liquids increases their endogenous levels. For example, Soda et al. (2009b) showed that a polyamine-enriched diet increased blood spermine level in mice (26 weeks) and humans (2 months). They could furthermore show that a long-term daily intake of 50 to 100g of natto (a traditional Japanese polyamine-rich fermented soybean product) for 2 months increased spermine blood levels in healthy humans. Eisenberg et al. (2009) observed an increase in intracellular spermidine concentrations in aging cells of yeast, flies, and mouse liver through spermidine supplementation.

Not only were polyamine levels shown to increase through dietary polyamine interventions, but other protective effects were also shown to be promoted. For example, mice fed with high-polyamine level chow showed lower mortality than those fed with low-polyamine chow in the first 88 weeks (Soda et al., 2009a). In addition, it was shown that polyamines exert a causative role in promoting longevity through autophagy induction, epigenetic modification, and necrosis suppression, in worms, flies, yeast, and in human immune cells (Eisenberg et al., 2009) and mice (Yue et al., 2017, Eisenberg et al., 2016).

1.5.1 Polyamines and cardiovascular health

As pointed out, polyamine levels decrease with age and the risk of cardiovascular diseases and death increases. In old mice, the oral administration of spermidine preserved diastolic function and reduced cardiac hypertrophy (Eisenberg et al., 2016). Cardioprotective aspects could also be shown in humans: reduced blood pressure and a lower risk for cardiovascular diseases (CVD) were associated with higher spermidine intake through daily nutrition, assessed through a food frequency questionnaire (Eisenberg et al., 2016). Furthermore, nutritional polyamines were negatively associated with CVD-caused mortality rates (Soda et al., 2012). Spermidine's cardioprotective effects may result from its autophagy and mitophagy induction, thus improving cardiomyocyte and mitochondrial function (Eisenberg et al., 2016) as well as from anti-inflammatory actions (Soda, 2010). In addition, increased polyamine intake has been shown to support the maintenance of vascular health by suppressing de novo polyamine synthesis from arginine. Therefore the amount of arginine increases and is available for synthesis of nitric oxide, important for normal vascular function preservation (Drexler et al., 1991, Cooke et al., 1992). These findings suggest that an increase in dietary polyamine intake may help decelerate the progression of CVD, which in turn might have a positive effect on higher-order brain function.

1.5.2 Polyamines, brain and cognitive health

Age-related decrease of the polyamine level can also be observed in the brain. Spermine and spermidine levels decreased in human basal ganglia during aging (Vivo et al., 2001). Moreover, Liu et al. (2008) also observed age-related polyamine concentration changes in different memory-associated brain regions in rats. In addition, hippocampal polyamine concentrations were shown to be linked to memory retrieval and formation in mice (Tiboldi et al., 2012). As polyamines are involved in neurogenesis in adult rodent brains (Malaterre et al., 2004), their decrease may contribute to the impairment in brain neurogenesis during aging. Furthermore, polyamine levels decreased in aging Drosophila brains, which was accompanied by a decline in memory (Gupta et al., 2013). Moreover, metabolism of polyamine and arginine were found to be disturbed in AD mouse models (Pan et al., 2016) and MCI patients (Graham et al., 2015).

Recent studies have shown beneficial effects of polyamine interventions on brain health. In mice, spermidine supplementation was shown to diminish frontotemporal lobe dementia based on autophagy inducement (Wang et al., 2012). The protection from age-induced memory impairment in old mice was achieved by an oral administration of arginine, thus up-regulating the colonic polyamines (Kibe et al., 2014). Moreover, 30 days of spermidine feeding restored the brain spermidine levels and protected memory from age-dependent impairment in aging fruit flies

(Gupta et al., 2013, Sigrist et al., 2014). The autophagic action of spermidine reduces specific proteins in the presynaptic active zone, which decreases the release of synaptic vesicles, essential for synaptic plasticity and flexibility (Gupta et al., 2016). Thus, synaptic dynamics are restored, which are increasingly restricted in old age and lead to memory deterioration (Madeo et al., 2018). In addition, in a 3-month placebo-controlled intervention trial spermidine supplementation, gained from a spermidine-rich plant extract (dosage: 1.2 mg/day), was shown to be safe and well-tolerated (Schwarz et al., 2018a) and enhanced hippocampus-dependent memory performance compared to the placebo group in older adults with SCD (Wirth et al., 2018b).

In summary, these findings suggest the involvement and neuroprotective effects of polyamines on brain and cognitive health.

1.6 Main questions and hypotheses

As the world population ages and grows, chronic age-related pathologies such as AD become more prevalent and represent a major psychological and social challenge as well as an economic burden that urgently requires appropriate action (de Cabo et al., 2014, Takizawa et al., 2015).

Well-functioning autophagy is a requirement for healthy aging. However, it is impaired in neurodegenerative diseases like AD. The autophagy enhancing polyamines suggest a promising protective dietary approach to promote brain and cognitive as well as cardiovascular health, as most thoroughly described in animal models.

However, the role of polyamines in brain health and cognition in humans needs to be better comprehended. Structural neuroimaging markers, such as brain atrophy in the hippocampus and cortical thickness, are sensitive to changes during healthy and pathological brain aging which allows us to determine whether dietary patterns are associated with age-related brain and cognitive changes.

In addition, dietary patterns strongly influence the daily polyamine intake and increased amounts of polyamines were described in Mediterranean food. To which extent these findings are replicable with a questionnaire of Mediterranean Diet adherence, should be further elucidated. Moreover, enlarging the scope of dietary health with a general healthy diet questionnaire would gain another quality check for the reliability of the food frequency assessment for polyamine intake used in the present study.

The current study aimed to investigate the association between self-reported dietary polyamine intake, assessed through a food frequency questionnaire, and brain, cognitive, and cardiovascular health as well as other well-known health promoting dietary habits in cognitively unimpaired older adults.

Four main hypotheses contribute to the framework of this study:

1. Dietary polyamine intake is associated with further health promoting diets such as a Mediterranean Diet and a generally healthy diet.
2. Higher polyamine intake is associated with larger hippocampal volume and structural brain measures such as cortical thickness and volume, i.a. in AD vulnerable areas, paralleled by a better cognitive performance.
3. Polyamine intake shows different associations with volume in individual hippocampal subfields, as they are differently affected by AD.
4. Higher polyamine intake is associated with a lower cardiovascular risk profile.

As the main risk factor for AD is progressive age, it is difficult to distinguish age-related changes from effects of preclinical disease (De Flores et al., 2015) and the boundary between healthy aging and neurodegeneration is still obscure (Jagust, 2013). In combination with the fact of this being the first analyses of dietary polyamine intake and brain structure in humans, these analyses were of exploratory nature.

2 MATERIAL AND METHODS

2.1 Study design

The present research was created within the ongoing SmartAge trial, a proof-of-concept study that investigates the effect of polyamines on memory performance and other health parameters through a 12-month supplementation in individuals with SCD (monocentric, double-blind, randomized and placebo-controlled phase IIb trial) (Wirth et al. (2019); ClinicalTrials.gov: NCT03094546, registered 29 March 2017). For baseline analyses, healthy controls (HC) were also recruited. Assessments took place on two different days. On the first visit, participants underwent an on-site screening and standardized medical as well as neuropsychological examination at the NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, Germany. On a second visit, a 3-Tesla cerebral magnetic resonance imaging (MRI) was conducted at the Berlin Center for Advanced Neuroimaging (BCAN, Charité - Universitätsmedizin Berlin). Questionnaires on diet, lifestyle and cognition were collected, partly on the day of the examination itself, partly by the participants at home. After 12 and 18 months, follow-up visits took place and the participants received an allowance after completing the study. For the present study, only the data acquired at baseline was analysed in a cross-sectional design.

The SmartAge trial was approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin and conforms to the declaration of Helsinki. Furthermore, it was compiled in accordance with the charter of the Charité – Universitätsmedizin Berlin to ensure good scientific practice. Before participating in the study, all participants provided written informed consent.

2.2 Study participants

At baseline, this study consisted of two different diagnostic groups, older adults with SCD and HC with no SCD. Study participants were recruited from memory clinics, neurologists and cooperating resident physicians as well as from the general German population through flyer, newspaper and internet advertisements. The possible study participants were pre-screened for inclusion and exclusion criteria, conducted over the telephone (supplementary material Table S12).

Fluent German-speaking older adults aged between 60 and 90 years without objectively measurable cognitive abnormalities were included. They had to be able to provide written informed consent and to be covered by health insurance in case incidental diagnosis throughout the study examination were found.

For the SCD-group, diagnostic criteria were:

- the expression of subjective cognitive complaints for at least 6 months
- related self-reported worries
- previously consulted or considered consulting a doctor due to these cognitive concerns
- normal cognitive performance (no neurological diagnosis, not even MCI)
- non-restricted activities of daily living

These criteria were in accordance with the established framework of Jessen et al. (2014a). With regard to the planned intervention, individuals with current polyamine intake and/or intolerance/allergies to wheat germs, gluten or histamine were excluded.

HC were included when subjects experienced no or age-appropriate subjective cognitive worsening with no explicit related worries, showed normal cognition and no functional impairment.

Exclusion criteria for all study participants comprised neurological diseases such as dementia, MCI, clinically manifest stroke, and epilepsy. Further exclusion criteria for study enrolment were severe or untreated diseases (advanced cardiac or respiratory disease, severe liver, kidney or metabolic diseases, untreated thyroid dysfunctions, or untreated diabetes mellitus), psychiatric disorders (untreated depression, psychosis), malignancies (current or on medical history), findings of brain pathologies, and MRI-contraindications (claustrophobia, metallic implants (e.g. intracranial metal clips), electronic devices (e.g. cardiac pacemakers) or permanent tattoos and make-up). Furthermore, subjects were excluded in cases of drug abuse and/or alcohol dependency and use of medication affecting neuropsychological performance.

If the potential subjects met all of these eligibility criteria captured in the telephone screening, they were invited to the first visit at the Charité – Universitätsmedizin Berlin. When informed written consent was provided on site, they were included in the SmartAge study and continued with the on-site screening.

At the time of analysis, 104 individuals, 53 SCD and 51 HC participants, were recruited and had provided informed consent. For the present analyses, the two diagnostic groups (SCD and HC) were taken together since both groups included older adults with normal cognition. At the first visit, 3 failed the on-site screening (described below) and were then excluded from the following examinations. Among the remaining 101 participants who continued with the study assessment, 1 participant could not provide blood samples, 1 participant withdrew from the study after the first visit and 4 participants could not provide MRI-data, due to missed MRI exclusion criteria or MRI processing problems. At the end, analyses included 101 older participants with cognitive data, 100 with dietary and cardiovascular information and 96 with MRI scans (Figure 3).

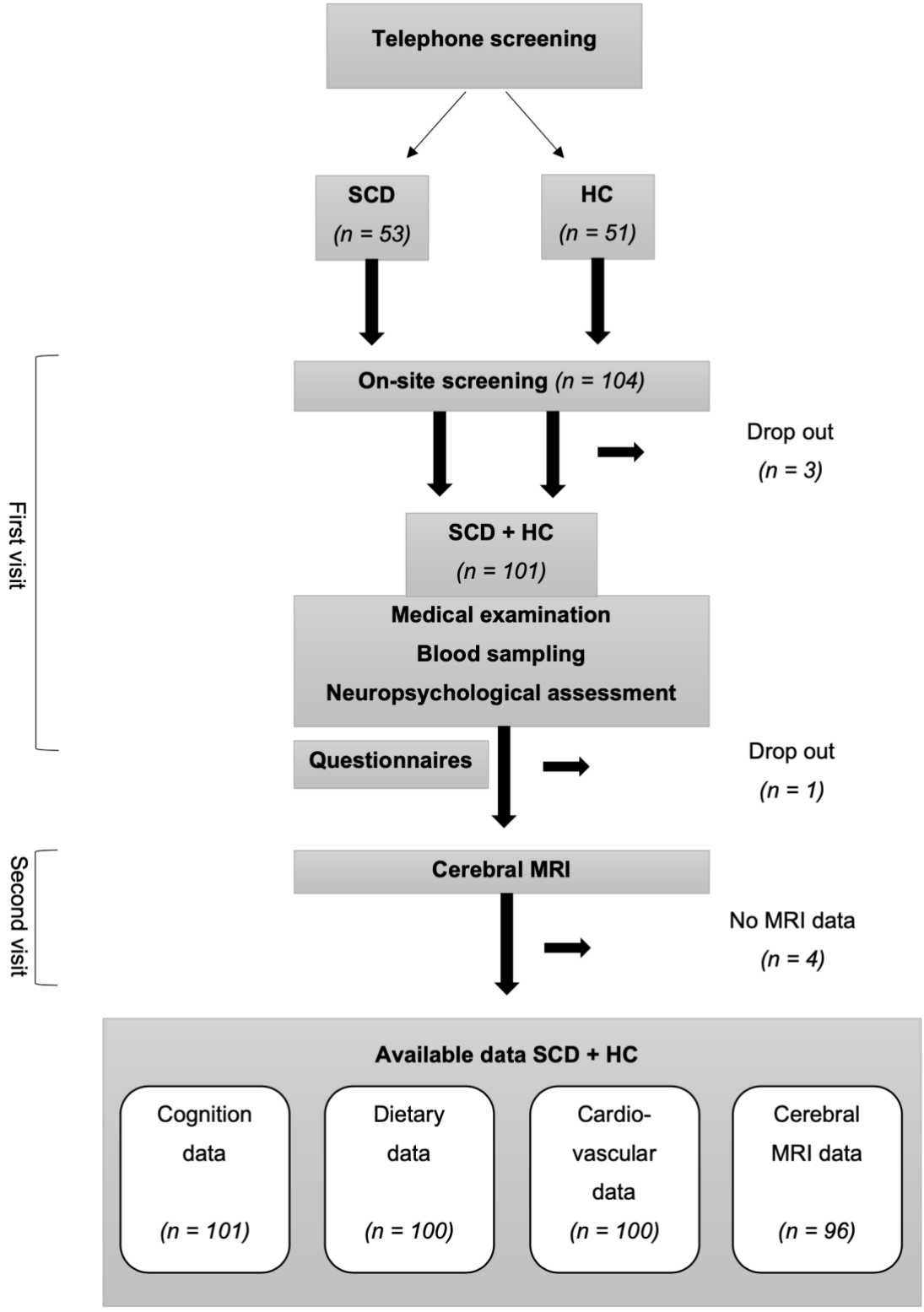

Figure 3: Study design and sample size
SCD: subjective cognitive decline group; HC: healthy controls group; MRI: magnetic resonance imaging. In total 104 individuals, 53 SCD and 51 HC participants, were recruited through telephone pre-screening. On the first visit, however, 3 failed the on-site screening, thus 101 participants underwent medical and neuropsychological examination and on the second visit a cerebral MRI was planned (n = 100; 1 drop out due to study withdrawal after first visit). However, 1 participant could not provide blood samples, 1 participant withdrew from the study after the first visit and 4 participants could not provide MRI-data. In the end, 101 participants remained with cognitive data, 100 with dietary and cardiovascular information, and 96 with MRI scans.

2.3 On-site screening

To ensure the absence of objective cognitive impairment and psychiatric disorder such as depression, a standardized on-site screening including the following neuropsychological tests and questionnaires was assessed (Wirth et al., 2019):

- **Mini-Mental State Examination** (MMSE) (Folstein et al., 1975) is a clinical screening procedure for dementia and Alzheimer's Disease. It examines central cognitive functions and study participants had to achieve at least 26 out of 30 points.
- **Logical Memory IIa subtest** from the Wechsler Memory Scale (LMS) (Wechsler, 1987) includes two stories A and B which the tester reads aloud and the participants must repeat and then memorize the content of the read story. After 20-30 minutes the stories are to be recalled (delayed recall), for each remembered and reproduced content, they get one point. A performance above -1.5 standard deviation (SD) of age-adjusted norms in the Logical Memory II subscale total delayed recall (Story A and B) must be achieved.
- **Trail Making Test** (TMT) (Reitan, 1955) is described in detail under 2.5 Neuropsychological assessment. For a successful on-site screening a performance in the TMT Part A above -1.5 SD of age-adjusted norms had to be achieved too.
- **Instrumental Activities of Daily Living Scale** (IADL) (Lawton and Brody, 1969) is a questionnaire used for detecting restrictions in everyday life. Participants must have no deficits on selected items of the IADL.
- **Geriatric Depression Scale** (GDS) (Yesavage et al., 1982), short version, is a questionnaire facilitating the detection of depression within 15 yes-or-no questions about the present mental mood state of the participants. In this study, a score > 10 points, indicating severe depression, lead to exclusion.

If the on-site screening was successfully completed, the participants continued with the baseline examinations.

2.4 Medical history and examination

Subjects underwent a medical history assessment (i.a. demographics, family history, current medication intake) and a routine medical examination including measures of systolic and diastolic blood pressure and heart rate at rest, weight and body height. A standardized internal and neurological examination was carried out to identify any previously undiscovered diseases or to quantify and document known disorders. In addition, biographical information such as profession and education was collected. Subsequently, fasting venous blood samples were taken for

scientific as well as diagnostic means. Various blood parameters e.g. haematological parameters, clinical chemistry, vitamin B12 metabolism were analysed by Labor Berlin according to standardized procedures (Schwarz et al., 2018a).

2.5 Neuropsychological assessment

Neuropsychological examination, conducted by experienced testers, included validated paper-and-pencil and computer-based neuropsychological tests as well as behavioural questionnaires. The following description of the neuropsychological test battery is limited to those relevant for the analyses of the present study. Extensive detail of the complete test battery is provided elsewhere (Wirth et al., 2019).

- The **Stroop interference test** (Bäumler and Stroop, 1985) consists of three subtests, measuring elementary information processing capabilities as selection, coding and decoding. Nomination, selectivity, alertness and reading speed are measured, considering the concentrative stress, so that sensorimotor speed and executive function can be calculated through the Stroop interference test. For the first subtest (Stroop A), the participants should read aloud the 72 black printed colour words on the card ("RED", "GREEN", "BLUE" and "YELLOW"). The second subtest (Stroop B) is implemented by naming the colour of the 72 represented blocks of colours on the card. For the third test (Stroop C), the interference test, the colour words are printed in a colour, which differs from the colour word itself (e.g. the word "BLUE" would be printed in green). The participants should read aloud the written colour word, ignoring the colour of the printed text, so they need to concentrate on resisting dominant reaction tendencies or the tendency to interfere. The time for each subtest is documented and mistakes are directly pointed out to the participants by the investigator.

- The German version of the **auditory verbal learning test** (AVLT) (Helmstaedter et al. (2001), verbaler Lern- und Merkfähigkeitstest), is a test for serial list learning that measure the declarative verbal memory in a learning paradigm. The AVLT consists of two different word lists, each including 15 independent words, and a recognition list containing the words of the two lists plus 20 further independent distractor words (total=50). The investigator reads the 15 words of the first list, which have to be immediately recalled by the participants. This happens in 5 consecutive trials (sum of correctly remembered words, learning). Then the second list, the interference list, is read and needs to be immediately recalled too. In the following, the participants have to recall again the 15 words of the first list and again after a 20-30 minutes' delay (delayed recall). Subsequently, the investigator

reads aloud the words of the recognition list (50 words) and the participants need to recognize the 15 words of the first list (recognition trial).

- The **TMT** (Reitan, 1955) is sensitive to impairment in multiple cognitive domains, such as executive function, working memory, task-switching, task inhibition and visuo-motor speed. It contains two parts, A and B. Part A consists of 25 circles randomly distributed on a sheet of paper. Under time measurement the participants need to draw lines to connect the circles, numbered 1 to 25, in ascending order, without lifting the pencil from the paper. In part B, some circles include numbers (1-13) and others include letters (A-L), which, as in part A, should be connected in an ascending pattern, but additionally alternating between the numbers and the letters (e.g. 1-A-2-B-3-C, etc.). Mistakes are indicated directly. The time and the errors are documented for each part by the investigator. The test is terminated after 150 seconds for part A, and after 5 minutes for part B.

- The **digit span test** (Wechsler, 1981) enhances both short-term and working memory performance as well as attention. It consists of two parts, a forward and a backward recognition part. The investigator reads aloud a digit sequence of growing length (maximum: 9 digits); it follows an immediate playback of the digit sequence by the participants (each trial consisting of two sequences of the same length). The test is aborted when the participant fails two consecutive times at the same level of difficulty. The number of correctly recalled sequences is summed. The second part is the same, except that the series of numbers must be reproduced backwards.

- The **digit symbol test** (Wechsler, 1981) tests the executive function, consisting of a list of 9 digit-symbol pairs (e.g. 1 and –, 2 and ⊥, etc.)**.** It is followed by a list of digits and under each digit the participants need to write down the corresponding symbol as fast as possible, within a specified time (90 seconds). The investigator calculates the sum of the correct corresponding symbols.

- The **verbal fluency test** (Morris et al., 1989) is used to measure language and executive function by evaluating phonemic and semantic fluency. Within 60 seconds, the subjects must name as many independent words starting with the given initial letter P. In the semantic part, participants need to name as many words of the same semantic category (animals) within 60 seconds. The investigator counts the number of correct unique words.

2.6 Questionnaires

Several questionnaires on diet, lifestyle, behaviour, and cognition were assessed. In the following, only the dietary questionnaires, relevant for the present study will be described in detail.

2.6.1 Food frequency questionnaire

The dietary polyamine intake was assessed through the food frequency questionnaire (FFQ) based on the gold-standard FFQ by Willett et al. (1985) and Eisenberg et al. (2016) including 89 food-items. A standard unit or serving size was specified for each item and participants had to fill in how often, in the past 12 months, they had consumed each listed item. The intake frequency was operationalized by 9 response categories which ranged from “never or <1 time/month” to “≥6 times/day” (0-8) (supplementary material: Table S13).

The FFQ’s validity and reproducibility are well reported in multiple large population-based cohorts (Willett et al., 1985, Eisenberg et al., 2016, Kiechl et al., 2018). They showed that the nutrition patterns stayed highly stable over time, which indicates that these calculated (surveyed) intakes reflect an average long-term dietary intake adequately.

The calculation of nutrient intakes was implemented in collaboration with Bruneck Study researchers using the same method applied in former studies (Kiechl et al., 2018, Eisenberg et al., 2016): Long-term average daily food portion intakes were multiplied by nutrient (i.a. polyamine) content of food per portion to obtain long-term average daily nutrient intake. Data on nutrient contents of foods were taken from the same database used previously by Kiechl et al. (2018) based on the United States Department of Agriculture database (U.S. Department of Agriculture, Agricultural Research Service, 2010, USDA National Nutrient Database for Standard Reference, Release 23) augmented by manually compiled data on polyamine content of foods from prior reports (Nishimura et al., 2006, Kalač et al., 2005, Kalač and Krausová, 2005, Kalac, 2009, Eliassen et al., 2002, Atiya Ali et al., 2011) (online supporting material of Kiechl et al. (2018): supplemental table 4 - Nutrient Database for Polyamine Intake).

To estimate nutrition intake (i.a. polyamine intake), weighted sums of all contributing foods were calculated by multiplying the frequency (e.g. 1 time/day equals a weight of 1) and the nutrient content of each item. Polyamine intake was given in nmol/day. To avoid confounding by total caloric intake, polyamine intakes were adjusted for caloric intake by forming the ratios of polyamine with total caloric intake for each subject (Willett and Stampfer, 1986). For further calculations, the ratio was used in log10-transformed form.

2.6.2 Mediterranean Diet Adherence Screener

The Mediterranean Diet Adherence Screener (MEDAS) is a 14-question, self-reported questionnaire analysing food consumption frequency and intake habits, which are characteristic for a Mediterranean Diet (Schröder et al., 2011). The examiners of the current study translated the questionnaire into German (supplementary material: Table S14), which is similar to the validated German version of Hebestreit et al. (2017). The items were coded with 0 or 1 for each question so that 1 is assigned for each favourable food component of a Mediterranean Diet. The MEDAS score was calculated for each participant by summing the items of the questionnaire, the score ranging from 0 to 14. Consequently, a higher MEDAS score indicates a better adherence to a Mediterranean Diet.

2.6.3 Qualitative food frequency list

The participants were asked to fill in the average frequency of consumption of 37 different foods and their dietary habits based on a short, qualitative food frequency list (FFL) used in several large-scale surveys in Germany (Floel et al., 2008, Winkler and Döring, 1998). The 6 frequency categories ranged from “every day or almost daily” to “never”, while for the use of oils and fats the 3 frequency categories ranged from “regularly” to “not at all” (supplementary material: Table S15).

Out of this information a diet score was created which ranged from 20 to 127 points (Floel et al., 2008). The consumption of the following food items was included in score calculation: meat, processed meat, poultry, fish, potatoes, pasta, rice, cooked vegetables, chocolates, cakes and cookies, sweets, salad and raw vegetables, fresh fruits, wholemeal bread, skimmed milk, fruit juice, mineral water, as well as salt eating habits and the use of oil and fats. The frequency categories were coded so that, for example, the daily intake of healthy food was coded as 6 and the daily intake of unhealthy food as 1. Thus, higher scores indicate a healthier diet (rich in raw vegetables and fruits; moderate sheer meat; low-fat, low-sugar and wholemeal products; fish, oils and margarines high in unsaturated fatty acids and sensible salting habits), while lower values represent unhealthier behaviour (rich in fatty products, sweets, soft drinks, processed meat, butter and salt, and low in vegetables and fruits).

2.7 Cerebral Magnetic Resonance Imaging

2.7.1 MRI acquisition

MRI scans of the head of every study participant were performed on a 3-Tesla MRI scanner using a 12-channel head coil (Siemens Magnetom Trio, Erlangen, Germany) at the Berlin Center for Advanced Neuroimaging (BCAN, Charité - Universitätsmedizin Berlin). For structural analysis, a 3-dimensional T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence was acquired (orientation = sagittal, repetition time = 1900 ms, echo time = 2.52 ms, inversion time = 900 ms, field-of-view = 256×256 mm^2, matrix = 256x256, 192 slices, 1 mm isotropic voxels, flip angle = 9 degrees).

2.7.2 Pre-processing of MRI measurements

The structural images were examined visually for quality and the presence of artefacts, and were then evaluated using the software packages FSL V.5.0 (FMRIB Software Library, Oxford, UK, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) and FreeSurfer V.6.0 (Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, USA, http://surfer.nmr.mgh.harvard.edu).

The processing of the images run on a Linux Server and each step was scripted, so that the processing pipeline is easily reproducible. The MPRAGE sequence provided a T1-weighted anatomical image in DICOM format (Digital Imaging and Communications in Medicine). Then it was converted to the required 4D analysis format NIFTI (Neuroimaging Informatics Technology Initiative). Subsequently, using FSL, the T1-weighted images were reoriented from LPS (x-left, y-posterior, z-superior) orientation to LAS (x-left, y-anterior, z-superior) orientation, remaining in native space.

The re-oriented native-space T1-weighted images were processed using FreeSurfer to estimate subcortical and cortical volume as well as cortical thickness. An automated stream (“recon-all”) run to perform the FreeSurfer cortical reconstruction process, including skull stripping by using a watershed algorithm, brain mask creation, subcortical segmentation, automatic cortical parcellation, and pial surface segmentation (more technical details are described in prior publications e.g. Fischl et al. (2002)). Results of the automated stream were overlaid on anatomic images for visual inspection to assure an error-free registration and region-of-interest identification (Figure 4).

For 3 MRI images, the automated stream of FreeSurfer was not able to generate the main output of recon-1 (= brain mask creation), probably due to skull stripping problems. Therefore, manual

correction was applied on these images. As skull and dura interfered with surface formation, they must be erased. For that step, the brain extraction tool (BET) was applied, generating a skull stripped mask. Its borders were visually checked using FSLview. When there was no clear separation between the BET mask and the skull of the T1-weighted image, the mask was edited by manually erasing the connection. Subsequently a subtraction of the T1-weighted image and the new manually modified mask was performed, thus creating a new brain mask for the automated FreeSurfer stream so that the further steps (recon-2 and recon-3) could be conducted. This procedure solved 2 of the 3 cases. One image remained with defects that FreeSurfer was unable to process and could therefore not be used for further volume and thickness analyses.

Subsequently, quality checks of the FreeSurfer outputs were performed and then volume as well as cortical thickness data were extracted by using the graphical interface of FreeSurfer, Qdec.

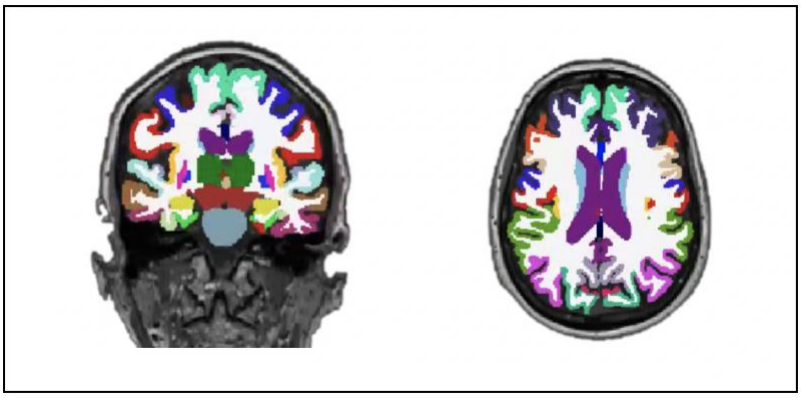

Figure 4: FreeSurfer segmentation
Output of the automated stream of FreeSurfer overlaid on anatomic images in FSLview is shown.

2.7.3 Volume analyses

The main region-of-interest for volume analyses was the hippocampus. Moreover, total grey matter volume (TGMV) was also of interest as a global volume measure. Therefore, the automatic subcortical segmentation (aseg) based on an atlas, which contains probabilistic information on the location of structures described by Fischl et al. (2002) was used (http://freesurfer.net/fswiki/SubcorticalSegmentation).

For explorative regional cortical volume analyses, an automated cortical parcellation (aparc) based on the included “Desikan-Killiany” gyral atlas (Desikan et al., 2006) was performed to subdivide the cerebral cortex into gyral-based regions-of-interest (https://surfer.nmr.mgh.harvard.edu/fswiki/CorticalParcellation).

The individual “Desikan-Killiany” parcellations were relabelled into the lobes assigning a set of gyri to each lobe, averaging volume values across hemispheres:

- **Frontal:** sum of superior frontal, rostral and caudal middle frontal, lateral and medial orbitofrontal and frontal pole, pars opercularis, triangularis and orbitalis, but excluding precentral and paracentral gyrus;
- **Parietal**: superior and inferior parietal, supramarginal and precuneus gyrus, but excluding the postcentral one;
- **Temporal**: superior, middle and inferior temporal gyrus, fusiform, transverse temporal, entorhinal and parahippocampal gyrus, banks of the superior temporal sulcus, temporal pole;
- **Cingulate**: rostral and caudal anterior frontal gyrus, posterior and isthmus parietal gyrus.

Consistent with former studies (Wirth et al., 2017), the following 5 neuroanatomical regions-of-interest preferentially affected in AD (Mattsson et al., 2014, Reiman et al., 2009, Wirth et al., 2013, Lacalle-Aurioles et al., 2014) were combined to create an AD-vulnerable template (Figure 5) for structural brain analyses: the precuneus gyrus, the isthmus of the cingulate gyrus, the entorhinal gyrus, the inferior parietal gyrus, and the inferior temporal gyrus.

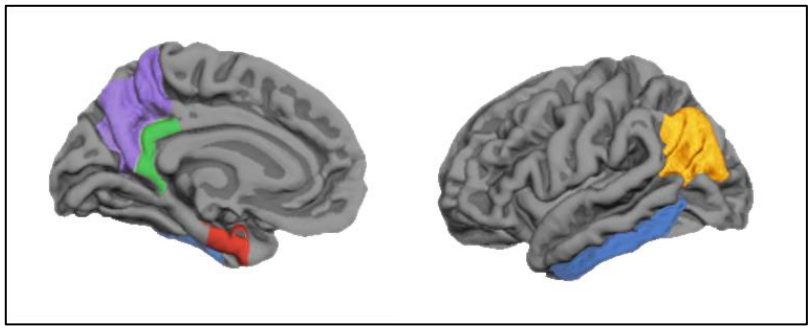

Figure 5: AD-vulnerable template
Purple: precuneus; Green: isthmus of the cingulate gyrus; Red: entorhinal; Blue: inferior temporal; Yellow: inferior parietal. Alzheimer’s Disease (AD) vulnerable regions-of-interest are projected on the 3-dimensional grey matter surface of the left hemisphere. The template is based on Wirth et al. (2017).

For adjustments in head size differences across study participants, each volume measure (mm^3) was adjusted by estimated total intracranial volume (eTIV), supplied by FreeSurfer (Buckner et al., 2004) using a simple ratio calculation, then multiplying it by 100 (Figure 6).

$$\textbf{ROI} = \frac{\text{ROI [volume]}}{\text{eTIV}} * 100 \qquad \text{or} \qquad \textbf{LOBE} = \frac{\text{ROI}_1\text{ [volume]} + \text{ROI}_2\text{ [volume]}}{\text{eTIV}} * 100$$

Figure 6: Volume adjustment by estimated total intracranial volume
ROI: region-of-interest; eTIV: estimated intracranial volume.

An automated hippocampal subfield segmentation was carried out according to Iglesias et al. (2015) to extract individual hippocampal subfield volumes. Therefore, a whole brain T1-weighted scan was included, which had been analysed with the main FreeSurfer stream ("recon-all") (https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala) (Figure 7). The hippocampal subfields of interest were the subiculum, the cornu ammonis (CA) fields 1-4 (CA2 is always combined with CA3, due to unclear contrast), the dentate gyrus (DG), and the hippocampal tail. DG and CA4 were added together. Both hemispheres were averaged and each subfield (mm^3) was adjusted by eTIV, supplied by FreeSurfer, using a simple ratio calculation to correct for head size differences across study participants (Figure 6).

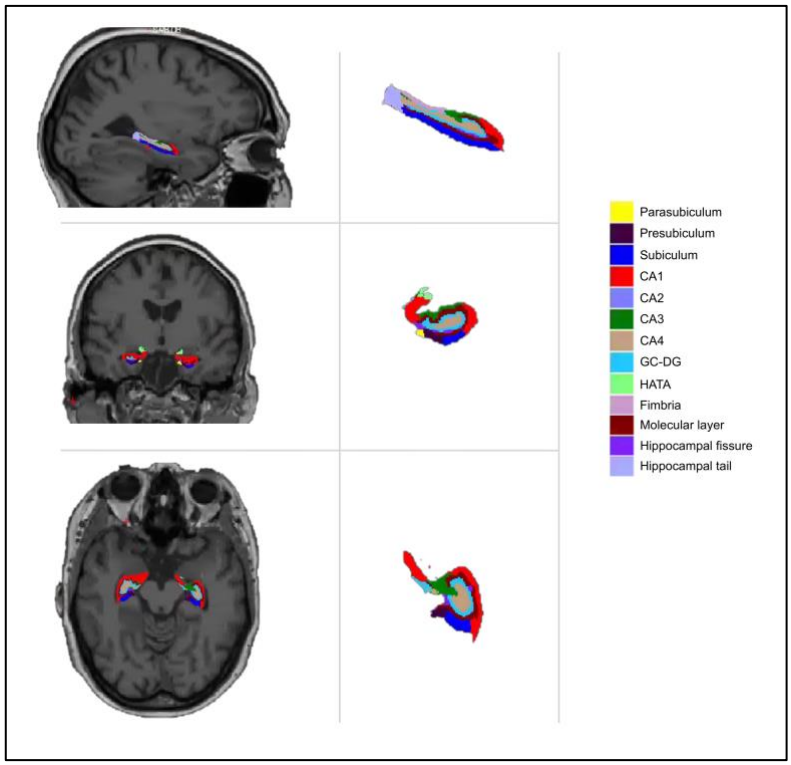

Figure 7: Hippocampal subfield segmentation
CA: Cornu Ammonis; DG: gyrus dentatus; GC: granule cell layer; HATA: hippocampal-amygdaloid transition region. T1-weighted images and magnified views of hippocampal subfields in the sagittal, coronal, and axial planes are shown. The hippocampus was automatically segmented according to Iglesias et al. (2015).

2.7.4 Cortical thickness analyses

Cortical thickness (mm) was calculated using the automated cortical parcellation data (aparc) and averaging the distance between the grey/white boundary and the pial surface. Mean cortical thickness across the whole brain within each study participant was extracted. For cortical thickness analyses, the same individual “Desikan-Killiany” regions-of-interest were used (frontal,

parietal, temporal, cingulate, and the AD-vulnerable template) and combined to the different lobes as for volume measurements (described above 2.5.3) averaging cortical thickness values across both hemispheres. Cortical thickness is only measurable on gryal regions and not on subcortical regions (= no hippocampus data). Cortical thickness is independent of eTIV. The area on the pial surface (mm^2) was measured for each region-of-interest. To take the different region-of-interest surface area sizes into account, weighted arithmetic means were calculated (Figure 8).

$$\text{LOBE} = \frac{(\text{ROI}_1\,[\text{cortical area}] * \text{ROI}_1\,[\text{cortical thickness}]) + (\text{ROI}_2\,[\text{cortical area}] * \text{ROI}_2\,[\text{cortical thickness}])}{\text{ROI}_1\,[\text{cortical area}] + \text{ROI}_2\,[\text{cortical area}]}$$

Figure 8: Cortical thickness adjustment by surface area size
ROI: region-of-interest.

2.8 Cognitive performance evaluation

Cognition was represented by three variables, memory, executive function, and mean performance. For the statistical evaluation of cognitive performance, the scores of the above described neuropsychological tests were standardized by being converted into z-scores (Figure 9), which are generated through calculating the raw value minus the mean of the population divided by the standard deviation of the population.

$$Z = \frac{x - \mu}{\sigma}$$

Figure 9: **Formula calculating standardized z-scores**
z = z-score, x = raw value, μ = mean of the population, σ = standard deviation of the population.

Subsequently, the z-scores were used to calculate the following composite scores according to van de Rest et al. (2008) (Figure 10). The individual composite scores thus summarize the neuropsychological tests that measure the same cognitive function. All cognitive tests are reversed; thus higher scores mean better performance.

Memory =

$$\frac{\text{z AVLT learning} + \text{z AVLT delayed recall} + \text{z AVLT recognition} + \text{z digit span backward}}{4}$$

Executive Function =

$$\frac{\text{z phonemic fluency} + \text{z semantic fluency} + \left(-\text{z TMT}\ \frac{B-A}{A}\right) + \left(-\text{z Stroop C} - \frac{A+B}{2}\right)}{4}$$

Figure 10: Composite scores for memory and executive function
TMT: Trail Making Test; AVLT: auditory verbal learning test. Composite scores were calculated according to van de Rest et al. (2008).

The following composite score "PACC5- Preclinical Alzheimer Cognitive Composite", being sensitive to early pathological changes, was calculated as mean performance according to Papp et al. (2017) and was adapted to the SmartAge testing (Figure 11).

PACC5 =

$$\frac{\text{z AVLT learning} + \text{z LMS total, delayed recall} + \text{z digit symbol} + \text{z MMSE} + \text{z phonemic fluency}}{5}$$

Figure 11: Preclinical Alzheimer Cognitive Composite 5
PACC5: Preclinical Alzheimer Cognitive Composite 5; AVLT: auditory verbal learning test; LMS: logical memory subtest; MMSE: Mini-Mental State Examination. Composite score calculation was based on Papp et al. (2017).

2.9 Cardiovascular risk calculation

Cardiovascular health was assessed using the Framingham risk score for CVD (heart failure, myocardial infarction, coronary insufficiency, haemorrhagic stroke, coronary death, angina, ischemic stroke, transient ischemic attack, peripheral artery disease) estimating the risk (in %) of developing a CVD within the next 10 years, based on the sex-specific multivariable formula of D'Agostino et al. (2008). The following risk factors information was required to calculate the CVD Risk Score: sex (male/female), age, total cholesterol (TotalChol; mg/dl), high-density lipoprotein cholesterol (HDLChol; mg/dl), systolic blood pressure (SysBP; mmHg), antihypertensive medication use (yes/no), current smoking (Cig; self-reported: yes/no) and diabetes mellitus status (DM; insulin or antidiabetic medication: yes/no). Cox regression model of proportional hazards forms the base of CVD risk score assessment. The risk period factor represents the baseline hazard and the risk factor value is a sum of the values of the influencing variables multiplied by the respective estimated regression coefficient β. Continuous variables are logarithmed and dichotomous variables are evaluated as 0 = no or 1 = yes. The use of antihypertensive medication

is taken into account in the formula by adjusting the influence of systolic blood pressure. The CVD risk score was calculated as follows (Figure 12).

Risk Factors =
(ln(Age) ∗ AgeFactor) + (ln(TotalChol) ∗ TotalCholFactor) + (ln(HDLChol) ∗ HDLCholFactor) + (ln(SysBP) ∗ SysBPFactor) + Cig + DM

Risk Score = $1 - \text{RiskPeriodFactor}^{\exp(\text{RiskFactors} - \text{AvgRisk})}$

Variables	Men	Women
Average Risk	23.9802	26.1931
10-year risk (RiskPeriodFactor)	0.88936	0.95012
	Beta Values / Factors	
Age	3.06117	2.32888
Total Cholesterol	1.12370	1.20904
HDL Cholesterol	-0.93263	-0.70833
SBP untreated	1.93303	2.76157
SBP treated	1.99881	2.82263
Smoking	0.65451	0.52873
Diabetes	0.57367	0.69154

Figure 12: Formulas calculating the Framingham Risk Score for cardiovascular diseases
TotalChol: total cholesterol (mg/dl); HDLChol: high-density lipoprotein cholesterol (mg/dl); SysBP: systolic blood pressure (mmHg); Cig: current smoker (yes/no); DM: diabetes mellitus (yes/no); AvgRisk: average risk. 10-year risk score according to D'Agostino et al. (2008).

2.10 Statistical analysis

Data were collected using Microsoft Excel and all statistical analyses were performed using IBM SPSS statistics software package V.25 (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 25.0. Armonk, NY: IBM Corp.). For the statistical evaluation all participants (SCD and HC) were taken together. Normal distribution was verified using the Shapiro-Wilk test, analyses of skewness and kurtosis as well as visual information (scatterplots). Regarding the characteristics of the study population, mean and SD of age, body mass index (BMI), years of education, MMSE, and GDS were calculated for the whole sample.

Linear regressions using general linear models (GLM) (univariate, main effects), were performed to analyse the associations between polyamine intake (spermidine and spermine, independent variables) and dietary, brain, cognitive, and cardiovascular health (dependent variables).

Spermidine and spermine intake were analysed separately and were added as independent variables into the GLMs. As dependent variable the different parameters were added subsequently into the models (every score and every brain region-of-interest separately): dietary health measures (Mediterranean Diet, FFL), brain health (structural brain measures of cortical thickness, cortical and subcortical volume), cognitive health (memory, executive function, PACC5), and cardiovascular health (CVD risk score).

First, unadjusted GLMs with spermidine and spermine intake were performed separately. In a second step, adjusted GLMs were conducted and differentiated between the dependent variables and the selection of the individual confounders, as followed. For **dietary health** only unadjusted GLMs were performed as there were no covariates considered to influence these associations. For **brain health** the following confounding factors were added to the model all at once: age, sex, education, CVD, and group status (SCD or HC); and in a third step Mediterranean Diet as well. GLMs for **cognitive health** included the following confounders: age, sex, and education; and in a further step Mediterranean Diet was added. In the GLMs for **cardiovascular health** only Mediterranean Diet was considered as a covariate, as age and sex are already part of the CVD risk score calculation and the other covariates are not considered to influence the relationship. Categorical variables such as sex and group status were defined as fixed factors in the particular models; the other confounders as independent covariates.

The significance level was set at alpha = 0.05 and the confidence interval at 95%. No correction for multiple testing was conducted, due to the exploratory nature of these analyses. Thus, all analyses have to be understood non-confirmatory.

3 RESULTS

3.1 Demographics

The demographic characteristics of the study population and the average value (± SD) achieved in the MMSE and GDS are shown in Table 1. In total, 50 cognitively normal elderly women (70 ± 6 years) and 51 elderly men (71 ± 6 years) were included in the analyses.

Table 1: Characteristics of study participants

Parameter	SCD + HC
N (women) [n]	101 (50)
Age [years]	70 ± 6
BMI [kg/m²]	26.2 ± 3.9
Education [years]	16 ± 3
MMSE [score]	29.1 ± 0.8
GDS [score]	1.3 ± 1.4

Notes: Data are given as mean ± standard deviation. SCD: subjective cognitive decline group; HC: healthy controls; BMI: Body Mass Index; MMSE: Mini-Mental State Examination; GDS: Geriatric Depression Scale (15-item).

3.2 Dietary health

As shown in Table 2, dietary spermidine intake was associated with adherence to a Mediterranean Diet (B = 4.536, $p < 0.01$, CI = (2.421, 6.652)) and with a generally healthy diet assessed through the FFL (B = 9.191, $p < 0.01$, CI = (3.739, 14.643)). There was an association between spermine and a healthy diet (B = 7.644, $p = 0.029$, CI = (0.818, 14.470)), but no statistically significant relationship with Mediterranean Diet (B = 2.529, $p = 0.071$, CI = (-0.224, 5.282)).

Table 2: Association between polyamine intake and dietary habits

Spermidine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: MeDi							
Step 1	4.536	1.066	4.256	**0.000***	2.421, 6.652	0.156	0.147
2: FFL							
Step 1	9.191	2.747	3.346	**0.001***	3.739, 14.643	0.103	0.093
Spermine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: MeDi							
Step 1	2.529	1.387	1.823	0.071	-0.224, 5.282	0.033	0.023
2: FFL							
Step 1	7.644	3.440	2.222	**0.029***	0.818, 14.470	0.048	0.038

Notes: Step 1: unadjusted, N = 100
General linear models were used to examine the association between polyamine intake and adherence to a Mediterranean and a generally healthy diet. Polyamine intake was adjusted for total caloric intake and log10-transformed. Asterisks indicate $p < 0.05$. MeDi: Mediterranean Diet adherence; FFL: food frequency list, healthy diet; CVD: cardiovascular disease risk; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

3.3 Brain health

3.3.1 Hippocampal and hippocampal subfield volumes

Higher spermidine intake was associated with larger hippocampal volume (B = 0.066, p = 0.008, CI = (0.018 to 0.114)), even after adjusting for age, sex, education, CVD, group, and in a third step for Mediterranean Diet (Table 3). In contrast, there was no statistically significant relationship between dietary spermine and hippocampal volume (all p's > 0.1, all 95% CI's include 0).

Table 3: Association between polyamine intake and hippocampal volume

Spermidine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
Hippocampus							
Step 1	0.066	0.024	2.704	**0.008***	0.018, 0.114	0.072	0.062
Step 2	0.065	0.024	2.666	**0.009***	0.017, 0.113	0.193	0.138
Step 3	0.059	0.027	2.164	**0.033***	0.005, 0.113	0.196	0.131
Spermine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
Hippocampus							
Step 1	0.048	0.030	1.579	0.118	-0.012, 0.109	0.026	0.015
Step 2	0.042	0.030	1.410	0.162	-0.017, 0.102	0.147	0.089
Step 3	0.034	0.031	1.100	0.274	-0.027, 0.095	0.164	0.097

Notes: Step 1: unadjusted, N = 96; Step 2: adjusted for control variables: sex, age, education, CVD and group, N = 95; Step 3: adjusted for control variables AND Mediterranean Diet adherence, N = 95
General linear models were used to examine the association between polyamine intake and hippocampal volume. Polyamine intake was adjusted for total caloric intake and log10-transformed. All volumes were adjusted for total intracranial volume. Asterisks indicate $p < 0.05$. CVD: cardiovascular disease risk; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

Dietary spermidine showed a positive association with CA1 (B = 0.009, p = 0.046, CI = (0.000, 0.018)), the subiculum (B = 0.007, p = 0.036, CI = (0.000, 0.014)), and the hippocampal tail (B = 0.011, p = 0.007, CI = (0.003, 0.020)) (Table 4). Adjustment for sex, age, education, CVD, and group did not attenuate the association substantially, however adding Mediterranean Diet to the model diminished the statistical significance of the association. Spermine intake did not show any statistically significant association with volumes of the hippocampal subfields (all p's > 0.1, all 95% CI's include 0) (Table 5).

Table 4: Association between spermidine intake and hippocampal subfield volumes

Spermidine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: CA1							
Step 1	0.009	0.004	2.023	**0.046***	0.000, 0.018	0.042	0.032
Step 2	0.009	0.004	2.065	**0.042***	0.000, 0.018	0.165	0.108
Step 3	0.008	0.005	1.707	0.091	-0.001, 0.018	0.166	0.099
2: CA3							
Step 1	0.000	0.002	0.183	0.855	-0.003, 0.003	0.000	-0.010
Step 2	0.000	0.002	-0.104	0.917	-0.003, 0.003	0.086	0.023
Step 3	0.000	0.002	0.128	0.899	-0.003, 0.004	0.088	0.015
3: CA4/DG							
Step 1	0.005	0.004	1.269	0.208	-0.003, 0.012	0.017	0.006
Step 2	0.004	0.004	1.042	0.300	-0.003, 0.011	0.166	0.109
Step 3	0.003	0.004	0.796	0.428	-0.005, 0.011	0.167	0.100
4: Subiculum							
Step 1	0.007	0.003	2.128	**0.036***	0.000, 0.014	0.046	0.036
Step 2	0.008	0.003	2.249	**0.027***	0.001, 0.015	0.171	0.115
Step 3	0.006	0.004	1.632	0.106	-0.001, 0.014	0.179	0.113
5: HPC Tail							
Step 1	0.011	0.004	2.771	**0.007***	0.003, 0.020	0.075	0.066
Step 2	0.011	0.004	2.565	**0.012***	0.002, 0.019	0.179	0.123
Step 3	0.009	0.005	1.902	0.061	0.000, 0.018	0.188	0.122

Notes: Step 1: unadjusted, N = 96; Step 2: adjusted for control variables: sex, age, education, CVD and group, N = 95, Step 3: adjusted for control variables AND Mediterranean Diet adherence, N = 95
General linear models were used to examine the association between spermidine intake and hippocampal subfield volumes. Spermidine intake was adjusted for total caloric intake and log10-transformed. All volumes were adjusted for total intracranial volume, multiplied by 100. Asterisks indicate $p < 0.05$. CVD: cardiovascular disease risk; CA: cornu ammonis; DG: gyrus dentatus; HPC: hippocampus; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

Table 5: Association between spermine intake and hippocampal subfield volumes

Spermine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: CA1							
Step 1	0.007	0.005	1.327	0.188	-0.004, 0.018	0.018	0.008
Step 2	0.006	0.005	1.178	0.242	-0.004, 0.017	0.138	0.080
Step 3	0.005	0.005	0.948	0.346	-0.006, 0.016	0.147	0.079
2: CA3							
Step 1	0.001	0.002	0.581	0.562	-0.003, 0.005	0.00	-0.007
Step 2	0.000	0.002	0.243	0.808	-0.003, 0.004	0.086	0.024
Step 3	0.001	0.002	0.359	0.721	-0.003, 0.005	0.090	0.016
3: CA4/DG							
Step 1	0.004	0.004	0.933	0.353	-0.005, 0.013	0.009	-0.001
Step 2	0.003	0.004	0.638	0.525	-0.006, 0.011	0.160	0.102
Step 3	0.002	0.004	0.490	0.626	-0.007, 0.011	0.163	0.096
4: Subiculum							
Step 1	0.005	0.004	1.111	0.269	-0.004, 0.013	0.013	0.002
Step 2	0.005	0.004	1.136	0.259	-0.004, 0.013	0.136	0.078
Step 3	0.003	0.004	0.785	0.434	-0.005, 0.012	0.160	0.093
5: HPC Tail							
Step 1	0.007	0.005	1.355	0.179	-0.003, 0.017	0.019	0.009
Step 2	0.006	0.005	1.219	0.226	-0.004, 0.016	0.133	0.073
Step 3	0.004	0.005	0.839	0.404	-0.006, 0.015	0.161	0.093

Notes: Step 1: unadjusted, N = 96; Step 2: adjusted for control variables: sex, age, education, CVD and group, N = 95, Step 3: adjusted for control variables AND Mediterranean Diet adherence, N = 95
General linear models were used to examine the association between spermine intake and hippocampal subfield volumes. Spermine intake was adjusted for total caloric intake and log10-transformed. All volumes were adjusted for total intracranial volume, multiplied by 100. Asterisks indicate $p < 0.05$. CVD: cardiovascular disease risk; CA: cornu ammonis; DG: gyrus dentatus; HPC: hippocampus; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

3.3.2 Cortical volume

Higher dietary spermidine intake was associated with larger TGMV (B = 2.343, p = 0.033, CI = (0.189, 4.498)) and this association was maintained after controlling for confounding factors in step 2 and 3 (Table 6). There was no statistically significant association between spermidine as well as spermine and cortical grey matter volume in AD-vulnerable, frontal, temporal, parietal and cingulate regions in unadjusted analyses (all p's > 0.5, all 95% CI's include 0) (Table 6 and Table 7). After adjustment for confounders in step 2, an association between spermidine intake

and the volume of the frontal and temporal region was revealed, which, however, diminished again after additional adjustment for Mediterranean Diet in step 3. For the remaining regional cortical volumes, both adjustment steps (2 and 3) did not attenuate the associations substantially.

Table 6: Association between spermidine intake and cortical volume

	Spermidine						
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: TGMV							
Step 1	2.343	1.085	2.160	**0.033***	0.189, 4.498	0.049	0.039
Step 2	2.323	0.928	2.503	**0.014***	0.478, 4.167	0.386	0.345
Step 3	2.406	1.035	2.325	**0.022***	0.349, 4.464	0.387	0.337
2: AD-regions							
Step 1	0.143	0.171	0.837	0.405	-0.197, 0.484	0.007	-0.003
Step 2	0.118	0.156	0.755	0.452	-0.192, 0.428	0.282	0.223
Step 3	0.038	0.173	0.220	0.827	-0.305, 0.381	0.291	0.234
3: Frontal							
Step 1	0.493	0.283	1.741	0.085	-0.069, 1.055	0.031	0.021
Step 2	0.568	0.263	2.164	**0.033***	0.046, 1.090	0.253	0.202
Step 3	0.576	0.293	1.968	0.052	-0.006, 1.159	0.253	0.193
4: Temporal							
Step 1	0.380	0.222	1.713	0.090	-0.060, 0.820	0.030	0.020
Step 2	0.418	0.193	2.161	**0.033***	0.034, 0.802	0.351	0.307
Step 3	0.306	0.214	1.433	0.155	-0.118, 0.731	0.361	0.310
5: Parietal							
Step 1	0.392	0.221	1.768	0.080	-0.048, 0.831	0.032	0.022
Step 2	0.291	0.214	1.358	0.178	-0.135, 0.716	0.208	0.155
Step 3	0.207	0.238	0.869	0.387	-0.266, 0.680	0.214	0.151
6: Cingulate							
Step 1	0.045	0.073	0.616	0.539	-0.100, 0.191	0.004	-0.007
Step 2	0.082	0.071	1.143	0.256	-0.060, 0.223	0.176	0.120
Step 3	0.113	0.079	1.432	0.156	-0.044, 0.271	0.184	0.119

Notes: Step 1: unadjusted, N = 96; Step 2: adjusted for control variables: sex, age, education, CVD and group, N = 95; Step 3: adjusted for control variables AND Mediterranean Diet adherence, N = 95
General linear models were used to examine the association between spermidine intake and cortical volume. Spermidine intake was adjusted for total caloric intake and log10-transformed. All volumes were adjusted for total intracranial volume. Asterisks indicate $p < 0.05$. CVD: cardiovascular disease risk; TGMV: total grey matter volume; AD: Alzheimer's Disease; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

Table 7: Association between spermine intake and cortical volume

Spermine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: TGMV							
Step 1	2.049	1.337	1.533	0.129	-0.606, 4.704	0.024	0.014
Step 2	1.702	1.136	1.498	0.138	-0.556, 3.960	0.359	0.315
Step 3	1.556	1.167	1.333	0.186	-0.764, 3.876	0.362	0.310
2: AD-regions							
Step 1	0.073	0.209	0.348	0.729	-0.343, 0.489	0.001	-0.009
Step 2	-0.003	0.187	-0.017	0.987	-0.375, 0.369	0.277	0.228
Step 3	-0.057	0.191	-0.299	0.766	-0.437, 0.322	0.291	0.234
3: Frontal							
Step 1	0.400	0.348	1.150	0.253	-0.291, 1.091	0.014	0.003
Step 2	0.429	0.320	1.343	0.183	-0.206, 1.064	0.229	0.176
Step 3	0.388	0.328	1.182	0.240	-0.264, 1.041	0.232	0.170
4: Temporal							
Step 1	0.132	0.274	0.481	0.632	-0.412, 0.675	0.002	-0.008
Step 2	0.110	0.237	0.466	0.643	-0.361, 0.582	0.318	0.271
Step 3	0.011	0.239	0.048	0.962	-0.464, 0.487	0.346	0.294
5: Parietal							
Step 1	0.348	0.272	1.280	0.204	-0.192, 0.888	0.017	0.007
Step 2	0.233	0.258	0.903	0.369	-0.280, 0.746	0.199	0.145
Step 3	0.168	0.264	0.638	0.525	-0.356, 0.692	0.211	0.148
6: Cingulate							
Step 1	0.068	0.089	0.768	0.444	-0.109, 0.245	0.006	-0.004
Step 2	0.057	0.086	0.658	0.512	-0.114, 0.227	0.168	0.111
Step 3	0.066	0.088	0.745	0.458	-0.110, 0.241	0.170	0.104

Notes: Step 1: unadjusted, N = 96; Step 2: adjusted for control variables: sex, age, education, CVD and group, N = 95; Step 3: adjusted for control variables AND Mediterranean Diet adherence, N = 95
General linear models were used to examine the association between spermine intake and cortical volume. Spermine intake was adjusted for total caloric intake and log10-transformed. All volumes were adjusted for total intracranial volume. Asterisks indicate $p < 0.05$. CVD: cardiovascular disease risk; TGMV: total grey matter volume; AD: Alzheimer's Disease; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

3.3.3 Cortical thickness

Higher spermidine intake was associated with overall greater mean cortical thickness, cortical thickness within AD-vulnerable as well as frontal, temporal and parietal regions (AD-regions: B = 0.166, p = 0.013, CI = (0.035, 0.297); mean: B = 0.148, p = 0.015, CI = (0.029, 0.266); frontal:

B = 0.142, p = 0.032, CI = (0.013, 0.271); temporal: B = 0.199, p = 0.009, CI = (0.051, 0.347); parietal: B = 0.206, p = 0.002, CI = (0.077, 0.335)) (Table 8). However, no statistically significant association with cingulate cortical thickness was observed (all p's > 0.1, all 95% CI's include 0). These associations were maintained after adjustment for age, sex, education, CVD, and group, except for the diminished association with frontal cortical thickness (p = 0.054). Further adjustments for Mediterranean Diet (step 3) did not attenuate these relationships substantially. There were no statistically significant associations between spermine intake and cortical thickness both unadjusted and after controlling for confounding factors (step 2 and 3) (all p's > 0.08, all 95% CI's include 0) (Table 9).

Table 8: Association between spermidine intake and cortical thickness

Spermidine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: Mean							
Step 1	0.147	0.060	2.465	**0.016***	0.029, 0.265	0.061	0.051
Step 2	0.132	0.056	2.371	**0.020***	0.021, 0.243	0.277	0.227
Step 3	0.151	0.062	2.436	**0.017***	0.028, 0.275	0.281	0.223
2: AD-regions							
Step 1	0.166	0.065	2.531	**0.013***	0.036, 0.296	0.064	0.054
Step 2	0.140	0.063	2.230	**0.028***	0.015, 0.265	0.248	0.197
Step 3	0.156	0.070	2.235	**0.028***	0.017, 0.296	0.251	0.190
3: Frontal							
Step 1	0.140	0.065	2.154	**0.034***	0.011, 0.268	0.047	0.037
Step 2	0.122	0.062	1.955	0.054	-0.002, 0.246	0.225	0.173
Step 3	0.132	0.070	1.903	0.060	-0.006, 0.271	0.226	0.164
4: Temporal							
Step 1	0.198	0.074	2.661	**0.009***	0.050, 0.345	0.070	0.060
Step 2	0.183	0.068	2.668	**0.009***	0.047, 0.319	0.312	0.265
Step 3	0.209	0.076	2.745	**0.007***	0.058, 0.360	0.317	0.262
5: Parietal							
Step 1	0.205	0.065	3.169	**0.002***	0.077, 0.334	0.097	0.087
Step 2	0.174	0.064	2.727	**0.008***	0.047, 0.300	0.238	0.186
Step 3	0.194	0.071	2.736	**0.008***	0.053, 0.335	0.242	0.181
6: Cingulate							
Step 1	0.074	0.092	0.799	0.426	-0.109, 0.256	0.007	-0.004
Step 2	0.079	0.091	0.863	0.391	-0.103, 0.260	0.148	0.090
Step 3	0.080	0.102	0.788	0.433	-0.122, 0.283	0.148	0.080

Notes: Step 1: unadjusted, N = 96; Step 2: adjusted for control variables: sex, age, education, CVD and group, N = 95; Step 3: adjusted for control variables AND Mediterranean Diet adherence, N = 95
General linear models were used to examine the association between spermidine intake and cortical thickness. Spermidine intake was adjusted for total caloric intake and log10-transformed. Regional cortical thickness was adjusted for surface area size. Asterisks indicate $p < 0.05$. CVD: cardiovascular disease risk; AD: Alzheimer's Disease; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

Table 9: Association between spermine intake and cortical thickness

Spermine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: Mean							
Step 1	0.108	0.074	1.466	0.146	-0.038, 0.255	0.022	0.012
Step 2	0.081	0.068	1.179	0.242	-0.055, 0.217	0.242	0.191
Step 3	0.079	0.070	1.118	0.267	-0.061, 0.219	0.242	0.182
2: AD-regions							
Step 1	0.111	0.082	1.365	0.175	-0.051, 0.273	0.019	0.009
Step 2	0.076	0.077	0.983	0.328	-0.077, 0.229	0.214	0.161
Step 3	0.071	0.079	0.899	0.371	-0.086, 0.229	0.215	0.152
3: Frontal							
Step 1	0.079	0.080	0.984	0.328	-0.081, 0.239	0.010	0.000
Step 2	0.052	0.076	0.687	0.494	-0.099, 0.204	0.196	0.141
Step 3	0.046	0.078	0.586	0.559	-0.110, 0.202	0.197	0.133
4: Temporal							
Step 1	0.181	0.092	1.963	0.053	-0.002, 0.363	0.039	0.029
Step 2	0.145	0.084	1.734	0.086	-0.021, 0.312	0.280	0.231
Step 3	0.144	0.086	1.672	0.098	-0.027, 0.316	0.280	0.223
5: Parietal							
Step 1	0.144	0.082	1.760	0.082	-0.018, 0.306	0.032	0.022
Step 2	0.113	0.079	1.435	0.155	-0.043, 0.269	0.192	0.137
Step 3	0.108	0.081	1.338	0.184	-0.052, 0.269	0.193	0.128
6: Cingulate							
Step 1	0.008	0.113	0.068	0.946	-0.216, 0.231	0.000	-0.011
Step 2	-0.005	0.110	-0.043	0.966	-0.223, 0.214	0.141	0.083
Step 3	-0.013	0.113	-0.118	0.906	-0.238, 0.211	0.142	0.073

Notes: Step 1: unadjusted, N = 96; Step 2: adjusted for control variables: sex, age, education, CVD and group, N = 95; Step 3: adjusted for control variables AND Mediterranean Diet adherence, N = 95
General linear models were used to examine the association between spermine intake and cortical thickness. Spermine intake was adjusted for total caloric intake and log10-transformed. Regional cortical thickness was adjusted for surface area size. Asterisks indicate $p < 0.05$. CVD: cardiovascular disease risk; AD: Alzheimer's Disease; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

3.4 Cognitive health

As shown in Table 10, there were no statistically significant associations between higher spermidine or spermine intake with memory, executive function, and PACC5 score performance (all p's > 0.1, all 95% CI's include 0). After controlling for confounding factors, the associations remained statistically non-significant.

Table 10: Association between polyamine intake and cognitive health

Spermidine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: Memory							
Step 1	0.295	0.392	0.752	0.454	-0.483, 1.073	0.006	-0.004
Step 2	0.133	0.375	0.354	0.724	-0.611, 0.877	0.147	0.111
Step 3	0.286	0.408	0.701	0.485	-0.525, 1.097	0.155	0.110
2: Executive Function							
Step 1	0.208	0.285	0.730	0.467	-0.358, 0.775	0.005	-0.005
Step 2	0.139	0.279	0.497	0.620	-0.415, 0.692	0.109	0.071
Step 3	-0.061	0.301	-0.204	0.839	-0.658, 0.535	0.135	0.089
3: PACC5							
Step 1	0.143	0.319	0.448	0.655	-0.491, 0.777	0.002	-0.008
Step 2	-0.039	0.288	-0.137	0.891	-0.612, 0.533	0.236	0.204
Step 3	-0.129	0.315	-0.410	0.683	-0.754, 0.496	0.240	0.200
Spermine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
1: Memory							
Step 1	0.319	0.477	0.669	0.505	-0.627, 1.265	0.005	-0.006
Step 2	0.064	0.454	0.142	0.887	-0.837, 0.965	0.146	0.110
Step 3	0.130	0.463	0.282	0.779	-0.788, 1.049	0.151	0.106
2: Executive Function							
Step 1	0.450	0.345	1.305	0.195	-0.234, 1.134	0.017	0.007
Step 2	0.292	0.336	0.869	0.387	-0.376, 0.960	0.114	0.076
Step 3	0.191	0.339	0.563	0.575	-0.483, 0.865	0.138	0.091
3: PACC5							
Step 1	0.070	0.389	0.180	0.857	-0.701, 0.841	0.000	-0.010
Step 2	-0.188	0.349	-0.538	0.592	-0.880, 0.504	0.238	0.206
Step 3	-0.235	0.356	-0.661	0.510	-0.941, 0.471	0.242	0.202

Notes: N = 100 for Memory and PACC5; N = 99 for Executive Function, as one participant was color-blind and therefore could not pass the required Stroop test; Step 1: unadjusted; Step 2: adjusted for control variables: sex, age, education; Step 3: adjusted for control variables AND Mediterranean Diet adherence
General linear models were used to examine the association between polyamine intake and cognitive performance (composite scores). Polyamine intake was adjusted for total caloric intake and log10-transformed. PACC5: Preclinical Alzheimer Cognitive Composite 5; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

3.5 Cardiovascular health

No statistically significant association between spermidine and spermine with the CVD risk score was observed in both unadjusted and adjusted analyses (all p's > 0.1, all 95% CI's include 0) (Table 11).

Table 11: Association between polyamine intake and cardiovascular risk

Spermidine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
CVD Risk Score							
Step 1	-9.241	6.203	-1.490	0.140	-21.551, 3.070	0.022	0.012
Step 2	-7.081	6.763	-1.047	0.298	-20.506, 6.344	0.029	0.009
Spermine							
Model	*B*	*SE*	*t*	*p*	*CI*	R^2	*Adj.* R^2
CVD Risk Score							
Step 1	-4.646	7.613	-0.610	0.543	-19.755, 10.463	0.004	-0.006
Step 2	-2.923	7.720	-0.379	0.706	-18.246, 12.401	0.019	-0.001

Notes: N = 99; Step 1: unadjusted; Step 2: adjusted for Mediterranean Diet adherence
General linear models were used to examine the association between polyamine intake and cardiovascular 10-year risk. Polyamine intake was adjusted for total caloric intake and log10-transformed. Asterisks indicate significance at $p < 0.05$. CVD: cardiovascular disease; B: unstandardized regression coefficient; SE: Standard Error; CI: 95% Confidence Interval for B; R^2: explained variance; Adj. R^2: adjusted explained variance.

4 DISCUSSION

The modification of lifestyle practices, such as dietary patterns, is one possible starting point for developing strategies that delay or prevent the pathological AD processes in a timely manner before the onset of symptoms. The aim of this cross-sectional study was to examine whether self-reported dietary polyamine intake is associated with brain, cognitive and cardiovascular health as well as other well-known health promoting dietary habits in older adults with and without SCD. There were three main findings of this research. **First**, a positive association was found for dietary intake of both polyamines and a generally healthy diet as well as for spermidine intake and adherence to a Mediterranean Diet. **Second**, results showed that only the higher intake of spermidine was associated with brain structure, more precisely larger hippocampal volume (i.a. CA1 and subiculum), TGMV, and greater cortical thickness in almost all brain areas, among others in an AD-vulnerable template, but not with cognition. **Third**, no relationship between polyamine intake and cardiovascular risk could be observed. The results were mainly independent of a Mediterranean Diet adherence, suggesting that spermidine acts as an independent nutritional component.

4.1 Discussion of results

4.1.1 Dietary health

Dietary spermine and spermidine intake showed an association with a generally healthy dietary pattern. Further, spermidine intake was associated with adherence to a Mediterranean Diet, while spermine intake showed only a trend. These findings validate the quality of the FFQ used to quantify polyamine intake.

In fact, it is not surprising that polyamine intake was linked to a healthy nutrition, as assessed by the FFL. Taking a look at the amount of polyamine content in different foods, it becomes clear that most of the food with high content of polyamines are generally known to be part of a healthy diet, e.g. vegetables and fruits. However, a notable difference to the Mediterranean Diet is the consumption of red meat, which is one of the main sources for daily polyamine intake (Atiya Ali et al., 2011, Zoumas-Morse et al., 2007), but is not considered a beneficial food of the Mediterranean Diet (Willett et al., 1995). Especially, spermine is most abundant in animal-derived products, while spermidine is the main polyamine in plant-based food (Atiya Ali et al., 2011, Zoumas-Morse et al., 2007). This might be one explanation for the present findings, in which dietary spermine showed a weaker association with Mediterranean Diet than spermidine did.

In some aspects, the present analyses replicate the findings of former studies showing a relationship between polyamine intake and Mediterranean food (Binh et al., 2011). However, Binh and colleagues showed a specific relationship between the intake of spermine and many foods from the Mediterranean Diet, whereas in the present sample there was no significant but only a trend relationship. Methodologically, it is important to note that there are differences between the two studies regarding dietary assessment, which could explain the present findings. In this research, polyamine intake and adherence to a Mediterranean Diet were quantified using self-reported questionnaires, whereas Binh and colleagues obtained statistical data on food supply from the online database of the Statistics Division of the Food and Agriculture Organization of the United Nations. Thus, the type of approach to analyse these relationships were different.

Although spermidine intake comes along with a Mediterranean Diet, the results did show a correlation between spermidine intake and brain structure even when being adjusted for adherence to a Mediterranean Diet. Thus, these results suggest that associations of dietary spermidine with structural brain measures are for the most part not a proxy of Mediterranean Diet but rather underline the importance of spermidine intake through that well-known brain-healthy diet. However, mediation analyses that investigate whether spermidine mediates the direct relationship between Mediterranean Diet and brain structure could be a more accurate statistical approach to confirm this hypothesis in the future. Moreover, it is likely that spermidine-rich food also comprises other health beneficial nutrients which may interact with spermidine and might be associated with structural brain measures. Whether it is spermidine alone that promotes these positive effects, or whether it is a complex interaction of many useful compounds, should be the focus of further studies. However, there is evidence that the different food nutrients of the Mediterranean Diet contribute to additive effects and present diverse interactions which ultimately offers increased health benefits (Ortega, 2006).

Previous studies have shown that following a Mediterranean Diet reduces risk (Scarmeas et al., 2006, Scarmeas et al., 2009, Singh et al., 2014) and mortality in AD (Scarmeas et al., 2007). As cognitive decline, especially in dementia, is associated with brain atrophy, it was suggested that the maintenance of brain structure or the delay of brain aging atrophy may be one of the positive effects of a Mediterranean Diet (Gu et al., 2015). Another possible mechanism contributing might be the protective effect of Mediterranean Diet and polyamines through the maintenance of vascular health. Through the external supply of polyamines, enzymatic activities of polyamine synthesis are suppressed, leading to an increase in arginine (starting substance of polyamine synthesis). Thus, the latter is more available for nitric oxide synthesis which helps to maintain vascular function (Drexler et al., 1991, Cooke et al., 1992). Although the beneficial role of a

Mediterranean Diet is still largely unexplained, this may hint at a potential mediation of polyamines on brain health through a Mediterranean Diet.

Furthermore, dietary assessment needs to be discussed. The evaluation was based solely on self-reported questionnaires which may not accurately reflect actual dietary habits, e.g. through over or underestimation (Kroke et al., 1999). Beneficial results on brain health could be influenced by the propensity of well-educated and cognitively healthy people to participate in research studies who, in turn, follow a healthier and more responsible lifestyle than less educated and cognitively impaired individuals. However, structural brain analyses were adjusted for education which should correct this potential influence. When interpreting differing results from other studies on dietary patterns, it is important to keep in mind that diet strongly depends on cultural, religious, and socio-economic backgrounds. In this study, only individuals from Germany were included. Therefore, future studies conducted in different countries or continents should be compared with caution.

In addition, it is very important to mention that dietary patterns as in the Mediterranean Diet are mostly stable over time. Mosconi et al. (2014) showed that around 90% of their study participants maintained their dietary lifestyle for at least 5 years. In the present study, participants were older adults without notable disorders, thus disease-related dietary pattern changes and recall bias are very unlikely. However, the cross-sectional nature of the present analyses does not allow us to assess longitudinal dietary pattern constancy, and thus cannot exclude potential recent changes in dietary habits with certainty. Further investigations should elucidate if short-term spermidine-rich dietary patterns are sufficient to preserve brain health or if a lifelong adherence is necessary. Collectively, the present findings suggest that spermidine is not a Mediterranean Diet proxy but may play a crucial role as an abundant component of the Mediterranean Diet.

4.1.2 Brain health

In line with the hypothesis, there was an association between dietary spermidine intake, total grey matter and hippocampal volume in older adults, as well as volume of the CA1, the subiculum and the hippocampal tail. However, spermidine intake was not associated with regional cortical volume, suggesting that the volume relationship is limited to global and subcortical regions (hippocampus) only. Further analyses of the present study on regional cortical thickness showed associations between spermidine intake and mean, frontal, parietal, and temporal, as well as AD-vulnerable cortical thickness (but no association with cingulate cortical thickness).

Contrary to expectations, the key associations with structural brain measures could not be observed for both polyamines, but only for dietary spermidine. The different findings between these two polyamines might be explained by the fact that spermidine is more easily absorbed from the human intestine without metabolization (40-80%) than spermine (Kiechl et al., 2018). Polyamines are indeed involved in the same strictly regulated pathway but should not be considered as identical agents. The two polyamines are regulated separately and are involved in diseases in different or even opposite ways (Minois et al., 2011, Igarashi and Kashiwagi, 2010). Thus, it is conceivable that spermine may not be as strongly involved in the maintenance of brain structure as spermidine. Nevertheless, the present findings do not exclude an involvement of spermine in brain health. Spermine can be re-converted to spermidine by the enzyme spermine-oxidase (Figure 2) and could therefore affect brain structure indirectly or through other effects not considered yet. The results cannot be fully explained by these hypotheses and the underlying mechanisms of the polyamines' differences in dietary intake, uptake, effects, and sides of action cannot be clarified in the context of the present study design. In addition, recent research has focused more on the promising spermidine in terms of age, health, and diseases, than on spermine (Madeo et al., 2018), which explains the gap of knowledge. Further research is therefore needed to investigate their differing effects on structural brain measures in humans and the dietary long-term effects of each polyamine.

The contribution of peripheral sources for central nervous system polyamine levels is seemingly small (Guerra et al., 2016). This could explain the limited observed effect of dietary polyamine intake on brain structure. They do in fact pass the blood-brain barrier (as shown in rats), but only to a limited extent with a brain uptake index of about 5% (Shin et al., 1985). However, several studies have suggested that the systemic, peripheric polyamine administration may increase cerebral levels of polyamines to biologically active concentrations (Signor et al., 2014, Ribeiro et al., 2013, Camera et al., 2007).

In conjunction with earlier studies, the present associations between spermidine intake and cortical thickness as well as total grey matter and hippocampal volume suggest a potential involvement of spermidine in the normal process of brain aging, with brain atrophy as a central process. However, there are no studies to date that investigate the effect of polyamine intake on brain structure in humans, so results cannot be compared with former findings or applied methods for the time being. Taking this into consideration, the individual results of this present study need to be discussed with caution and should be considered as a basis for further investigations.

Hippocampal and hippocampal subfield volumes

The association between spermidine intake and hippocampal volume strengthens the findings of previous studies. Wirth et al. (2018b) showed improved memory performance in a task sensitive for hippocampal function after a 3-month spermidine supplementation versus placebo in individuals with SCD. Previous studies in animal models had further observed protection from age-induced memory impairment through spermidine feeding (Gupta et al., 2013) as well as the involvement of hippocampal polyamine levels in memory formation and retrieval (Tiboldi et al., 2012). Thus, the present findings provide a possible pathophysiological substrate to the insufficient data to date, especially in humans. Without additional supplementation, it may be possible to influence hippocampus structure by dietary patterns alone, but supplementation or longitudinal interventions may be necessary in order to capture cognitive effects.

It has been reported that N-methyl-D-aspartate (NMDA) receptors in the hippocampus are affected during aging (Pelleymounter et al., 1990, Clayton and Browning, 2001). Polyamines interact with the polyamine binding side at the NMDA receptor (Signor et al., 2014, Camera et al., 2007), thus modulating learning and memory and enhancing synaptic plasticity (Guerra et al., 2016)**.** Moreover, polyamine levels change with age and could potentially contribute to the dysfunction of the NMDA receptors (Liu et al., 2008).

The distinct hippocampal subfields exert specific functions, connect to different brain regions and are differentially affected by aging (Aggleton, 2012, Maruszak and Thuret, 2014, Small et al., 2011). This may explain the differing results between the subfield volumes where only CA1, subiculum, and hippocampal tail were associated with spermidine intake. In addition, hippocampal subfields differ in their vulnerability to AD, with CA1 being the earliest and most strongly affected subfield followed by the subiculum, with regard to neuronal cell loss (West et al., 1994) and NFT targeting (Schönheit et al., 2004, Lace et al., 2009). Even in very early stages of AD, CA1 and subiculum atrophy were detectable in cognitively intact subjects that later developed MCI or clinical AD (Apostolova et al., 2010, Csernansky et al., 2005). After a 3-month spermidine supplementation, Wirth et al. (2018b) observed memory improvement in a memory task that is sensitive to specific hippocampus subfields. These findings suggest differential effects of increased spermidine levels on hippocampus subfields even in elderly without cognitive impairment. This can be explained by their different anatomical and regional molecular profiles (Thompson et al., 2008), which contributes to diverse vulnerabilities. Regarding the association between spermidine intake and hippocampal tail, to date no former explaining literature was found. Moreover, former studies showed different concentrations of polyamine levels in the various subfields (Liu et al., 2008), indicating a potential difference in affinity, uptake or regulation of polyamines in the different subfields.

Hippocampal subfields findings should be interpreted with caution. The sensitivity of the subfield segmentation tool in FreeSurfer V.6.0 has recently been addressed by some authors (Yushkevich et al., 2015, De Flores et al., 2015, Wisse et al., 2014, Pluta et al., 2012), questioning the program's ability to correctly distinguish such minute subfields in low-resolution images. Therefore, the use of more accurate imaging approaches should be considered in future research. There are new techniques to define hippocampal subfield volumes with high resolution T2-weighted scans, which may be more precise than the standard T1-weighted images used in the present study. Evaluating the microstructure of the hippocampus by mean diffusivity estimated using Diffusion Tensor Imaging would also further improve the exploration of the associations found. Moreover, it would be interesting to examine the volume of other subcortical structures like putamen or amygdala in further analyses.

In summary, these results suggest that dietary spermidine may be differentially involved in brain aging across the subregions of the hippocampus, even if the exact details of underlying mechanisms are still unknown, and more precise imaging tools should be used in future analyses.

Cortical volume and thickness

Higher spermidine intake was indeed associated with a greater mean and regional cortical thickness in almost all brain areas, suggesting that it was specific not only for AD-prone regions, possibly due to the unclear boundary between healthy and pathological aging (Jagust, 2013). There was an association between spermidine intake and total grey matter but not regional grey matter volume (except for frontal and temporal grey matter volume after adjusting for confounding factors in step 2, but this relationship was diminished after adding Mediterranean Diet to the model). This might indicate additive statistical effects of the different regional cortical volumes.

There were no associations between polyamine intake and the cingulate lobe, although the cingulate cortex is vulnerable to grey matter loss during the aging process (Mann et al., 2011). The anterior and posterior cingulate gyrus are involved in different functions and are connected to different brain areas, and are also differently affected by age (Raz et al., 2004). The cingulate cortex is an important intermediate link with complex connectivity to other brain areas. When re-evaluating the involvement of polyamines in the cingulate cortex, it may therefore be of interest to consider both anterior and posterior separately. In general, functional MRI measurements of brain networking would allow a broader understanding of the involvement of polyamines in brain health.

Furthermore, associations with AD-vulnerable, parietal, and temporal cortical thickness were the most stable results after confounding factor adjustments. This may indicate that brain regions

involved in AD pathology may benefit most from the potential neuroprotective effects of spermidine, for example due to autophagy stimulation, which counteracts neurotoxic aggregates, like Aβ and NFTs, accumulated in these brain regions rather than in others.

The methods used for the cortical imaging analyses are well established and validated through post mortem studies (Cardinale et al., 2014). Nevertheless, given the differing regional cortical volume and thickness results, some aspects should be specified. Cortical thickness and volume measures represent different properties. Volume measurement of cortical grey matter is basically a construct of both cortical thickness and surface area, but seems to be more closely related to surface area (Winkler et al., 2010). Cortical thickness has been shown to be a more sensitive method of assessing age-related grey matter decline compared to the volume of grey matter (Hutton et al., 2009). However, Hutton and colleagues also suggested considering both volume and thickness measurements as complementary. Thus, it was useful to examine regional cortical volume as well, in order to be able to assess non-specific effects on the brain, which might not influence thickness or surface area directly. As volume takes into account more potential interference effects than cortical thickness, differing results of spermidine's relationship on regional cortical volume and thickness might be explained this way.

Potential mechanisms of action

The exact mechanisms of action through which spermidine might influence brain health are unknown. Spermidine levels throughout the entire organism as well as hippocampal volume and cortical thickness decrease with age. Hippocampal volume is known to decrease during human aging and to be related to memory alterations and neurodegenerative diseases like AD. In the following several potential mechanisms of action are discussed.

The most discussed underlying mechanism of action of spermidine is its capacity to induce or restore efficient autophagy**.** Autophagy supports the recycling of damaged and potentially harmful cell material (Rubinsztein et al., 2011), thus playing a beneficial role in slowing the process of aging (Yamaguchi and Otsu, 2012). The explorative analyses of regional cortical volume in contrast to the findings with subcortical volume of the hippocampus, could be explained by the different vulnerability of certain brain areas to autophagy decline during aging. Through spermidine's autophagy induction, higher external intake of spermidine could prevent neuronal cell loss, which could result in preserved hippocampal volume as well as greater cortical thickness. For example, in invertebrate models, spermidine administration prevented a-synuclein neurotoxicity thus rescuing the loss of dopaminergic neurons (Buttner et al., 2014), it is therefore conceivable that neurotoxic aggregates of AD pathology, namely Aβ and NFTs, may also be cleared through spermidine-enhanced autophagy, but this has not yet been proven in humans.

Alternatively, spermidine's neuroprotection may also result from down-regulation of inflammatory cytokines and immune cells thus suppressing maladaptive inflammation (Madeo et al., 2018). For instance, spermidine's protection from axon demyelination (Guo et al., 2011) is induced by suppression of autoimmune-reactive demyelinating T cells (Yang et al., 2016).

Another approach of explanation is that spermidine is known to mimic caloric restriction and to interact with deoxyribonucleic acid. Caloric restriction is related to neural plasticity to the extent of inducing adaptive cellular stress response pathways by upregulating specific genes. Thereby, cytoprotective proteins, enzymes, and neurotrophic factors are translated, which results in neural plasticity enhancement and stronger neural networks (Yu and Mattson, 1999, Maswood et al., 2004, Arumugam et al., 2010). To what extent spermidine is involved in such positive effects on neural plasticity should be evaluated in future interventional approaches.

Thus, the neuroprotective property of spermidine supplementation is based on its autophagy enhancement in neuronal or glial cells but also its autophagy-independent transcriptional and metabolic effects in vivo. These mechanisms might thus be involved in the anti-aging and disease-modulatory effects of spermidine (Madeo et al., 2018). The described mechanisms of action may explain the present findings to some extent, but it definitely requires more focused research to deeply understand the underlying mechanisms.

In conclusion, the present neuroimaging findings suggest a potential effect of spermidine on brain structure. The biological mechanisms for the reported positive associations between spermidine intake and hippocampal volume, TGMV as well as cortical thickness still need to be clarified. Adjustments for possible confounders such as age, sex, education, CVD, and group affiliation, effectively did not change the associations. Thus, these results suggest that dietary spermidine is a protective factor that is independent of conventional risk factors. Findings still need to be re-evaluated in a prospective longitudinal setting and in different study samples.

4.1.3 Cognitive health

In the present study, contrary to the hypothesis, there were no associations of spermidine and spermine with cognitive performance, neither for memory, executive function nor global cognition as measured using the PACC5.

In conclusion, a transfer of the animal model to humans could not be confirmed for the time being. Former studies analysed polyamine levels in the brains of fruit flies and showed that its decrease was associated with memory decline (Gupta et al., 2013). However, they measured spermidine

in the brain of fruit flies, which differs from the present analyses in humans where polyamine information was assessed through a FFQ. In addition, most of the studies suggesting a memory enhancing effect of polyamines were performed in animal models and they analysed the effect of polyamine supplementation over time. Recently, the only human study by Wirth et al. (2018b) showed an improvement in memory function by oral polyamine supplementation over 3 months compared to placebo in older adults with SCD. Wirth and colleagues assessed memory performance by a very sensitive pattern separation task that was not evaluated for this exploratory analysis. However, they also observed no positive effects on memory or executive function as measured by standard neuropsychological tests, which is consistent with the present findings.

In the present study, cognitive performance of the participants was still within the normal range, ensured by the complex neuropsychological test battery. The variance is very limited because of the specific cut-off applied to screening scores, which might have led to a “ceiling effect” in testing. Thus, the variance of the neuropsychological test scores might not be large enough to detect correlations with dietary habits. For example, in further research this ceiling-effect could be circumvented by including MCI patients and thus enlarging the range of test scores. Besides the limited variance, the tests used might not be sensitive enough to uncover subtle changes of cognition in cognitively unimpaired adults (Jessen et al., 2014a). Wirth et al. (2018b) underline this idea, as they only observed positive effects in a test highly sensitive to hippocampal function and no improvement in traditional cognitive measures. In addition, Berlese et al. (2005) reported that spermidine’s facilitatory effect on memory is limited to the acquisition and early consolidation phases of memory, at least in rats. Consequently, these findings indicate that tests implicated in the composite scores may not have been quantifying spermidine sensible memory features.

Structural brain changes usually occur before clinically objectively measurable alterations. This might explain why higher dietary spermidine intake showed no relationship with cognitive performance, while there were in fact associations between spermidine intake and hippocampal volume as well as cortical thickness and TGMV. Furthermore, cognition can be maintained through compensatory mechanisms such as cognitive reserve (Stern, 2002), while structural changes are already measurable. This capacity, which varies greatly between individuals, makes it challenging to investigate dietary associations with cognition in pre-clinical conditions. In contrast, neuroimaging biomarkers sensitive to the detection of structural changes in the aging brain allow us to examine associations between dietary patterns and age-related brain changes, which may not be detectable by clinical evaluation (Gu and Scarmeas, 2013).

4.1.4 Cardiovascular health

In the present cross-sectional study, contrary to the hypothesis, higher dietary polyamine intake was not associated with lower CVD risk, quantified by the validated Framingham CVD risk score of D'Agostino et al. (2008).

Cardiovascular factors have a significant impact on brain health e.g. in the development of AD as one of the main risk factors (Baumgart et al., 2015). Therefore, the prevention of cardiovascular risk factors through polyamines might have in turn a positive effect on the brain. Thus, it was conceivable that the observed associations of dietary spermidine and brain structure could be explained by the involvement of spermidine in cardiovascular health (Eisenberg et al., 2016, Soda et al., 2012). However, in the present study there was no association between higher polyamine intake and a lower CVD risk. Nevertheless, determining CVD risk has been helpful to correct for potential confounding on the relationships between polyamine intake and structural brain measures (which maintained mostly stable). In conclusion, these results suggest that the beneficial effect of spermidine on the brain may be independent of CVD risk and may rather be based on other underlying mechanisms.

The present results do not converge with the findings of Eisenberg et al. (2016) and Soda et al. (2012), who observed a protective effect of higher spermidine intake against CVD and cardiac death risk in humans. Moreover, Kiechl et al. (2018) observed a reduced incidence of cardiovascular diseases, which was accompanied by reduced all-cause mortality in individuals with a high total dietary intake of spermidine. In the present sample, subjects with severe cardiovascular disorders were excluded from the study, which restricts the variation in CVD score levels and could explain the differing results. Furthermore, Eisenberg and colleagues analysed the data of a considerably larger sample size in a prospective population-based survey, where they assessed detailed information about cardiovascular health of study participants over decades. Regarding this, the present cross-sectional study design with a one-time visit and a pure risk score calculation might not have been suitable to assess overall cardiovascular health. To determine polyamine intake the same FFQ and the same calculation methods were used as by Eisenberg et al. (2016) and Kiechl et al. (2018).

However, score calculation can be affected by imprecise measurements. The value with the largest potential source of error is blood pressure measurement, which may differ depending on whether or not participants took their hypertensive medications before the visit, on how they felt on that day, and on their excitement due to the examinations. The diabetes mellitus status was derived from the information about medication use, whereby unknown but existing, untreated

diabetes was not assessed and thus represents a possible source of error. The other values are considered more reliable. Moreover, cardiovascular risk also depends on many other factors that were not considered in the risk score calculation of D'Agostino and colleagues. For example, additional information about alcohol consumption, family history, blood glucose levels, or even socioeconomic background as well as stress, could contribute to a more precise risk estimation. However, the CVD risk score calculator has been used in many former studies and has been validated for predictive power and accuracy in various populations (Khalili et al., 2012, D'Agostino et al., 2013).

Importantly, the Framingham CVD risk score estimated the 10-year risk of developing CVD, so it may not have been the best way to assess cardiovascular health in general. However, several studies on animals as well as on humans have provided evidence of cardioprotective effects of polyamines of (Eisenberg et al., 2016, Soda et al., 2012). Thus, the present cross-sectional findings should not be interpreted too strictly for the time being. Future studies should address the limits of risk score calculation and include a less healthy and a more heterogeneous study population.

4.2 Limitations of the study

This exploratory cross-sectional study is one of the first polyamine studies in humans and the first to investigate the relationship between polyamine dietary intake and brain structure in older adults taking into account cardiovascular health and cognition. Multiple levels and types of brain measures, provided by a 3-Tesla MRI, were analysed with a well-established brain imaging protocol, allowing for an overall view of the brain. Standardized neuropsychological tests were conducted and various composite scores were calculated, which is a qualitative way of representing cognitive performance. Participants with MCI or dementia or other severe health impairments were excluded, so diseases were unlikely to have an influence on dietary patterns, and potential recall bias due to cognitive deficits was reduced.

Nevertheless, some limitations have to be discussed. Given the limited sample size, statistical power was low, which might explain the small effects on structural brain measures and the contrary findings on CVD with spermidine intake. However, with a limited sample size, it is more difficult to observe statistically significant associations, therefore the significant results reported in the present study may have good accuracy.

When interpreting the results, it should be considered that the study population (HC and SCD) might not be representative of the general population. The participants were exclusively German,

so that the available results may not be applicable to other ethnic groups with potentially different demographic characteristics, socio-economic and cultural backgrounds, strongly influencing dietary habits. Moreover, these findings apply to polyamines from dietary amounts and sources characteristic of Western diet. However, as mentioned before, the inclusion of a more heterogenous study population regarding diet-influencing backgrounds has to be compared with caution. In addition, the recruitment of participants (HC and SCD) through study advertisement, was more likely to attract well educated individuals interested in brain health. However, advertisements comprised very different strategies (via newspaper, radio, internet, healthcare facilities, flyers), which made it possible to reach people from different areas of society. Nevertheless, the results are not yet suitable for drawing general conclusions about extreme diets or polyamine supplementation and should be reanalysed in a more general population, regarding origin, education, and health status.

Moreover, in the general population a much larger effect of dietary spermidine on brain health might be expected, as the present results in a more homogeneous sample are a conservative approximation. Due to strict exclusion and inclusion criteria as well as screening thresholds, the group of subjects is homogeneous in terms of health status and memory performance. However, homogeneity in age and ethnicity reduces associated confounding with brain changes and diet. Furthermore, the cross-sectional sample also offers sufficient non-uniformity as there are adults with SCD and without. Moreover, SCD itself is a heterogenous and non-specific state with diverse underlying causes beside the onset of AD pathology and thus includes not only adults prone to AD but also adults who remain cognitively stable over many years (Jessen et al., 2014b). Finally, this was a good sample for cross-sectional analysis as it is a homogeneous group but not too uniform, but accuracy of the estimate of the general population might be affected.

In the present study data has been adjusted for several potential confounding factors like age, sex, education, CVD, group affiliation, and Mediterranean Diet. Noticeably, the associations maintained after controlling for education suggesting that spermidine intake does not merely reflect a function of healthier life choices in more educated individuals. Adjustments for Mediterranean Diet have been helpful not only to ensure that polyamine intake is not a proxy of the Mediterranean Diet, but also to control for other beneficial nutrients contained in a Mediterranean Diet, besides polyamines. Despite the adjustment for potential confounding factors, residual confounding cannot be excluded. There may be confounding variables which may exert influence on the association of polyamine intake and structural brain measures not yet considered (e.g. genetic disposition, other beneficial nutrients). A former study, using a similar FFQ and calculation, additionally adjusted for multiple features of diet to minimalize the chance of residual confounding by dietary pattern and lifestyle linked to polyamine intake, such as indexes

of healthy or unhealthy diet, individual foods, composite categories of food item or macronutrients (Kiechl et al., 2018). Adherence to a Mediterranean Diet was considered in the present study, nevertheless some covariates may not have been fully addressed and could be included in future analyses. However, given the exploratory nature of the present study, only the most prominent confounders were included to avoid overfitting of the statistical models.

Furthermore, some limitations about the FFQ should be addressed. Since measures of polyamine intake are provided through a self-reported questionnaire, accuracy may be limited and measurement error is possible as self-reported questionnaires tend to over or underestimation (Kroke et al., 1999). Direct estimation, i.e. daily recording of food intake, would be more ideal, but was not feasible in the present study. However, it should be pointed out that self-reported lifestyle habits have been described as highly valid and such food frequency questionnaires are the standard method for estimating food intake in nutritional epidemiology. Studies have shown that individual dietary patterns stay highly stable over time. Polyamine intake calculations have been performed thoroughly in previous studies and were shown to be validated (Willett et al., 1985, Eisenberg et al., 2016, Kiechl et al., 2018). They showed that these calculated intakes, assessed through the FFQ, are representative of average long-term intakes and are in accordance with other more detailed assessments. In addition, in the present study the FFQ assessment could be verified through the two other independent food questionnaires MEDAS and FFL. Thus, the obtained values for polyamine intake can be considered as reliable. Nevertheless, it is only an indirect method of quantifying polyamine intake that does not take into account food processing, storage and preparation, which could affect polyamine content, but exceeds the scope of FFQs.

Moreover, the assessment of polyamine intake by the FFQ could have been supported by some endogenous parameters. Within the SmartAge trial polyamine levels and proxy-biomarkers such as autophagy will also be measured in peripheral blood and muscle tissue (as a proxy of neuronal cells). At the time of analysis, these data were not available. Though, it would have been interesting to examine whether the polyamine levels measured in blood and tissue can be represented by the FFQ. However, it should be considered that blood polyamine levels may not be fully representative of brain levels anyway. The brain-blood barrier (Shin et al., 1985) and the low polyamine blood concentration compared to tissue levels (Magnes et al., 2014) can confound this representation. Thus, blood polyamine levels might not be the best proxy for future analyses investigating the effects of polyamines on brain structure.

The question whether the findings should have been corrected for multiple testing has to be asked. To date, there are no studies that examine the main questions addressed in this study, so it is legitimate to have carried out the analyses on an exploratory level. This is an important first

step in order to generate hypotheses for future, interventional studies, as correction may have deemed some of the results non-significant which could lead to overlooking some important associations. Nevertheless, the present findings lack this correction and must be confirmed or disproved in another sample.

Finally, the cross-sectional, epidemiologic design of the study did not allow confident conclusions to be drawn about causalities. The chance of reverse causality cannot be fully excluded, i.e. behavioral changes including dietary habits alterations could result from brain structure changes. However, since there are no prospective studies on polyamine intake and brain health in humans, the results obtained form the basis for larger prospective trials. Moreover, more than just adjustments in regression analyses are required to analyse the complex relationship between dietary polyamine intake, brain structure, cognition, and cardiovascular risk factors. Further interventional trials are needed to provide more information about the underlying relationships between polyamine intake and brain structure.

4.3 Future prospects

The present research has only considered the cross-sectional baseline data of the ongoing SmartAge study. Follow-up examinations after one year of polyamine supplementation versus placebo are necessary to re-evaluate its hypotheses. Unanswered questions remain, for example, if spermidine intake through diet or supplementation preserves hippocampal volume and cortical thickness over time. Moreover, it will be interesting to see whether this in turn improves cognition over time or whether spermidine effects on cognition are still not observable. For example, functional MRI measurements, PET data, and polyamine levels in tissues will complement the present findings and allow a broader understanding of polyamines in brain health.

So far, the present results suggest positive influences on brain structure through a spermidine-rich diet, by favouring the consumption of spermidine-rich food such as broccoli, cauliflower, peas, mushrooms, green pepper, wheat germs, soybeans (natto), nuts, some types of cheese,... (Zoumas-Morse et al., 2007, Nishimura et al., 2006, Ali et al., 2011). Another strategy to increase spermidine uptake could be achieved by supplementing spermidine, using spermidine-rich natural plant extracts, or administrating pre- and probiotics to promote the intestinal microbial synthesis of polyamines (Madeo et al., 2018). Another very controversial and futuristic topic would be genetic engineering, which could create foods rich in spermidine, for instance, transgenic spermidine rich potatoes seem possible (Farriol et al., 2000). But for this, further evidence of positive effects of spermidine on the human organism should clearly be present.

In general, the findings concerning brain structure as biomarkers can be useful for future research. For example, a randomized intervention in which participants are assigned to a spermidine-rich diet versus standard diet over a certain period of time, could define the change of cortical thickness and hippocampal volume as an outcome measure. Further, larger sample sizes and longitudinal designs are required to determine whether cortical thickness and hippocampal volume predict cognitive decline in individuals with a spermidine-rich versus spermidine-low diet and to a greater extent if the association between dietary spermidine, brain structure, and cognition differs depending on the degree of brain pathology, group status (SCD or HC), and age.

Some aspects of dietary assessment could be improved in future dietary pattern investigations. A more direct daily record of food intake, e.g. with diet apps suitable for older participants, could be used to better track dietary information. Portion sizes of different foods in the FFQ could be supplemented with pictures to help participants understand how much, e.g. 100g of a particular food really represents. In addition, the above-mentioned limitations should be addressed by evaluating information on food processing and cooking habits. Thus, the estimation of nutrient intake could gain precision. Further longitudinal observations of dietary patterns are necessary to distinguish between beneficial lifelong or short-term effects of a spermidine-rich diet on brain health.

In future, spermidine implication may be more aimed at delaying the onset of clinical symptoms than at the final treatment or cure of the underlying pathology of AD. Even the delay of only five years would have immense consequences, namely of reducing the number of AD patients as well as the associated health care costs by nearly 50% (Alzheimer's Association, 2015). In addition, the already described questionable boundaries between healthy and pathological aging and the long preclinical phase of AD require a preventive delaying approach in any case. Moreover, Minois et al. (2011) points out that spermidine "can be beneficial for aging in healthy organisms but that they may be harmful when disease appears". Among other things, this can be underlined by the autophagy down-regulation during normal brain aging, which is in contrast to compensatory autophagy up-regulation in AD patients' brains (Lipinski et al., 2010) as well as increased production of spermidine in MCIs converting to AD subjects (Graham et al., 2015).

Finally, by interpreting these and future results on spermidine an important note is that it is an endogenous metabolite which was shown to be safe and well-tolerated (Schwarz et al., 2018a) in contrast to the other few exogenous substances found to protect against age-dependant impairments, with potential adverse side effects. This makes spermidine a promising agent for non-pharmacological interventional trials with supplementation.

4.4 Conclusion

In summary, this study provides the first evidence for an association between higher dietary spermidine intake with larger hippocampal and total grey matter volume as well as greater mean cortical thickness including frontal, parietal, temporal, and AD-vulnerable regions in cognitively unimpaired older adults. However, there were no associations between polyamine intake and cognition or cardiovascular risk. Although the underlying mechanisms remain unclear, these findings complement those of previous studies that suggested a beneficial effect of spermidine on brain health.

From a prevention point of view, a modifiable factor such as dietary behaviour is of interest, which is even cost-effective, safe, and easy to implement. Additional longitudinal and interventional research is needed to confirm whether the reported cross-sectional associations are reliable. If this is the case, the present study may have implications for health education supporting high spermidine content, abundant in the Mediterranean Diet, as a new feature of a brain-healthy diet. To a larger extent, supplementation may help to preserve brain health during aging in individuals at higher risk for AD as well as in healthy older adults, a hypothesis to be evaluated in future. In conclusion, the present findings suggest that dietary intake of spermidine may maintain healthy brain aging and may even contribute to counteracting the destructive effects of AD, thereby bringing health and life span closer together.

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SUPPLEMENTARY MATERIAL

Table S12: Telephone screening (German)

Einschlußbefähigung (Telefonscreening) - SmartAge

Name: Herr Frau _____

Telefon: _____

Email: _____

Anschrift: _____

Wie alt sind Sie? _____ Jahre (zwischen 60-90)

Wann haben Sie Geburtstag? ____/_____/_____

Tag/Monat/Jahr

wie haben Sie von der Studie erfahren? _____

Nehmen Sie schon Weizenkeimlinge, Gluten- oder Histaminkapseln ein? **ja** nein

Diagnostische Fragen SCD *Die folgenden Fragen dienen der Identifizierung/Definierung von SCD. Für den Einschluss der Probanden **müssen** die **Fragen 1, 3 & 4** mit „**JA**“ beantwortet werden, sowie die **Frage 2 mit >6 Monaten** beantwortet werden (bei >10 Jahre und sonstigen Unsicherheiten Rücksprache)!*

1. Haben Sie das Gefühl, dass Ihre geistige Leistungsfähigkeit schlechter geworden ist?	☐ **JA (Einschlusskriterium)** ☐ NEIN
2. Wenn ja, seit wann hat sich Ihre geistige Leistungsfähigkeit verschlechtert?	**6 Monate bis 10 Jahre**
3. Bereitet Ihnen diese Verschlechterung Sorgen?	☐ **JA (Einschlusskriterium)** ☐ NEIN
4. Würden Sie diesbezüglich einen Arzt aufsuchen bzw. haben Sie dies bereits getan?	☐ **JA (Einschlusskriterium)** ☐ NEIN
5. Haben Sie das Gefühl, dass Ihre geistige Leistungsfähigkeit schlechter ist als bei anderen Leuten Ihres Alters ist?	☐ JA ☐ NEIN
6. Ist das Gedächtnis betroffen?	☐ JA ☐ NEIN
7. Sind andere Funktionen betroffen?	**Antwort optional**

Ausschluss MCI/Demenz:

Haben Sie in letzter Zeit Gedächtnis-Verschlechterungen bei sich festgestellt (MCI)?	**ja**	nein
Haben Sie auch andere Beschwerden (Orientierung, Rechnen, Sprache, Bewegung)?	ja	nein
Können Sie sich im Alltag noch recht gut zurechtfinden?	ja	**nein**
- Wurden „leichte kognitive Einschränkung (MCI)“ diagnostiziert, wenn ja wo?	**ja**	nein
- Wann? _____ (>2 Jahre: neuer Termin Gedächtnissprechstunde)		
- Wurde bereits eine Demenz oder ein dementielles Syndrom bei Ihnen diagnostiziert?	**ja**	nein
Ist Deutsch Ihre Muttersprache?	ja	nein

Wie groß sind Sie? _____ m

Wie viel wiegen Sie? _____ kg

BMI (25-35)? _____ kg/m²

Leiden Sie an einer Zuckerkrankheit (Diabetes)?	ja	nein
Leiden Sie an einer Stoffwechselkrankheit?	ja	nein

Welche? _____

(z.B. Schilddrüsenüber-/-unterfunktion, Morbus Wilson, VitaminB12-/Folsäure-Mangel)

Die Nahrungsergänzungsmittel-Einnahme soll über 12 Monate erfolgen. Könnten Sie sich vorstellen, in dieser Zeit täglich Kapseln einzunehmen? (Nur für SCD-Probanden)	ja	**nein**
Planen Sie im nächsten Jahr (bzw. „in der nächsten Zeit“ bei *Probanden ohne SCD*) einen sehr langen Urlaub bzw. steht eine Operation an? Wenn ja, wann/wie lange? _____	ja	nein
Wurde bei Ihnen momentan oder früher eine Essstörung diagnostiziert (z.B. Magersucht, Ess-/Brech-Sucht) oder hat sich Ihr Gewicht in den letzten 3 Monaten stark geändert (>3 kg)?	**ja**	nein
Nehmen Sie bereits an einer anderen Studie teil, bzw. planen Sie dies?	ja	nein
Beinhaltet die Studie ionisierende Strahlung? Wenn ja, an welcher, worum geht es dort?	ja	nein

Medizinische Fragen:

Haben Sie einen Herzschrittmacher oder eine Medikamentenpumpe?	**ja**	nein
Haben Sie eine schwere unbehandelte arterielle Hypertonie (behandelt: dauerhaft systolischer Blutdruck > 140)?	**ja**	nein
Haben Sie eine schwere unbehandelte Herz- oder Gefäßerkrankung?	**ja**	nein
Leiden Sie an Gelenkerkrankungen oder Rheuma? (z.B. Vaskulitis, systemischer Lupus, Sarkoidose, Morbus Wegener)	**ja**	nein
Hatten Sie jemals Durchblutungsstörungen des Gehirns (Schlaganfall)?	**ja**	nein
Leiden Sie an einer schweren unbehandelten Leber- oder Niereninsuffizienz?	**ja**	nein
Wurden Sie schon mal wegen einer Einschränkung der Nierenfunktion behandelt?	ja	nein
Leiden Sie an Platzangst?	**ja**	nein
Sind in Ihrem Körper Metallteile (Künstliche Herzklappe, Cochlea-Implantat, Künstliche Linse, Stents (in Gefäßen), Shunt, Gefäßclips, Metallsplitter, Port, Permanent Make-Up/sonstiges, Tattoos)? Welche?	ja	nein
Bei MRT-tauglichen Metallteilen: Besitzen Sie einen Pass dafür? Wenn ja, was steht dort drin (MRT, welche Tesla-Zahl, genaue Beschreibung des Implantats)?	ja	**nein**
Bei Tattoos: Wie groß? Wo? Schwarz-Weiß oder Farbe?		
Sind bei Ihnen Allergien bekannt? (vorrangig Gluten- und/oder Histamin Unverträglichkeit?)	ja	nein
Allergien bei lokalen Betäubungsmittel (insbesondere Lidocain)? (relevant für Muskelbiopsie bei SCD-Probanden)	ja	nein
Haben sie eine schwere unbehandelte Atemwegserkrankung? (z.B. Asthma, chronische Bronchitis, Lungenemphysem, COPD)	**ja**	nein

Leiden Sie an einer neurologischen Erkrankung? (z.B. Multiple Sklerose, M. Parkinson, Chorea Huntington, Epilepsie, Hirnhautentzündung, Migräne)	**ja**	nein
Hatten Sie schon einmal eine Krebserkrankung (Ausnahme: Basaliom)? Wenn ja, welche/wann?	**ja**	nein
Bei Frauen: Hatten Sie schon einmal Brustkrebs?	**ja**	nein
Wurde jemals ein Tumor in Ihrem Kopf festgestellt?	**ja**	nein
Haben Sie jemals eine Kopfverletzung mit Bewusstseinsstörung oder nachgewiesener Hirnschädigung erlitten?	**ja**	nein
Hatten Sie jemals Epilepsie/ epileptische Anfälle?	ja	nein
Leidet jemand in Ihrer Familie an Epilepsie?	ja	nein
Nehmen Sie regelmäßig Medikamente ein? *Wenn ja, würden wir Sie bitten eine Liste zur ersten Untersuchung mitzubringen.*	ja	nein
Bestehen bei Ihnen Gerinnungsstörungen, Marcumar Therapie, oder ASS-Therapie? (relevant für Muskelbiopsie bei SCD-Probanden)	ja	nein
Sind Sie zurzeit in psychiatrischer Behandlung (Depression, Manie, Schizophrenie, etc.)?	**ja**	nein
Haben Sie eine Erkrankung, die eine generelle Einschränkung der Aufmerksamkeitsleistung beinhaltet?	**ja**	nein
Haben Sie eine andere akute Erkrankung? _____	ja	nein
Besteht eine Drogen- / Medikamenten - oder Alkohol**abhängigkeit** bei Ihnen?	**ja**	nein
Trinken Sie **Alkohol**? *Wenn ja*, wie viele Gläser regelmäßig pro Tag? ____	ja	nein
(mehr als ½ l Wein oder 1 l Bier)? *(mehr als 50g = ja = möglicher Ausschluss)*		
Rauchen Sie? *Wenn ja*, wie viele Zigaretten pro Tag? ____	ja	nein
(mehr als 10 = ja = möglicher Ausschluss)		
Nur bei Frauen um die 60 Jahre: sind Sie in der Menopause?	ja	nein

Notes: SCD: Subjective Cognitive Decline; MCI: Mild Cognitive Impairment; BMI: Body Mass Index; MRT: Magnetresonanztomographie (magnetic resonance Imaging).

Table S13: Food Frequency Questionnaire (FFQ; German)

1. Milch und Milchprodukte	Nie od <1/MO	1-3x pro MO	1x pro WO	2-4x pro WO	5-6x pro WO	1x pro Tag	2-3x pro Tag	4-5x pro Tag	6x pro Tag
Vollmilch (0.25 l)									
Entrahmte Milch (0.25 l)									
Joghurt (125 g)									
Topfen/Quark (100 g)									
Käse (100 g)									
Sahne (20 g)									
2. Früchte	**Nie od <1/MO**	**1-3x pro MO**	**1x pro WO**	**2-4x pro WO**	**5-6x pro WO**	**1x pro Tag**	**2-3x pro Tag**	**4-5x pro Tag**	**6x pro Tag**
Rosinen oder Trauben (100 g)									
Äpfel oder Birnen (1 Stück)									
Pfirsich, Marille, Pflaume (1 Stück)									
Orangen (1 Stück)									
Zitronen (1 Stück)									
Wassermelone (1 Scheibe)									
Zuckermelone (1/4)									
Erdbeeren (100 g frisch oder Marmelade)									
Schwarzbeeren (100 g oder Marmelade)									
Pflaumen (100 g)									
Ananas (1 Scheibe)									
Mango (1 Stück)									
Kirschen (50 g)									
Bananen (1 Stück)									
3. Gemüse	**Nie od <1/MO**	**1-3x pro MO**	**1x pro WO**	**2-4x pro WO**	**5-6x pro WO**	**1x pro Tag**	**2-3x pro Tag**	**4-5x pro Tag**	**6x pro Tag**
Tomaten (1 Stück)									
Tomatensauce (1 Portion)									
Zucchini, Gurken (100-150g)									
Salat (100-150 g)									
Kraut (100-150 g)									
Broccoli (100-150 g)									

Karfiol/Blumenkohl (100-150 g)									
Karotten (1 Stück)									
Rüben (100-150g)									
Erbsen (100 g, gekocht)									
Bohnen (100 g, gekocht)									
Linsen (100 g)									
Spinat (im Salat)									
Spinat (gekocht)									
Sellerie (1 Stück)									
Knoblauch (1 Zehe)									
Pilze (100g)									
Keimgemüse(Weizenkeime, Leinsamen, usw.)									

4. Tierisches Eiweiß	**Nie od <1/MO**	**1-3x pro MO**	**1x pro WO**	**2-4x pro WO**	**5-6x pro WO**	**1x pro Tag**	**2-3x pro Tag**	**4-5x pro Tag**	**6x pro Tag**
Eier (1 Stück)									
Geflügel (100-150 g)									
Rind- und Kalbfleisch (100-150g)									
Schweinefleisch (120-150g)									
Wild (200-250 g)									
Hammel und Kitzfleisch (150-200g)									
Wurst (Salami, Mortadella, Schinken,) 100-150 g									
Geräuchertes Fleisch (Speck, Rohschinken) 50-100 g									
Innereien (Leber, Milz, Niere)									
Dosenfisch (Thunfisch, Sardinen)									
Süßwasserfisch (Forelle) 200-250g									
Salzwasserfisch (Seezunge,..) 250 g									
Soja (1 Portion)									
Nüsse (50g)									

5. Süßigkeiten	**Nie od <1/MO**	**1-3x pro MO**	**1x pro WO**	**2-4x pro WO**	**5-6x pro WO**	**1x pro Tag**	**2-3x pro Tag**	**4-5x pro Tag**	**6x pro Tag**
Schokolade (1 Riegel)									
Mehlspeisen (1 Stück)									

gemeint ist z.B. 1 Stück Torte									
Eis (2 Kugel)									
Bonbons und Pralinen									
Honig (25g)									
Kekse									
6. Kohlenhydrate	**Nie od <1/MO**	**1-3x pro MO**	**1x pro WO**	**2-4x pro WO**	**5-6x pro WO**	**1x pro Tag**	**2-3x pro Tag**	**4-5x pro Tag**	**6x pro Tag**
Weißbrot (1 Semmel bzw. 1 Scheibe)									
Vollkornbrot (1 Scheibe)									
Teigwaren (100 g) (Nudeln, Spatzln..)									
Knödeln (1 Stück)									
Müsli (1 Portion)									
Kartoffeln (150-200 g)									
Reis (100g)									
Polenta (100g)									
Omeletten (1 Stück)									
Pizza (1 Stück) hier ist nur der Teig gemeint – Auflagen gehen in andere Items ein									
Pommes frites (1 Portion)									
Kartoffelchips									
7. Fette	**Nie od <1/MO**	**1-3x pro MO**	**1x pro WO**	**2-4x pro WO**	**5-6x pro WO**	**1x pro Tag**	**2-3x pro Tag**	**4-5x pro Tag**	**6x pro Tag**
Butter (15 g)									
Schweinefett									
Margarine (15g)									
Olivenöl (1 Esslöffel)									
Samenöl (1 Esslöffel)									
Mayonnaise (1 Portion)									
8. Getränke	**Nie od <1/MO**	**1-3x pro MO**	**1x pro WO**	**2-4x pro WO**	**5-6x pro WO**	**1x pro Tag**	**2-3x pro Tag**	**4-5x pro Tag**	**6x pro Tag**
Cola (1 kleine Flasche)									

Fanta, Sprite u.a. (1 kleine Flasche)									
Apfelsaft (1 kleine Flasche)									
Zitronensaft (1 kleine Flasche)									
Orangensaft (1 kleine Flasche)									
Tee (0.2 L)									
Mineralwasser (1/4 L)									
Rotwein (1/4 L)									
Weißwein (1/4 L)									
Bier (0.33 L)									
Liquore, Schnaps (2 cl)									
Kaffee (1 Tasse)									
Zuckerkonsum Kaffee/Tee (1 Würfel)									

Notes: MO: Monat (month); WO: Woche (week). Food frequency questionnaire based on the gold-standard FFQ by Willett et al. (1985) and Eisenberg et al. (2016).

Table S14: Mediterranean Diet Adherence Screener (MEDAS; German)

1. Verwenden Sie als Speisefett vorwiegend Olivenöl?	**Nein**	**Ja**
2.Wie viel Olivenöl verbrauchen Sie an einem Tag (berücksichtigen Sie auch Öl zum Braten, für Salate, beim Essen außer Haus usw.)?	Weniger als 4 Esslöffel	4 oder mehr Esslöffel
3.Wie viel Gemüseportionen essen Sie pro Tag? *(1 Portion = 200 g – Beilagen gelten als 1/2 Portion)*	Weniger als 2	2 oder mehr *(wenigstens 1 Portion roh oder als Salat)*
4.Wie viele Früchte (einschließlich natürlicher Fruchtsäfte) essen Sie pro Tag?	Weniger als 3	3 oder mehr
5.Wie viele Portionen rotes Fleisch, Hamburger oder Fleischprodukte (Schinken, Würstchen usw.) essen Sie pro Tag? *(1 Portion = 100-150 g)*	1 oder mehr	Weniger als 1
6.Wie viele Portionen Butter, Margarine oder Sahne essen Sie pro Tag? *(1 Portion = 12 g)*	1 oder mehr	Weniger als 1
7.Wie viele süße/kohlensäurehaltige Getränke trinken Sie pro Tag?	1 oder mehr	Weniger als 1
8.Wie viel Wein trinken Sie pro Woche?	Weniger als 7 Gläser	7 oder mehr Gläser
9.Wie viele Portionen Hülsenfrüchte essen Sie pro Woche? *(1 Portion = 150 g)*	Weniger als 3	3 oder mehr
10.Wie viele Portionen Fisch oder Meeresfrüchte essen Sie pro Woche? *(1 Portion: 100-150 g Fisch oder 4-5 Stück oder 200 g Meeresfrüchte)*	Weniger als 3	3 oder mehr
11.Wie oft in der Woche essen Sie gekaufte Süßigkeiten oder süße Backwaren (nicht selbstgemacht), z. B. Kuchen, Gebäck, Kekse oder Vanillesoße?	3 oder mehr	Weniger als 3
12.Wie viele Portionen Nüsse (auch Erdnüsse) essen Sie pro Woche? *(1 Portion = 30 g)*	Weniger als 3	3 oder mehr
13.Essen Sie vorzugsweise Hühnchen-, Enten- oder Kaninchenfleisch anstelle von Rind, Schwein, Hamburger oder Würstchen?	Nein	Ja
14.Wie oft in der Woche essen Sie Gemüse, Nudeln, Reis oder andere Gerichte, die mit Sofrito (einer Soße aus Tomaten, Zwiebeln, Lauch oder Knoblauch, in Olivenöl erhitzt) gewürzt sind?	Weniger als 2	2 oder mehr

Notes: The left column lists food not attributed to a Mediterranean Diet and the right column lists food pertaining to the Mediterranean Diet. The examiners of the current study translated the questionnaire into German, which is similar to the validated German version of Hebestreit et al. (2017).

Table S15: Qualitative food frequency list (FFL; German)

. Wie häufig verwenden Sie in Ihrem Haushalt folgende Fette bzw. Öle zum Kochen, Backen oder Braten?

Butter:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
Margarine:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
↳ Markenname:				
Biskin/Palmin:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
Sonnenblumenöl:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
Olivenöl:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
Mayonnaise:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
Sahne/Crème fraîche:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
Sonstiges:	☐ regelmäßig	☐ gelegentlich	☐ gar nicht	☐ weiß nicht
↳ was genau?				

Salzen Sie Ihr Essen nach?

☐ Wenn das Essen nicht genügend gesalzen ist
☐ Immer, bevor ich gekostet habe
☐ Nie

Welches Speisesalz verwenden Sie zu Hause?

☐ Normales Salz
☐ Meersalz
☐ Jodiertes Salz
☐ Natriumarmes Salz (Kaliumsalz)
☐ Ich weiß nicht

Wenn Sie auswärts essen, schmeckt das Essen dann verglichen mit dem zu Hause in der Regel weniger salzig, genauso salzig, oder salziger?

☐ Weniger salzig
☐ Genauso salzig
☐ Salziger

	Täglich bzw. fast täglich	Mehrmals in der Woche	Etwa einmal in der Woche	Mehrmals im Monat	Einmal im Monat oder seltener	Nie
Fleisch (ohne Wurstwaren)	☐	☐	☐	☐	☐	☐
Wurstwaren, Schinken	☐	☐	☐	☐	☐	☐
Geflügel	☐	☐	☐	☐	☐	☐
Fisch	☐	☐	☐	☐	☐	☐
Kartoffeln (auch Pommes)	☐	☐	☐	☐	☐	☐
Teigwaren	☐	☐	☐	☐	☐	☐
Reis	☐	☐	☐	☐	☐	☐
Salat oder Gemüse, roh zubereitet	☐	☐	☐	☐	☐	☐
Gemüse, gekocht	☐	☐	☐	☐	☐	☐
Frisches Obst	☐	☐	☐	☐	☐	☐
Schokolade, Pralinen	☐	☐	☐	☐	☐	☐
Kuchen, Gebäck, Kekse	☐	☐	☐	☐	☐	☐
Sonstige Süßwaren (Bonbons, Kompotte u.ä.)	☐	☐	☐	☐	☐	☐
Zitrusfrüchte (Orangen, Grapefruit)	☐	☐	☐	☐	☐	☐
Soja-Produkte (Sojasauce, Sojamilch, Tofu)	☐	☐	☐	☐	☐	☐
Sauerkraut	☐	☐	☐	☐	☐	☐
Salzige Knabbereien wie gesalzene Erdnüsse, Chips etc.	☐	☐	☐	☐	☐	☐
Weißbrot, Mischbrot, Toastbrot	☐	☐	☐	☐	☐	☐
Vollkornbrot, Schwarzbrot, Knäckebrot	☐	☐	☐	☐	☐	☐
Haferflocken, Müsli, Cornflakes	☐	☐	☐	☐	☐	☐
Quark, Joghurt, Dickmilch	☐	☐	☐	☐	☐	☐
Käse	☐	☐	☐	☐	☐	☐
Eier	☐	☐	☐	☐	☐	☐
Milch einschl. Buttermilch und Vollmilch-produkte (Joghurt, Quark)	☐	☐	☐	☐	☐	☐
Fettarme Milchprodukte bis zu 1,5 % Fett-gehalt (Joghurt, Milch, Quark etc.)	☐	☐	☐	☐	☐	☐
Obstsäfte	☐	☐	☐	☐	☐	☐
sonstige Erfrischungsgetränke (Limonade, Cola u.ä.)	☐	☐	☐	☐	☐	☐
Mineralwasser	☐	☐	☐	☐	☐	☐
Diätlimonaden, sonstige Diätgetränke	☐	☐	☐	☐	☐	☐
Alkoholfreies Bier	☐	☐	☐	☐	☐	☐

STATUTORY DECLARATION

“I, Nora Horn, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Dietary polyamine intake and its association with brain structure, cognition, and cardiovascular risk in older adults” / “Diätetische Polyaminzufuhr und ihr Zusammenhang mit der Gehirnstruktur, der Kognition und dem kardiovaskulären Risiko bei älteren Menschen”, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date 04.05.2020 Signature

Declaration of your own contribution to any publications

Nora Horn contributed the following to the below listed publications:

Publication 1:
Horn N., Schwarz C., Benson G., Pechlaner R., Wirth M. & Flöel A. (2019). Dietary Spermidine Intake is Associated with Hippocampal Volume and Cortical Thickness in Older Adults. *Alzheimer’s & Dementia*, 15, P1207-P08. (Abstract, DOI: 10.1016/j.jalz.2019.06.3641)

Contribution: Major role in the acquisition of data, recruitment of study participants, medical examination, MRI-acquisition and -analyses, data entry, data analyses, statistical analyses, interpretation of data. Drafting of the abstract, presentation of the poster at the conference.

_____

Signature, date and stamp of first supervising university professor

_____

Signature of doctoral candidate

CURRICULUM VITAE

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

PUBLICATIONS

1. Schwarz C.*, **Horn N.*,** Benson G., Wrachtrup Calzado I., Wurdack K., Pechlaner R., Grittner U., Wirth M. & Flöel A. Spermidine Intake is Associated with Cortical Thickness and Hippocampal Volume in Older Adults. Submitted at *Neuroimage* in April 2020 (under review, manuscript number: NIMG-20-901).

2. Wirth M.*, Schwarz C.*, Benson G.*, **Horn N.**, Buchert R., Lange C., Köbe T., Hetzer S., Maglione M., Michael E., Märschenz S., Mai K., Kopp U., Schmitz D., Grittner U., Sigrist S.J., Stekovic S., Madeo F. & Floel A. (2019). Effects of spermidine supplementation on cognition and biomarkers in older adults with subjective cognitive decline (SmartAge) - study protocol for a randomized controlled trial. *Alzheimer's Research & Therapy*, 11**,** 36.

Abstracts:

1. **Horn N.**, Schwarz C., Benson G., Pechlaner R., Wirth M. & Flöel A. (2019). Dietary Spermidine Intake is Associated with Hippocampal Volume and Cortical Thickness in Older Adults. *Alzheimer's & Dementia*, 15, P1207-P08. *Poster presentation at the Alzheimer's Association International Conference (AAIC),* Los Angeles, U.S.A. (2019).

2. Schwarz C., Lange C., Benson G., **Horn N.**, Wurdack K., Wrachtrup Calzado I., Lukas M., Buchert R., Wirth M. & Flöel A. (2019). Amyloid-β and its Association with Cortical Thickness, Memory, and Cognitive Complaints in Older Adults. *Alzheimer's & Dementia,* 15, P428-P29. *Poster AAIC* (L.A., 2019).

3. Schwarz C., Benson G., **Horn N.**, Wurdack K., Wrachtrup Calzado I., Kuhn E., Chetelat G., Wirth M. & Flöel A. (2019). Reduction of Hippocampal Subfield Volumes in Older Adults with Subjective Cognitive Decline. *Alzheimer's & Dementia*, 15, P1114-P15. *Poster AAIC* (L.A., 2019).

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5. Benson G., Schwarz C., **Horn N.**, Marchant N., Flöel A. & Wirth M. (2018). Beyond anxiety and depression: rumination, stress coping, and quality of life in subjective cognitive decline. *Alzheimer's & Dementia*, 14, P416. *Poster AAIC* (Chicago, 2018).

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