Cardiovascular risk factors in ageing brains:

Functional and structural correlates of modifiable risk factors of brain ageing and Alzheimer's disease among older individuals

Dissertation
zur Erlangung des akademischen Grades
Dr. rer. med.

an der Medizinischen Fakultät
der Universität Leipzig

eingereicht von:

M.Sc. Shahrzad Kharabian Masouleh
19.09.1985, geboren in Tehran, Iran

angefertigt am:

Max-Planck-Institut für Kognitions- und Neurowissenschaften, Abteilung Neurologie, Leipzig

Betreuer:

Prof. Dr. med. Arno Villringer

Dr. A. Veronica Witte

Beschluss über die Verleihung des Doktorgrades vom: 16.04.2019

Content

List of Abbreviations

AD	Alzheimer's disease
ARD	Automatic relevance determination
BMI	Body mass index
BOLD	Blood-oxygen level dependent
DMN	Default mode network
EPI	Echo-planar imaging
FC	Functional connectivity
GICA	Group independent component analysis
GM	Grey matter
ICA	Independent component analysis
MeDi	Mediterranean style diet
MRI	Magnetic resonance imaging
NMV	Net magnetization vector
PCA	Principle component analysis
RF	Radio-frequency
Rs-fMRI	Resting state Functional magnetic resonance imaging
T1	Longitudinal relaxation time
TE	Echo times
TR	Rrepetition times
VAT	Visceral adipose tissue
VBM	Voxel-based morphometry

List of Figures

List of Tables

1. Introduction

The world is on the brink of a demographic milestone. We soon will have more elderly people than children and more people at extreme old age than ever before. Driven by falling fertility rates and remarkable increases in life expectancy, population ageing will continue and even accelerate (Figure 1) (WHO, 2011).

At a biological level, ageing results from the accumulation of a wide-variety of molecular and cellular damage over time. In the brain, this leads to structural and functional alterations and gradual decrease in cognitive abilities.

Normal ageing, which is experienced by the majority of individuals, prompts subtle cognitive changes, mainly in domains of processing speed, memory and executive functions (Harada et al., 2013). However, it is known that there is considerable variability among individuals in the apparent rate of ageing, and only a loose association of these changes to the person's chronological age has been observed. This indicates importance of other factors modulating the effects of ageing on the brain.
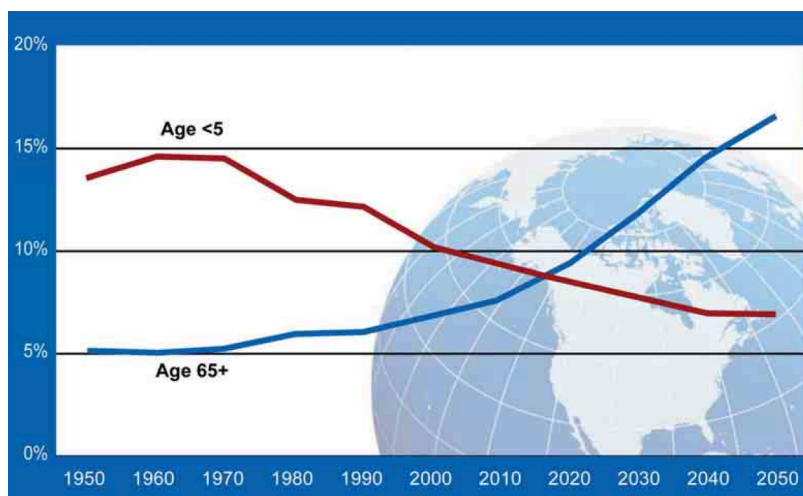

Figure 1. Young children and older people as a percentage of global population: 1950-2050.
Source: http://www.who.int/ageing/publications/global_health.pdf

Furthermore, as the population gets older, the number of individuals developing neurodegenerative diseases such as dementia and mild cognitive impairment increases. Although, by definition these pathologic conditions are not part of a normal ageing process, observations show that almost all aged brains show characteristic changes that are linked to neurodegeneration (Wyss-Coray, 2016). This raises the possibility that fundamental mechanisms of ageing may display early disease changes or contribute to the pathogenesis of neurodegenerative disorders (Bartzokis, 2011; Bishop et al., 2010; Raz, 2005).

Due to related high global social and economic costs and lack of causal therapeutic options for neurodegenerative diseases, risk-reduction has been increasingly recognised as the key prevention strategy. Therefore, studies on identification of possible "modifiable" risk factors have become a research priority. Most studies focus on healthy elderly and aim to identify genetic as well as environmental factors that accelerate ageing on both the brain and the behavioural level and the possible link between the accelerated ageing-associated changes and an increased risk of neurodegenerative diseases.

In this doctoral thesis, I focus on major modifiable, life-style related risk factors associated with increased risk of the most prevalent ageing-associated neurodegenerative disease, namely Alzheimer's disease (AD). The aim is to identify alterations associated with these risk-factors among the elderly population and their influence on an accelerated brain- as well as cognitive-ageing (Barnes and Yaffe, 2011).

In section 1.1, I briefly review the current knowledge on typical ageing-associated alterations in human brain structure and function, assessed with

in-vivo magnetic resonance imaging (MRI).

The major modifiable risk factors of brain ageing and their estimated impact on prevalence of AD, are briefly covered in section 1.2.

Section 1.3, covers a basic description of imaging modalities and processing algorithms, which are used to assess structural and functional properties of the brain at an individual level.

Based on this information, I will derive the rationale and main questions (section 1.4) that have been addressed in our 4 published papers, which form the basis of the present cumulative dissertation (section 2).

1.1: "Normal" cognitive ageing

As individuals get older, they will experience individual levels of alterations in their cognitive abilities, which can be attributed to a normal ageing process. These often subtle changes in cognitive abilities are particularly represented by difficulties in episodic memory and in tasks that represent different types of high level functions, such as tasks that involve working memory (Mccabe et al., 2010), attention, and task switching behavior (Grady, 2012). Older adults also have generally slower processing speed (Salthouse and Madden, 2013). Nevertheless, some aspects of cognition, such as crystallized intelligence, referring to skills and knowledge that is overlearned and familiar, are resilient to ageing (Harada et al., 2013). Understanding neurobiological mechanisms that underlie these alterations in cognitive abilities, is a challenge, on which structural and functional neuroimaging studies of ageing have focused.

1.1.1. Ageing-associated changes in brain structure and function

In-vivo assessment of structural properties of brain's grey matter (GM) is commonly determined by-means of T1-weighted MRIs, (*see section 1.3.1, for definition*). Starting from middle age, decreased total GM volume and widespread cerebral cortical thinning, spanning cortical regions in primary as well as in association cortices, have been shown (Salat et al., 2004). Despite this global pattern, several lines of evidence suggest differential vulnerability to ageing in different parts of the brain (Raz, 2005). More specifically, in older individuals, accelerated decrease in volume and thickness have been predominantly documented in the prefrontal cortex, inferior temporal lobe, posterior association cortices, hippocampus and in subcortical structures such

as the striatum (Fjell et al., 2014; Fjell and Walhovd, 2010; Salat et al., 2004; Sowell et al., 2003).

Higher vulnerability of these areas to ageing processes is also supported by evidence of pronounced age-related decline in certain cognitive abilities such as speed of processing, episodic and working memory, cognitive control including response inhibition and interference suppression, all of which rely on the integrity of these specific brain areas (Raz et al., 1998; West, 1996).

Recent developments in data-driven analyses of MRI have immensely improved our understanding of brain structural and functional organization. It has been shown that the "structure" of the brain is organized in networks of distinct regions and morphological properties of areas that belong to the same structural network, co-vary together and follow the same developmental trajectories over the life span (Alexander-Bloch et al., 2013). It has also been shown that the strength of these brain-wide co-variance pattern is correlated with individuals' scores on different cognitive tests (Brickman et al., 2008, 2007) (even in healthy young subjects (Steffener et al., 2013)), emphasizing the practical significance of these network structures. Furthermore, regions belonging to the same structural network, are functionally connected, as defined using resting state functional magnetic resonance imaging (rsfMRI), see section 1.3.1, for details on rsfMRI and definition of functional connectivity (FC).

The default mode network (DMN), comprised of the medial prefrontal cortex (MPFC), the posterior cingulate cortex (PCC), the precuneus, the anterior cingulate cortex (ACC) and the bilateral parietal cortices as well as the hippocampal formation, is one such network (Raichle et al., 2001), which has

gained particular importance as an early biomarker for cognitive decline and AD (Damoiseaux et al., 2012; Petrella et al., 2011; Sorg et al., 2007). Among healthy individuals, the volume of GM and FC among sub-regions (nodes) of the DMN, correlates with episodic memory and executive function performance and declines over the lifespan (Andrews-Hanna et al., 2007) (Koch et al., 2010) (Damoiseaux et al., 2007; Douaud et al., 2014). In AD patients, regional atrophy is predominantly documented in DMN (e.g. (Fjell et al., 2015, 2014)), and FC among its nodes is also shown to be disrupted in advanced stages of the disease (Buckner et al., 2008).

In their publication in 2009, Seeley et al. showed several other examples of such convergent patterns of structural and functional covariance networks, identified in healthy individuals and that are targeted by specific neurodegenerative conditions.

Such coordinated change within spatially separated regions could hint towards shared susceptibility of regions within one network to selective pathologies or a network-based spread of toxic agents (Zhou et al., 2012).

Through similar mechanisms, factors modulating risk of neurodegenerative conditions and cognitive decline may also affect GM structure and functional properties in large-scale networks, rather than in independent regions.

In the next section, I will briefly introduce the current knowledge about major modifiable factors that accelerate brain ageing and their estimated impact on AD-prevalence.

1.2. Modifiers of brain ageing and AD

As briefly discussed in section 1.1, structural and functional properties of brain alters as individuals get older. However, previous research has shown that the

rate of brain ageing and its associated cognitive decline is not inevitably fixed but is plastic and possibly open to modification (Bishop et al., 2010).

In the last decades, several observational studies have identified a wide range of such potentially modifiable risk factors including cardiovascular risk factors (eg. obesity, diabetes and hypertension) and unhealthy behaviour (eg. low level of mental activity and smoking) (Barnes and Yaffe, 2011; Buchman et al., 2012; Fotuhi et al., 2012). In the same line, protective factors that reduce cardiovascular risk, namely regular exercise and a healthy diet seem to have beneficial effects on brain health and on the preservation of cognitive abilities in elderly (WHO, 2009).

Furthermore, although age is known as the strongest predictor of neurodegenerative conditions (e.g. late-onset AD), this important risk factor should be considered in the context of genetic predisposition, lifestyle and environmental factors. It has been estimated that one in three late-onset AD cases worldwide is attributable to one or more of seven key risk factors, namely, midlife obesity, diabetes, midlife hypertension, smoking, depression, physical inactivity, and low educational attainment (Barnes and Yaffe, 2011; Norton et al., 2014). It has also been estimated that a relative reduction of 10% per decade in the prevalence of each of these seven risk factors could reduce the prevalence of late-onset AD in 2050 by 8.3% worldwide (Norton et al., 2014).

In this context, many studies in the past decade have focused on structural and functional changes in the brain, underlying this manipulated risk of cognitive decline and AD. Obesity, as a major epidemic of the twentieth

century, is one of the most studied factors. Worldwide, the prevalence of obesity has nearly doubled since 1980 (WHO, 2014). A recent study, pooling data from 1698 population-based studies, estimating trends in mean body-mass index (BMI) and prevalence of different categories of BMI, revealed that the trend is present in 200 countries, both in men and women (NCD Risk Factor Collaboration, 2016). Based on their prediction, if the post-2000 trends continue, by 2025, global obesity prevalence will reach 18% in men and surpass 21% in women, with severe obesity exceeding 6% of individuals of both gender (NCD Risk Factor Collaboration, 2016).

These results are alarming, as obesity has been closely associated with higher prevalence of other cardiovascular risk factors such as type-2 diabetes, hypertension, and fatal outcomes such as coronary heart disease and ischemic stroke.

Additionally, numerous epidemiological studies have found that obesity in midlife has a high predictive value for cognitive impairments later in life, and can thus be regarded as an important risk factor (e.g., (Anstey et al., 2011; Beydoun et al., 2008; Fitzpatrick et al., 2009; Gustafson, 2006; Kivipelto et al., 2005; Whitmer et al., 2005); for review see (Emmerzaal et al., 2015); but see (Qizilbash et al., 2015) and (Kivimäki et al., 2017) for recent discussions.).

In the central nervous system, the trajectory of increased BMI over the life course has been associated with brain atrophy, white matter changes, disturbances of blood–brain barrier integrity, and an increased risk of late-onset dementia and AD (Kiliaan et al., 2014). One recent review, accounting for other associated risk factors, estimated 2% of AD cases worldwide being

potentially attributable to midlife obesity defined by BMI above 30 (Norton et al., 2014).

However, effects of obesity in later life on cognitive performance in non-demented individuals are equivocal. Some studies show lower performance in most cognitive domains including executive functions and memory (e.g. (Benito-León et al., 2013; Walther et al., 2010)), in association to higher BMI, while others did not find a reliable effect, partly dependent on sex and age range studied (e.g., (Elias et al., 2005); for a review, see (Smith et al., 2011)).

In addition, obesity-related cardiovascular risk factors such as type 2 diabetes mellitus and hypertension, have been associated with general lower GM volume and cortical thickness. These changes, found in some but not in all studies, were specifically located in vulnerable regions in the frontal and the medial temporal lobes (reviewed in (Friedman et al., 2014)). Furthermore, variability in blood pressure has also been associated with changes in GM structure, although the literature suffers a substantial discrepancy on this topic with respect to location and extent of the effects and pattern of the associations (Foster-Dingley et al., 2015; Friedman et al., 2014), specifically in older ages. Norton et al. in their review in 2014 predicted that 3% and 5% of AD cases worldwide could be attributed to type2 diabetes and midlife hypertension, respectively (Norton et al., 2014).

Interestingly, more favourable metabolic profiles such as lower glucose levels, even within normal ranges, have been shown to exert protective effects on AD-risk and microstructure of related temporal brain areas (Crane et al., 2013; Kerti et al., 2013; Villeneuve et al., 2014), suggesting significant effects of

body composition. Further, widespread cortical thinning in frontal and temporal lobes have been associated with lower levels of high-density lipoprotein (HDL) (Villeneuve et al., 2014).

Smoking, as an important independent cardiovascular risk factor, with a high prevalence of ~27% worldwide, is also associated with general cortical thinning (Karama et al., 2015), increased severity of some abnormalities typical for AD, including amyloidogenesis, neuroinflammation and tau phosphorylation (Moreno-Gonzalez et al., 2013) and eventually increased risk of AD, with around 14% of AD cases worldwide being attributable to smoking (Norton et al., 2014).

Physical inactivity as well as lower levels of education and mental activity, due to their high prevalence worldwide (~18% and 40%, respectively), serve as other major contributors of AD, attributed to 13% and 19% of cases worldwide, respectively. Beneficial effects of physical activity on brain structure, mainly increased cortical thickness and volume in the frontal lobe, and on cognitive function, especially memory performance, have been shown in several cross-sectional studies (Buchman et al., 2012; Colcombe et al., 2006; de Bruijn et al., 2013; Erickson et al., 2014; Flöel et al., 2010; Ruscheweyh et al., 2011).

These findings have already led to the initiation of promising large-scale longitudinal controlled trials (Ngandu et al., 2015). One example is the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), a randomised controlled trial that assessed effectiveness of a multi-domain intervention (diet, exercise, cognitive training, vascular risk

monitoring) to prevent cognitive decline in ~1200 at-risk elderly individuals from the general population. Findings of this large-scale study show a modest, yet significant, improvement in total neuropsychological tests, as the result of intervention, in a two-year follow-up assessment. Similar modest improvement in cognition was reported in the French Multidomain Alzheimer's Prevention Trial (MAPT) (Vellas et al., 2014). However, results of the six-year Prevention of Dementia by Intensive Vascular Care (PreDIVA) trial, questioned translation of such intervention to protection against Alzheimer's disease or other dementias in broad population (Moll van Charante et al., 2016). However, possible physiological and structural changes resulted by these interventions are yet to be reported.

In sum, there is a global intensified effort to identify causes and cures for neurodegenerative diseases, specifically the late-onset AD. Given that to this date no effective treatment has been found, risk factors identification and reduction is considered as the most promising approach to delay onset and potentially reduce the number of new AD cases (Barnes and Yaffe, 2011; Norton et al., 2014). In-vivo MRI gives precious insight into the neuronal correlates of these risk factors. However, as briefly reviewed in this section, there is a considerable amount of discrepancies in timing, location and extent of the observed effects and previous studies could neither establish a consistent pattern of regional changes, nor answer the question to what extent these changes could affect cognitive performance. This might be partly due to smaller sample sizes and different characteristics of the samples under study. Moreover, important confounders have not consistently been considered, rendering potential independent effects of these risk factors in

older age still debatable (Friedman et al., 2014; Prickett et al., 2015).

This doctoral thesis aims to overcome many of the above-mentioned shortcomings. The goal is to assess the independent effects of each of the major cardiovascular risk factors on structure and function of brain, assessed using state-of-the-art methods on high-resolution MRI at 3Tesla, among yet cognitively intact older individuals from the population-based LIFE-Adult-Study (Loeffler et al., 2015).

1.3. Methods

In this thesis brain's functional and structural properties were investigated using in-vivo magnetic resonance imaging.

1.3.1. Imaging protocols

For assessment of brain's anatomy, T1-weighted MRI is the standard protocol. It relies on longitudinal relaxation times of tissue's net magnetization vector (NMV) after application of a transverse (90 degree) radio-frequency (RF) pulse in a strong external magnetic field (3 Tesla, in case of our study). By incorporation of appropriate scanning parameters, such as short repetition times (TR) of the RF pulses and short delays before detecting the signal (TE), this protocol provides very good contrast between grey and white matter in most parts of the brain. Therefore, it is used for qualitative and quantitative assessment of brain's anatomy in healthy and pathologic conditions.

The activity of the brain is assessed using blood-oxygen level dependent (BOLD) functional MRI (fMRI), first introduced by (Ogawa et al., 1990). BOLD fMRI is an indirect measure of neuronal activity, based on relationship between neural activity, energy demands and blood flow (Logothetis, 2002).

It relies on detecting the changes in blood oxygenation and blood flow that occur in response to neuronal activity, usually as a result of engaging in a task. What we measure with functional MRI is the changes in magnetic field caused by difference in the magnetic susceptibility of oxygenated (diamagnetic) and deoxygenated (paramagnetic) blood, as an indirect measure of energy consumption. However, there are further parameters that contribute to the alteration in blood oxygenation, including oxygen consumption dynamic, cerebral blood flow and volume (Logothetis, 2002).

In 1995, Biswal et al. measured BOLD fMRI at rest (no task). They observed intrinsic spontaneous slow oscillations that were correlated within functionally coupled networks (Biswal et al., 1995). They concluded that correlation of low frequency fluctuations in different brain regions, which may arise from fluctuations in blood oxygenation or flow, is a manifestation of functional connectivity (FC) between brain regions.

Later, other researchers identified several large-scale networks of such correlated activity at rest, which resembled different task-based co-activation maps (Smith et al., 2009).

1.3.2. Network Identification

For extraction of different networks from resting-state fMRI (rsfMRI) and structural MRI, we use independent component analysis (ICA). ICA is a particularly efficient model for finding meaningful spatially independent components in an unsupervised setting, as it searches for non-Gaussian spatial sources that are likely to represent real features of the data. Unlike in a principal component analysis (PCA), the mixing matrix vectors of an ICA are not forced to be orthogonal to each other, and thus can explain common variance of variables external to the ICA. Because of different types of the inputs and different assumptions about the independent sources, we use different implementations of ICA for resting-state and GM structural data.

1.3.2.1. Resting-state fMRI network extraction

For estimation of resting-state ICA components, we used group independent component analysis (GICA) algorithm implemented in Matlab within GIFT toolbox (http://mialab.mrn.org/software/#gica).

GICA, involves two data reduction steps at subject and group level, using PCA. Here one begins with pre-processing of each participant's scans. The pre-processing step includes removal of the first few volumes, co-registration of the functional scan with participant's own anatomical image, motion and Echo-planar imaging (EPI) distortion and slice-time correction, normalization to the template space (MNI) and eventually reslicing to 3 mm isotropic voxels and smoothing with a gaussian kernel of 6 mm full-width-at- half-maximum. Each participant's pre-processed and intensity normalized rsfMRI scans undergo the first PCA, which reduces the number of time points for each individual separately. Then all subjects' PCA-reduced data are concatenated in time and a second level PCA is applied on this aggregate data to reduce the dimensionality of the data to the number of components to be estimated by ICA. A noise-free spatial ICA (based on Infomax-ICA implementation (Bell and Sejnowski, 1995)) is then used to extract the group-level spatial independent sources and a mixing matrix. The subject-specific spatial maps and time courses are then calculated using GICA3 back-projection algorithm method, which is based on PCA compression and projection. GICA and GICA3 back-projection algorithms are introduced in more detail in (Calhoun et al., 2001; Erhardt et al., 2011).

1.3.2.2. Grey matter structural network extraction

For estimation of structural networks, we used T1-weighted MRIs and incorporated information from three complementary types of GM image processing: GM volume as assessed using optimized voxel-based morphometry (VBM) in FSL software package (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM; FSL 4.1), vertex-wise cortical

surface thickness and area measures calculated using FreeSurfe (http://surfer.nmr.mgh.harvard.edu/; FreeSurfer 5.0.0). In VBM, T1-weighted images are matched up in a common space and segmented to classify grey matter, white matter and cerebrospinal fluid. By accounting for regional stretching and compression at each voxel, final voxel values could be considered as measure of local absolute volume.

On the other hand, cortical thickness measurements involve identification of the inner and outer cortical surfaces, achieved using surface geometry to construct a representation of the grey and white matter surfaces (Fischl and Dale, 2000; MacDonald et al., 2000; Miller et al., 2000). Thickness at each point on the GM surface is then given by a distance measure between corresponding points on the two surfaces. As by-product of the above-mentioned analysis, the surface area at the interface between grey and white matter can also be computed.

These measures are then smoothed with Gaussian kernels, accordingly and used in Linked-ICA framework, which derives spatially independent components from input data, consisting of more than one modality (input type) per participant.

Linked-ICA is a data-driven approach that can co-model multiple imaging modalities and extracts linked (Spatial) components based on Bayesian-ICA.

Unlike ICA algorithm used on the functional data, Bayesian-ICA incorporates a data reduction step in the ICA method itself by use of "automatic relevance determination (ARD)" on components. Furthermore, it models an explicitly "parameterized" non-Gaussian source model.

In this thesis, we used Linked flat ICA, which stacks maps of all subjects for each modality (input type) and applies Bayesian ICA on the stacked data.

In this model, the same subject-loading matrix is shared between all modalities (input types), so each resulting component consists of a single subject-course (loading) and one spatial map per modality (i.e. GM volume, cortical thickness and surface area). Linked-ICA is introduced in more detail in (Groves et al., 2011) (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLICA).

1.4. Rationale of the work

Epidemiological studies, as stated earlier, have identified several modifiable parameters, which might have substantial effect on accelerated brain ageing and AD prevalence. Even eating patterns in different countries have been shown to have causal influence on altered risk of AD and other types of dementia.

In our first study, we review evidence available in the literature on positive impact of "mediterranean style diet" (MeDi) on cognitive functioning and brain structure in ageing (Huhn et al., 2015). More specifically, we focus on two major components of MeDi, which have attracted increasing interest in the last years, namely long chain omega-3 fatty acids (LC-n3-FA), derived from fish, and plant polyphenols, which occur mainly in fruit, tea and red wine. Adherence to MeDi has been associated with better metabolic profiles and lowered cardiovascular risk score, two major factors that in turn are associated with decreased risk of AD worldwide.

Despite an increasing number of interventional studies aiming at manipulation of AD risk, by means of risk factors reduction, the mechanisms underlying this manipulated risk remain elusive.

In the next step, we choose a mechanistic approach, focusing on neuronal correlates of obesity as a major component of metabolic syndrome and an important cardiovascular risk factor. Thus, we investigated the influence of higher body mass index on brain structure and function, using T1-weighted and resting state functional MRIs, in a large cross-sectional sample of yet cognitively intact older adults. Furthermore, we explored the pathways by

which higher obesity could influence cognitive functioning in older individuals. Independence of these effects from a large set of possible confounders including other cardiovascular risk factors, depression and genetic predisposition to AD and cognitive decline, is an important aspect of our research.

In our next investigation, we followed-up our previous approach and expanded the scope of modifiers of brain ageing. Here, we investigated the association of a large number of important risk factors, including obesity, smoking status, physical activity, systolic blood pressure, glucose and lipid metabolism with brain GM structure across a large cohort of community-dwelled older individuals. Inspired by our results on network-based FC alterations and in-line with the hypothesis of network-based spread of toxic agents in neurodegenerative diseases, in the later study, instead of focusing on traditional voxel-wise associations in one modality, we identified modifying effects of these risk factors on large-scale GM "networks". We believe that the spatial extent and composition of co-varying GM measures within the different networks enhance interpretability of the effects of these factors on the brain, especially with regard to underlying neurobiological mechanisms.

2. Publications

2.1. Publication1: Review: Huhn et al, 2015

Components of a Mediterranean diet and their impact on cognitive functions in aging

Huhn S., Kharabian Masouleh S., Stumvoll M., Villringer A., Witte A.V.

Components of a Mediterranean diet and their impact on cognitive functions in aging

Sebastian Huhn[1], Shahrzad Kharabian Masouleh[1], Michael Stumvoll[2,3], Arno Villringer[1,2] and A. Veronica Witte[1,2]*

[1] Department of Neurology, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany, [2] Collaborative Research Centre 1052 'Obesity Mechanisms', Subproject A1, Faculty of Medicine, University of Leipzig, Leipzig, Germany, [3] IFB Adiposity Diseases, University of Leipzig, Leipzig, Germany

Background: Adhering to the Mediterranean diet (MeDi) is known to be beneficial with regard to many age-associated diseases including cardiovascular diseases and type 2 diabetes. Recent studies also suggest an impact on cognition and brain structure, and increasing effort is made to track effects down to single nutrients.

Aims: We aimed to review whether two MeDi components, i.e., long-chain omega-3 fatty acids (LC-n3-FA) derived from sea-fish, and plant polyphenols including resveratrol (RSV), exert positive effects on brain health in aging.

Content: We summarized health benefits associated with the MeDi and evaluated available studies on the effect of (1) fish-consumption and LC-n3-FA supplementation as well as (2) diet-derived or supplementary polyphenols such as RSV, on cognitive performance and brain structure in animal models and human studies. Also, we discussed possible underlying mechanisms.

Conclusion: A majority of available studies suggest that consumption of LC-n3-FA with fish or fishoil-supplements exerts positive effects on brain health and cognition in older humans. However, more large-scale randomized controlled trials are needed to draw definite recommendations. Considering polyphenols and RSV, only few controlled studies are available to date, yet the evidence based on animal research and first interventional human trials is promising and warrants further investigation. In addition, the concept of food synergy within the MeDi encourages future trials that evaluate the impact of comprehensive lifestyle patterns to help maintaining cognitive functions into old age.

Keywords: cognition, plasticity, omega-3 fatty acids, polyphenols, resveratrol, memory, brain structure

Background: Health Benefits of the Mediterranean Diet

According to the "Global Strategy on Diet, Physical Activity and Health", a review developed by the World Health Organization (WHO), the Mediterranean Diet (MeDi) is a promising strategy to prevent from diseases and enhance quality of life (World Health Organization, 2009). The review aims specifically on interventions, that reduce the risk for non-communicable diseases like cerebro- and cardiovascular diseases, cancer, respiratory diseases, diabetes and neurodegenerative

diseases, which comprise the leading causes of death worldwide (World Health Organization, 2009). The MeDi was first investigated by Ancel Keys in the 1950s during his Seven Countries Study, a large-scale prospective cohort-study with more than 11,000 participants (Keys, 1970; Keys et al., 1986). Keys et al. (1986) observed a considerable difference in the eating pattern of Southern European countries, compared to Northern Europe and the USA. This Mediterranean eating pattern and related low intake percentage of total energy from saturated fatty acids correlated with lower serum cholesterol and lower blood pressure in Mediterranean countries, which were again associated with a lower coronary mortality and a lower risk for the above mentioned diseases in comparison to countries adhering to a Western-type diet (Keys et al., 1986).

Distinctive for the MeDi is the high consumption of fruits, vegetables, grains as well as sea-fish on regular basis, while the intake of meat and dairy products, just as sweets and convenience food is rather low (Trichopoulou et al., 1995; Gotsis et al., 2015). In addition, the regular consumption of red wine (mainly served with food) and olive oil (as principal source of fat) is characteristic for the MeDi (Willett et al., 1995). For a detailed description of the MeDi, often displayed as food pyramid, see Bach-Faig et al. (2011).

Over the last decades, epidemiologic studies supported and extended Keys' findings to a multitude of health benefits that are provided by the MeDi, e.g., with regard to cancer and cardiovascular diseases (Couto et al., 2011; Lopez-Garcia et al., 2014; Gotsis et al., 2015). More recently, research also focused on neurodegenerative diseases and the impact of MeDi on cognition. For reviews, see e.g., Lourida et al. (2013) and van de Rest et al. (2015). For example, Scarmeas et al. (2006) observed in a prospective cohort of 2258 community-based non-demented individuals that higher adherence to the MeDi is associated with a significant reduction in the risk for Alzheimer's disease (AD). In a systematic review, Lourida et al. (2013) described a reasonably consistent pattern of associations between adherence to the MeDi and related lower risks for AD, reduced rates of cognitive decline as well as better cognitive function. Most recently, Valls-Pedret et al. (2015) described positive results of a long-term randomized clinical trial (RCT) in 334 participants with high cardiovascular risk at a mean age of 67 years (PREDIMED study), providing an even stronger level of scientific evidence than results based on observational studies (Valls-Pedret and Ros, 2013): Here, a MeDi supplemented with either olive oil or nuts, in comparison to a control diet, was associated with improved cognitive functions at 4-year follow-up (Valls-Pedret et al., 2015).

These beneficial effects might be due to multiple biological mechanisms, such as lower concentrations of serum-cholesterol in Mediterranean areas and a related decrease of cardiovascular risk, which were among the first findings by Keys et al. (1986). More specifically, adherence to the MeDi is associated with a reduced risk for coronary heart diseases and metabolic syndrome including hypertension and dyslipidemia, which have been associated with the development of cognitive impairments (for review see e.g., van den Berg et al., 2009; Yates et al., 2012). Additionally, adhering to the MeDi might prevent from disturbances in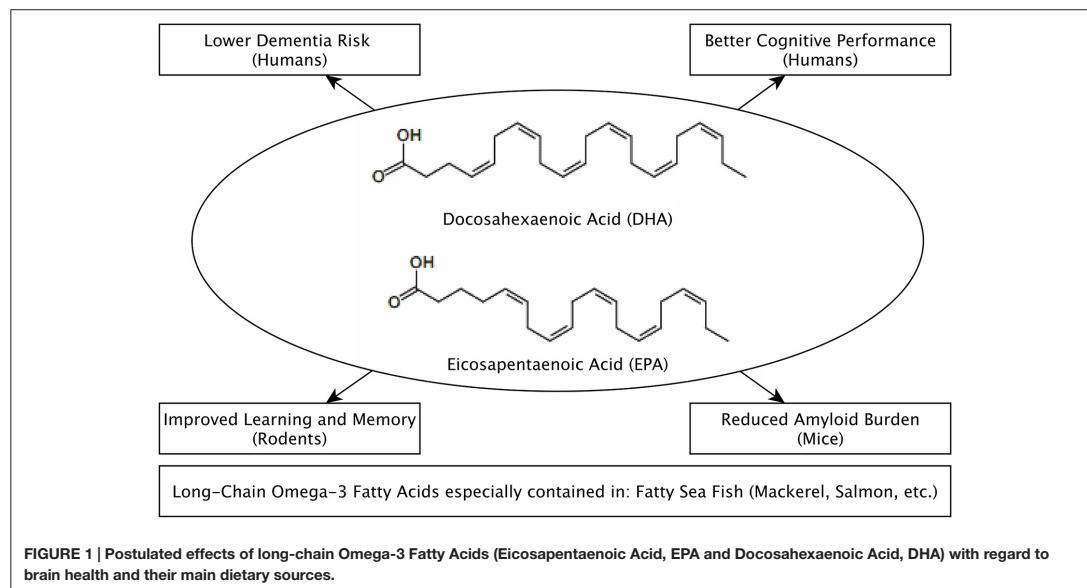 insulin/glucose metabolism that can result in type 2-diabetes mellitus (DM-2), which is associated with an increased risk for AD and cognitive impairments (Biessels et al., 2006; Hu et al., 2013). Even in the absence of manifest DM-2, chronically elevated levels of blood-glucose have shown to exert negative effects on AD risk and memory performance in older adults (Crane et al., 2013; Kerti et al., 2013).

In sum, the MeDi has been shown to exert positive effects on risk for AD and cognitive functions during aging, which is probably mediated through reductions in vascular risk factors and benefits on lipid and glucose metabolism. Moreover, based on animal research it has been postulated that specific nutrients could exert even more directly protective effects on the aging brain, e.g., considering amyloid-beta metabolism (Allès et al., 2012). As the MeDi is a complex eating pattern, though, a multitude of single components could cause beneficial effects (Jacobs et al., 2009; Gotsis et al., 2015). Understanding these underlying mechanisms and eventually develop preventive and therapeutic strategies based on those insights, are important issues for future research.

This review aims to evaluate recent findings concerning the effects of single components of the MeDi and their impact on cognition. Firstly, we focus on long chain omega-3 fatty acids (LC-n3-FA) derived from fish, as they distinguish the MeDi from other diets and are consumed with high frequency (Tangney et al., 2014). Secondly, our focus is on plant polyphenols (including resveratrol), which occur mainly in fruit, tea and red wine (Manach et al., 2004). The deliberate consumption of red wine is a well-known feature of the MeDi and especially resveratrol is assigned beneficial effects with regard to overall health, as well as cognition (Baur and Sinclair, 2006; Witte et al., 2014). Both nutrients attracted increasing research interest in the last years.

Impact of Omega-3 Fatty Acids on the Brain

One characteristic of the MeDi is a high intake of unsaturated fatty acids, including the long-chain omega-3 polyunsaturated fatty acids (LC-n3-FA) eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3; **Figure 1**). The main source of DHA and EPA in the human diet is fatty sea fish like mackerels or salmon (Max Rubner-Institut, 2011). DHA and EPA cannot be efficiently synthesized by human enzymes and are therefore regarded as semi-essential (Burdge and Calder, 2005; Burdge, 2006; Sala-Vila and Ros, 2011). Astrocytes in the brain are a major site for the processing of LC-n3-FA. They elongate and desaturate precursor fatty acids such as linoleic acid and the vegetable LC-n3-FA alpha-linolenic acid (ALA) to form EPA and DHA (Moore et al., 1991). Notably, not only the absolute amount of DHA and EPA might be important, but also the ratio of the precursors, as with different precursor ratios, different conversion rates to DHA and EPA occur (Kaur et al., 2014). In addition, intake of ALA, contained e.g., in nuts, might also directly contribute to the beneficial effects of the MeDi on cognition (Blondeau et al., 2009; Valls-Pedret et al., 2015; for a detailed discussion of possibly distinct effects of ALA, EPA and DHA please see Freemantle et al., 2006).

FIGURE 1 | Postulated effects of long-chain Omega-3 Fatty Acids (Eicosapentaenoic Acid, EPA and Docosahexaenoic Acid, DHA) with regard to brain health and their main dietary sources.

It is widely accepted, that LC-n3-FA are crucial for the growth and development of the infant brain during pregnancy and after birth (Kris-Etherton et al., 2009). The predominant LC-n3-FA DHA alone comprises 10–20% of total fatty acids of the brain and is thought to be important with regard to neuronal differentiation, synaptogenesis, and synaptic function (McNamara and Carlson, 2006). It has also been proposed that the access to DHA during hominid evolution played a key role in increasing the brain to body-mass ratio (Crawford et al., 1999, 2013). However, due to the easy availability of processed food in Western societies today, the consumption of saturated fatty acids and trans-fatty acids increased, while that of DHA decreased. This has been speculated to contribute to an increased incidence of brain disorders such as major depression (Su et al., 2003).

Considering the abundance of DHA in brain tissue and its importance for brain development and evolution, it is reasonable to suppose that DHA also contributes to the evolvement and maintenance of proper cognitive functioning in later life (Gómez-Pinilla, 2008). Indeed, several experimental animal studies demonstrated superior learning and better memory performance in rodents that received supplementary DHA with their diet (Morris et al., 2005). DHA might have a beneficial impact even during pathological conditions like AD. Lim et al. (2005) found in aged mice on a DHA-enriched diet a significant reduction of total amyloid β (A-β) by more than 70% when compared with low-DHA or control chow diets. This could be neuroprotective, given the probable downstream toxicity of A-β deposition and its implications in the development of AD (Lim et al., 2005). That is a further finding on the protective properties of DHA against synaptic loss, which is a critical issue in concerns of AD and seems to support the

hypothesis that DHA is protective against AD (Calon et al., 2004).

These findings are in line with human epidemiological studies that report associations between the consumption of fish in general (Barberger-Gateau et al., 2005; Morris et al., 2005), as well as LC-n3-FA e.g., as dietary fishoil supplement (McCann and Ames, 2005; Gómez-Pinilla, 2008), with better cognitive performances and lower risk of dementia (for a review, see Fotuhi et al., 2009). For example, a large-scale prospective cohort study with 6158 residents of a community in Chicago of 65 years and older, estimated that fish consumption was associated with slower cognitive decline with age, assessed using a global cognitive score (Morris et al., 2005).

The evidence for positive effects of LC-n3-FA fishoil supplementation on cognitive functions in normal and pathological aging based on placebo-controlled RCTs is less clear, see **Table 1** for an overview. In an early double-blind RCT in 204 AD patients, Freund-Levi et al. (2006) observed positive effects of LC-n3-FA in a small group of those with very mild AD who took supplementary LC-n3-FA over 6 months. These findings are in line with a 24-week RCT by Chiu et al. (2008) in 46 participants. Here the authors also concluded that LC-n3-FA improved general clinical function in patients with mild or moderate AD, as well as mild cognitive impairment (Chiu et al., 2008). In an own double-blind prospective interventional study, it was shown that LC-n3-FA improved executive functions and gray matter volume, as well as white matter microstructure in healthy older individuals, after 26 weeks of fish oil supplementation (Witte et al., 2013). Yurko-Mauro et al. (2010) observed in another RCT with 485 healthy subjects older than 55 years that 24 weeks of

28

TABLE 1 | Characteristics of studies reporting associations between fish-consumption or LC-n3-FA-supplementation and cognition.

Author (year)	Participants		Duration	Intervention		Measured outcome		Results	
	sample size/age (years)								
Chiu et al. (2008)	N = 46 memory complaints	I: 74.0 (70.1–77.8) P: 76.5 (71.8–81.1)	24 weeks	1.8 g Omega-3 PUFAs/d	Placebo	ADAS-cog		AD group: ○	MCI group: +
Dangour et al. (2010)	N = 867 healthy	I: 74.7 ± 2.5 P: 74.6 ± 2.7	24 months	200 mg EPA + 500 mg DHA/d	Placebo	Extensive NP test battery		Whole group: ○	
Freund-Levi et al. (2006)	N = 204 AD	I: 72.6 ± 9.0 P: 72.9 ± 8.6	6 months	1.7 g DHA/d and 0.6 g EPA/d	Placebo	ADAS-cog	MMSE	Whole group: ○	Sub-group: +
Morris et al. (2005)	N = 6185 healthy	I1: 74.6 I2: 74.2 I3: 73.9	6 years	Observational		Global cognitive score		Whole group: +	
Quinn et al. (2010)	N = 402 mild to moderate AD	I: 76 ± 9.3 P: 76 ± 7.8	18 months	2 g/d DHA	Placebo	ADAS-cog	Clinical Dementia Rating (CDR) sum of boxes	Whole group: ○	
Reddy et al. (2011)	N = 27 schizophrenia	18–45	24 weeks	2 g/d EPA		Wisconsin Card Sort Test		Whole group: +	
Tan et al. (2012)	N = 1575 healthy	67 ± 9	–	Observational (Red blood cell EPA + DHA)		Extensive NP test battery		Whole group: +	
van de Rest et al. (2008)	N = 302 healthy	I1800: 69.9 ± 3.4 I400: 69.5 ± 3.2 P: 70.1 ± 3.7	26 weeks	1800 mg/d EPA-DHA 400 mg/d EPA-DHA	Placebo	Extensive NP test battery		Whole group: ○	
Witte et al. (2013)	N = 65 healthy	I: 65 ± 6.3 P: 62.9 ± 6.8	26 weeks	2.2 g/d EPA-DHA	Placebo	Extensive NP test battery		Whole group: +	
Yurko-Mauro et al. (2010)	N = 485 healthy	I: 70 ± 9.3 P: 70 ± 8.7	24 weeks	900 mg DHA/d		CANTAB Paired Associate Learning		Whole group: +	

AD, Alzheimer's Disease; ADAS-cog, Alzheimer's Disease Assessment Scale, cognitive subscale; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; I, age of intervention group; MCI, Mild Cognitive Impairment; MMSE, Mini-Mental State Examination; NP, neuropsychological; P, age of placebo group; +, positive effect on cognition; ○, no effect on cognition.

supplementation with 900 mg/d DHA improved learning and memory function.

Supporting these findings, Pottala et al. (2014) observed in a cross-sectional analysis, that a higher LC-n3-FA intake (indicated by higher proportions of DHA and EPA in the membranes of blood erythrocytes, see Harris and Von Schacky, 2004) was correlated to higher total brain and hippocampal volume in 1111 postmenopausal women. In another cross-sectional study by Tan et al. (2012) in 1575 elderly participants, those with lower DHA had also lower scores on tests of executive function and abstract thinking. Similarly, executive functions could be improved after 24 weeks of supplementary LC-n3-FA intake (2 g EPA/d) in 27 schizophrenic patients in an open-label study (Reddy et al., 2011).

In contrast, other interventional studies in AD patients (Quinn et al., 2010) or healthy older adults (van de Rest et al., 2008; Dangour et al., 2010) did not support the positive effects of fish oil consumption. These inconsistent results might be explained due to differences in dosage and duration between studies, e.g., that LC-n3-FA intake might not have been sufficient to exert statistically significant effects on cognition. Furthermore studies might differ in intake instructions and cohort characteristics. It has also been noted that not only the amount of LC-n3-FA, but also the overall dietary fat-composition is considerably critical for brain functions (Morris et al., 2005). For example, an unfavorable fat composition might affect cognitive aging more than total fat intake itself (Okereke et al., 2012). Especially saturated fatty acids and trans-fatty acids are supposed to increase the risk of AD (Hooijmans et al., 2007; Studzinski et al., 2009; Ramassamy and Belkacémi, 2011) and affect cognition (Greenwood and Winocur, 2005), which could be due to decreased Brain-derived neurotrophic factor (BDNF) related synaptic plasticity (Molteni et al., 2002). Thus, it might be speculated that the positive effects of supplementary LC-n3-FA could be masked out by the negative effects of concurrent high saturated- and trans- fatty acid intake. According to the latest Cochrane reviews, it is not yet clear

29

that dietary or supplemental LC-n3-FA alter total mortality, combined cardiovascular events or cancers in people with, or at high risk of, cardiovascular disease or in the general population (Hooper et al., 2004). The same stated Sydenham et al. (2012) for LC-n3-FA and dementia. They could not state benefits for cognitive health for older people taking omega-3 supplements. However, none of the mentioned studies reported severe adverse effects of fish or fish oil consumption.

Underlying mechanisms of positive effects of LC-n3-FA on cognition could include a reduction of cardiovascular risk factors, e.g., by improving cerebral blood flow and lowering triacylglycerol levels as found in non-human primates and rats (Katayama et al., 1997; Tsukada et al., 2000; Fotuhi et al., 2009). More direct neuronal effects of LC-n3-FA are e.g., stimulation of neurogenesis and neurite outgrowth (Kawakita et al., 2006) and enhancement of synaptic membrane fluidity (Cansev and Wurtman, 2007). Also, LC-n3-FA have been found to increase the expression of myelin-related proteins (Salvati et al., 2008), which could contribute to improved axonal transmission and thus better neuronal signaling. In addition, LC-n3-FA are thought to upregulate several genes such as Sir2, involved in maintaining synaptic function and plasticity (Wu et al., 2007). A recent study in mice showed an increase of neuroprotectin D-1 (NPD-1) after fish oil treatment (Afshordel et al., 2015). NPD-1 represents a neuroprotective compound that is derived from unesterified DHA (Afshordel et al., 2015).

Moreover, LC-n3-FA play several roles with regard to inflammatory processes. DHA and EPA are capable of competing with arachidonic acid in the production of eicosanoids, which results in the production of biologically less active thromboxans and therefore in a better hemodynamic, vascular tone and inflammation (Mori and Beilin, 2004). LC-n3-FA might also upregulate the expression of antioxidant enzymes and downregulate genes associated with production of reactive oxygen species (ROS), such as peroxisome proliferator-activated receptors gamma (PPAR-γ; Takahashi et al., 2002; Mori and Beilin, 2004). Additionally, DHA has been implicated in reducing inflammation through fatty acid derivatives such as NPD-1 (Cole et al., 2010) and resolvin species (Kohli and Levy, 2009).

In sum, promising evidence indicates that LC-n3-FA, especially DHA, exert positive effects on brain structure and cognitive functions. Yet, more large-scale RCTs are needed before fish oil intake could be fully recommended as preventive strategy against cognitive decline in the older population.

Polyphenols and their Impact on the Brain

A further class of substances that is supposed to contribute to the beneficial effects of the Mediterranean Diet (MeDi) is that of polyphenols (**Figure 2**). Polyphenols are secondary metabolites of plants and characterized by the chemical structure of hydroxyl groups on aromatic rings (Manach et al., 2004). They are quite abundant in our diet and several thousand molecules have been identified to have polyphenol character (Manach et al., 2004). One polyphenol agent that came into research focus is resveratrol (RSV). It occurs naturally in the skin of red grapes, red wine, blueberries, peanuts and Japanese knotweed (Baur and Sinclair, 2006; Baur et al., 2006; Ingram et al., 2006). Another group, the flavonols, are part of the flavonoid family that is found in various fruits, cocoa, beans and the Ginkgo biloba tree (Gómez-Pinilla, 2008). Flavonols contain anti-inflammatory properties among several other complex actions (for review, see Gómez-Pinilla, 2008). Although polyphenols are somewhat heterogeneous regarding their chemical properties, they seem to have some effects in common with regard to cardiovascular health and (at least for some polyphenols) antioxidant capacity (Halliwell, 2007; Habauzit and Morand, 2012).
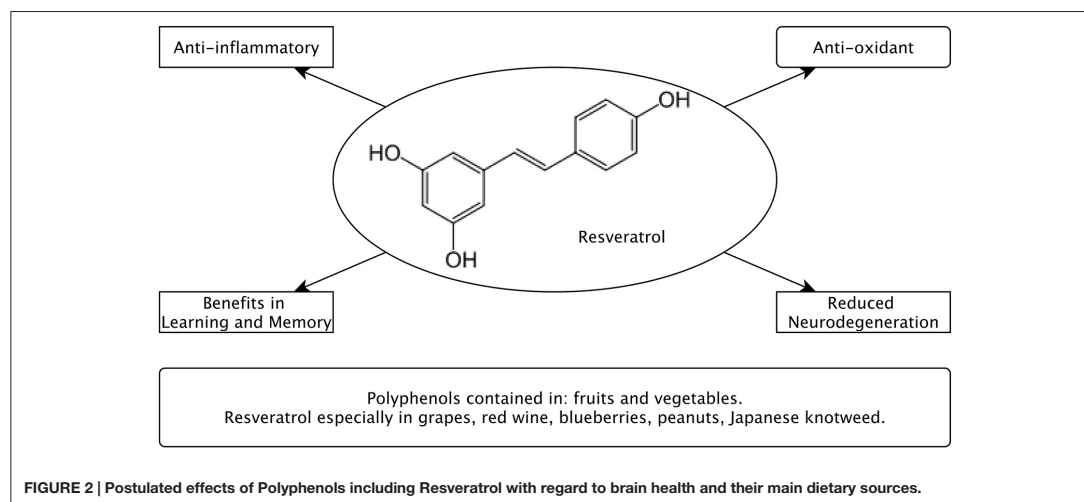

FIGURE 2 | Postulated effects of Polyphenols including Resveratrol with regard to brain health and their main dietary sources.

30

In vitro, several polyphenols including RSV eliminate a multitude of ROS, including hydroxyl radicals, peroxyl radicals hypochlorous acid and in part superoxide radical (Halliwell, 2007). ROS are considered to be toxic and ROS-induced cell damage is assumed to contribute to the process of aging (Liochev, 2013). In rats, polyphenols have been shown to increase heat-shock protein (HSP) 70 and insulin-like growth factor 1 (IGF-1) expression in the hippocampus, which protects against kainate-induced cell damage and benefits learning and memory performance (Casadesus et al., 2004; Galli et al., 2006). Reduced hippocampal neurodegeneration has also been shown after RSV administration in rodent models for AD/tauopathies (Kim et al., 2007). In addition, administration of RSV-containing red wine was found to preserve spatial memory, while reducing Aβ neuropathology (Wang et al., 2010). In a non-human primate study, supplementary RSV for 18 months increased spatial memory performance compared to placebo (Dal-Pan et al., 2011).

Considering human studies, there is a considerable heterogeneity in study quality, design and polyphenol formula/dosage (Crichton et al., 2013). See **Table 2** for an overview. In a cross-sectional study by Nurk et al. (2009) with 2,031 participants aged 70–74 years from the Hordaland Health Study in Norway, a diet over 1 year high in some flavonol-rich foods, such as chocolate, wine and tea, was associated with better performance in several cognitive abilities in a dose-dependent manner in comparison to a non-consumer group. Only a few placebo-controlled interventional studies are available to date, such as Kennedy et al. (2010). This study assessed the effects of 250 and 500 mg oral RSV on cognitive performance in a RCT crossover study in 22 healthy adults, with the result that even single doses of orally administered RSV can modulate cerebral blood flow variables, measured using MRI (Kennedy et al., 2010). In another study, blueberry supplementation (wild blueberry juice) improved paired associate learning and word list recall, as well as paired associate learning in a small sample of nine older adults after comparison with a matched, placebo-controlled sample (Krikorian et al., 2010). In a double-blind, clinical trial by Small et al. (2014) intake of a pill-based nutraceutical that contained a proprietary formulation of blueberry (including RSV), green tea, carnosine, vitamin D3 and biovin, resulted in significantly increased processing speed of 52 participants compared to placebo (N = 53). In an own study with 46 healthy overweight older individuals, a daily intake of 200 mg RSV (in a formula with quercetin) over 26 weeks compared to placebo intake significantly improved memory performance (Witte et al., 2014). In addition, glycated hemoglobin (HbA1c) in peripheral blood was significantly reduced after RSV treatment, and this reduction in HbA1c correlated with higher functional connectivity of the hippocampus, measured using resting-state functional MRI in the same subjects. Notably, changes in functional connectivity were found to correlate with the observed increases in memory, pointing to ameliorated glucose metabolism as one underlying mechanism of the positive effects of RSV on cognition (Witte et al., 2014). Also, Brickman et al. (2014) reported recently in a randomized study on flavonols with 37 healthy 50 69 year old subjects using functional MRI that a diet high in cocoa-flavanol over 3 months enhanced memory function and improved related activation in the dentate gyrus, the hippocampus region characterized by life-long neurogenesis, in comparison to a diet low in cocoa-flavanol.

Both RSV and flavonols could contribute to a better cognitive performance due to their protective effects against oxidative stress, which increases with age and is a risk factor for age-associated cognitive decline. Further possible neuroprotective mechanisms of polyphenols including RSV are reduced mitochondrial dysfunction, glucose toxicity, oxidative damage, and chronic inflammation, by improving glucose metabolism and vascular functions and by activating so-called longevity genes including the sirtuins. For further discussions see

TABLE 2 | Characteristics of studies reporting associations between flavonol or RSV consumption and cognition.

Author (year)	Participants sample size/age		Duration	Intervention		Measured outcome		Results (Polyphenol)
Kennedy et al. (2010)	N = 22	Healthy 20.17 y	Single dose	250 mg (RSV) 500 mg (RSV)	Placebo	Cognitive task	Cerebral blood flow	+
Krikorian et al. (2010)	N = 9, placebo N = 7	Healthy 76.2 ± 5.2 y	12 weeks	Daily consumption of wild blueberry juice		Paired associate learning	Word list recall	+
Nurk et al. (2009)	N = 2031	Healthy 70–74 y	Cross-sectional	Observational (Chocolate, Wine, Tea)		Extensive NP test battery		+
Small et al. (2014)	N = 52, placebo N = 53	Healthy I: 72.82 P: 74.34	2 months	Pill-based nutraceutical	Placebo	Extensive NP test battery		+
Witte et al. (2014)	N = 23, Placebo N = 23	Healthy, overweight I: 64.8 ± 6.8 P: 63.7 ± 5.3	26 weeks	200 mg/d RSV	Placebo	Auditory Verbal Learning Test		+
Brickman et al. (2014)	N = 37	Healthy 50–69 y	3 months	High cocoa flavonol-diet	Low flavonol-diet	ModBent task		+

NP, Neuropsychological; P, Age of placebo group; RSV, resveratrol; I, Age of intervention group; y, years of age; +, positive effect on cognition; ○, no effect on cognition.

31

e.g., Calabrese et al. (2008, 2009), Sun et al. (2011), Crichton et al. (2013) and Witte et al. (2014).

Conclusion and Outlook

A majority of available studies on the topic suggest that consumption of LC-n3-FA with fish or fish oil-supplements and plant polyphenols such as flavonols and RSV exerts positive effects on brain health and cognition in older humans. However, with regard to LC-n3-FA supplementation using fish oil, a final recommendation based on RCTs cannot be drawn, as some studies could not detect a positive effect. Here, more large-scale RCTs that, for example, also control for other fatty acid intake are needed to support a significant benefit of regular supplementary LC-n3-FA intake in maintaining cognitive performance. Considering polyphenols, the evidence based on high-quality RCTs is even less clear, given that only few reliable studies are available to date with different formulas and different duration of the intervention. Yet, those few studies were promising, and the animal literature provided convincing examples that polyphenols are highly potent in activating possible neuroprotective pathways, warranting the initiation of large-scale RCTs in humans on supplementary flavonol or RSV. Moreover, attempts to study in parallel the underlying mechanisms in humans, e.g., using high-resolution MRI, are especially important to further strengthen possible hypotheses that are mainly based on animal research. Future studies also need to address whether intervention-induced changes in LC-n3-FA or polyphenol intake relate to changes in fatty acid or polyphenol content at the brain level in humans, e.g., using post-mortem techniques.

Besides that, additive or synergistic effects between single dietary components come increasingly into focus. Diet is more than the sum of its components, which is considered in the concept of "food synergy". The assumption is that interactions and synergistic effects of the single food components occur as they are consumed in the framework of a balanced diet (Jacobs et al., 2009). For example, antioxidant nutrients can protect LC-n3-FA from peroxidation to which they are particularly susceptible due to their multiple double bounds (Barberger-Gateau, 2014). Also, even though studies on single nutrients

and their interactions might help to explain the beneficial effects of dietary patterns, there is an even greater framework. Yannakoulia et al. (2015) propose not only the additive and synergistic effects of single nutrients or foods, but also add other lifestyle behaviors like physical activity, social support, sharing food, having lengthy meals and post-lunch siestas to that explanatory approach. Regardless of all the modernization processes happening (Bach-Faig et al., 2011), the lifestyle of the Mediterranean countries remains an UNESCO World Cultural Heritage and could thus contribute to a multitude of insights regarding brain functioning and healthy aging (Bach-Faig et al., 2011). First publications of large-scale RCTs, such as Valls-Pedret et al. (2015) and Ngandu et al. (2015), provide a strong level of scientific evidence for the beneficial effects of the MeDi on cognitive functions. In addition, ongoing multidomain interventional trials like the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) will help to gain further insights into the beneficial effects of the MeDi-lifestyle and its components on cognition and brain function. The FINGER-study is a multi-center RCT and includes nutritional guidance, regular exercise, cognitive training and social activity, as well as management of metabolic and vascular risk factors, and might thus shed comprehensively further light on possible mechanisms of how modifiable lifestyle factors could help to maintain cognitive functions throughout age (Kivipelto et al., 2013).

Summing up, LC-n3-FA and polyphenols such as RSV are highly investigated substances in the framework of the MeDi. Even though, more studies are needed to clarify the main effects and their underlying mechanisms, they seem to be promising with regard to their impact on brain structure and function in aging.

Acknowledgments

References

Afshordel, S., Hagl, S., Werner, D., Röhner, N., Kogel, D., Bazan, N. G., et al. (2015). Omega-3 polyunsaturated fatty acids improve mitochondrial dysfunction in brain aging–impact of Bcl-2 and NPD-1 like metabolites. *Prostaglandins Leukot. Essent. Fatty Acids* 92, 23–31. doi: 10.1016/j.plefa.2014.05.008

Allès, B., Samieri, C., Féart, C., Jutand, M. A., Laurin, D., and Barberger-Gateau, P. (2012). Dietary patterns: a novel approach to examine the link between nutrition and cognitive function in older individuals. *Nutr. Res. Rev.* 25, 207–222. doi: 10.1017/s0954422412000133

Bach-Faig, A., Berry, E. M., Lairon, D., Reguant, J., Trichopoulou, A., Dernini, S., et al. (2011). Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr.* 14, 2274–2284. doi: 10.1017/s1368980011002515

Barberger-Gateau, P. (2014). Nutrition and brain aging: how can we move ahead? *Eur. J. Clin. Nutr.* 68, 1245–1249. doi: 10.1038/ejcn.2014.177

Barberger-Gateau, P., Jutand, M. A., Letenneur, L., Larrieu, S., Tavernier, B., Berr, C., et al. (2005). Correlates of regular fish consumption in French elderly community dwellers: data from the three-city study. *Eur. J. Clin. Nutr.* 59, 817–825. doi: 10.1038/sj.ejcn.1602145

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., et al. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337–342. doi: 10.1038/nature05354

Baur, J. A., and Sinclair, D. A. (2006). Therapeutic potential of resveratrol: the in vivo evidence. *Nat. Rev. Drug Discov.* 5, 493–506. doi: 10.1038/nrd2060

Biessels, G. J., Staekenborg, S., Brunner, E., Brayne, C., and Scheltens, P. (2006). Risk of dementia in diabetes mellitus: a systematic review. *Lancet Neurol.* 5, 64–74. doi: 10.1016/s1474-4422(05)70284-2

Blondeau, N., Nguemeni, C., Debruyne, D. N., Piens, M., Wu, X., Pan, H., et al. (2009). Subchronic alpha-linolenic acid treatment enhances brain plasticity and exerts an antidepressant effect: a versatile potential therapy for stroke. *Neuropsychopharmacology* 34, 2548–2559. doi: 10.1038/npp.2009.84

32

Brickman, A. M., Khan, U. A., Provenzano, F. A., Yeung, L. K., Suzuki, W., Schroeter, H., et al. (2014). Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults. *Nat. Neurosci.* 17, 1798–1803. doi: 10.1038/nn.3850

Burdge, G. C. (2006). Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot. Essent. Fatty Acids* 75, 161–168. doi: 10.1016/j.plefa.2006.05.013

Burdge, G. C., and Calder, P. C. (2005). Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* 45, 581–597. doi: 10.1051/rnd:2005047

Calabrese, V., Cornelius, C., Mancuso, C., Barone, E., Calafato, S., Bates, T., et al. (2009). Vitagenes, dietary antioxidants and neuroprotection in neurodegenerative diseases. *Front. Biosci. (Landmark Ed.)* 14, 376–397. doi: 10.2741/3250

Calabrese, V., Cornelius, C., Mancuso, C., Pennisi, G., Calafato, S., Bellia, F., et al. (2008). Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity. *Neurochem. Res.* 33, 2444–2471. doi: 10.1007/s11064-008-9775-9

Calon, F., Lim, G. P., Yang, F., Morihara, T., Teter, B., Ubeda, O., et al. (2004). Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* 43, 633–645. doi: 10.1016/j.neuron.2004.08.013

Cansev, M., and Wurtman, R. J. (2007). Chronic administration of docosahexaenoic acid or eicosapentaenoic acid, but not arachidonic acid, alone or in combination with uridine, increases brain phosphatide and synaptic protein levels in gerbils. *Neuroscience* 148, 421–431. doi: 10.1016/j.neuroscience.2007.06.016

Casadesus, G., Shukitt-Hale, B., Stellwagen, H. M., Zhu, X., Lee, H. G., Smith, M. A., et al. (2004). Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. *Nutr. Neurosci.* 7, 309–316. doi: 10.1080/10284150400020482

Chiu, C. C., Su, K. P., Cheng, T. C., Liu, H. C., Chang, C. J., Dewey, M. E., et al. (2008). The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32, 1538–1544. doi: 10.1016/j.pnpbp.2008.05.015

Cole, G. M., Ma, Q. L., and Frautschy, S. A. (2010). Dietary fatty acids and the aging brain. *Nutr. Rev.* 68(Suppl. 2), S102–S111. doi: 10.1111/j.1753-4887.2010.00345.x

Couto, E., Boffetta, P., Lagiou, P., Ferrari, P., Buckland, G., Overvad, K., et al. (2011). Mediterranean dietary pattern and cancer risk in the EPIC cohort. *Br. J. Cancer* 104, 1493–1499. doi: 10.1038/bjc.2011.106

Crane, P. K., Walker, R., Hubbard, R. A., Li, G., Nathan, D. M., Zheng, H., et al. (2013). Glucose levels and risk of dementia. *N. Engl. J. Med.* 369, 540–548. doi: 10.1056/NEJMoa1215740

Crawford, M. A., Bloom, M., Broadhurst, C. L., Schmidt, W. F., Cunnane, S. C., Galli, C., et al. (1999). Evidence for the unique function of docosahexaenoic acid during the evolution of the modern hominid brain. *Lipids* 34(Suppl.), S39–S47. doi: 10.1007/bf02562227

Crawford, M. A., Broadhurst, C. L., Guest, M., Nagar, A., Wang, Y., Ghebremeskel, K., et al. (2013). A quantum theory for the irreplaceable role of docosahexaenoic acid in neural cell signalling throughout evolution. *Prostaglandins Leukot. Essent. Fatty Acids* 88, 5–13. doi: 10.1016/j.plefa.2012.08.005

Crichton, G. E., Bryan, J., and Murphy, K. J. (2013). Dietary antioxidants, cognitive function and dementia–a systematic review. *Plant Foods Hum. Nutr.* 68, 279–292. doi: 10.1007/s11130-013-0370-0

Dal-Pan, A., Pifferi, F., Marchal, J., Picq, J. L., Aujard, F., and RESTRIKAL Consortium. (2011). Cognitive performances are selectively enhanced during chronic caloric restriction or resveratrol supplementation in a primate. *PLoS One* 6:e16581. doi: 10.1371/journal.pone.0016581

Dangour, A. D., Allen, E., Elbourne, D., Fasey, N., Fletcher, A. E., Hardy, P., et al. (2010). Effect of 2-y n-3 long-chain polyunsaturated fatty acid supplementation on cognitive function in older people: a randomized, double-blind, controlled trial. *Am. J. Clin. Nutr.* 91, 1725–1732. doi: 10.3945/ajcn.2009.29121

Fotuhi, M., Mohassel, P., and Yaffe, K. (2009). Fish consumption, long-chain omega-3 fatty acids and risk of cognitive decline or Alzheimer disease: a complex association. *Nat. Clin. Pract. Neurol.* 5, 140–152. doi: 10.1038/ncpneuro1044

Freemantle, E., Vandal, M., Tremblay-Mercier, J., Tremblay, S., Blachère, J.-C., Bégin, M. E., et al. (2006). Omega-3 fatty acids, energy substrates and brain function during aging. *Prostaglandins Leukot. Essent. Fatty Acids* 75, 213–220. doi: 10.1016/j.plefa.2006.05.011

Freund-Levi, Y., Eriksdotter-Jönhagen, M., Cederholm, T., Basun, H., Faxén-Irving, G., Garlind, A., et al. (2006). Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch. Neurol.* 63, 1402–1408. doi: 10.1001/archneur.63.10.1402

Galli, R. L., Bielinski, D. F., Szprengiel, A., Shukitt-Hale, B., and Joseph, J. A. (2006). Blueberry supplemented diet reverses age-related decline in hippocampal HSP70 neuroprotection. *Neurobiol. Aging* 27, 344–350. doi: 10.1016/j.neurobiolaging.2005.01.017

Gómez-Pinilla, F. (2008). Brain foods: the effects of nutrients on brain function. *Nat. Rev. Neurosci.* 9, 568–578. doi: 10.1038/nrn2421

Gotsis, E., Anagnostis, P., Mariolis, A., Vlachou, A., Katsiki, N., and Karagiannis, A. (2015). Health benefits of the Mediterranean diet: an update of research over the last 5 years. *Angiology* 66, 304–318. doi: 10.1177/0003319714532169

Greenwood, C. E., and Winocur, G. (2005). High-fat diets, insulin resistance and declining cognitive function. *Neurobiol. Aging* 26(Suppl. 1), 42–45. doi: 10.1016/j.neurobiolaging.2005.08.017

Habauzit, V., and Morand, C. (2012). Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: an update for clinicians. *Ther. Adv. Chronic Dis.* 3, 87–106. doi: 10.1177/2040622311430006

Halliwell, B. (2007). Dietary polyphenols: good, bad, or indifferent for your health? *Cardiovasc. Res.* 73, 341–347. doi: 10.1016/j.cardiores.2006.10.004

Harris, W. S., and Von Schacky, C. (2004). The Omega-3 index: a new risk factor for death from CHD? *Prev. Med.* 39, 212–220. doi: 10.1016/j.ypmed.2004.02.030

Hooijmans, C. R., Rutters, F., Dederen, P. J., Gambarota, G., Veltien, A., van Groen, T., et al. (2007). Changes in cerebral blood volume and amyloid pathology in aged Alzheimer APP/PS1 mice on a docosahexaenoic acid (DHA) diet or cholesterol enriched Typical Western Diet (TWD). *Neurobiol. Dis.* 28, 16–29. doi: 10.1016/j.nbd.2007.06.007

Hooper, L., Harrison, R. A., Summerbell, C. D., Moore, H., Worthington, H. V., Ness, A., et al. (2004). Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database Syst. Rev.* CD003177. doi: 10.1002/14651858.CD003177.pub2

Hu, N., Yu, J. T., Tan, L., Wang, Y. L., Sun, L., and Tan, L. (2013). Nutrition and the risk of Alzheimer's disease. *Biomed. Res. Int.* 2013:524820. doi: 10.1155/2013/524820

Ingram, D. K., Zhu, M., Mamczarz, J., Zou, S., Lane, M. A., Roth, G. S., et al. (2006). Calorie restriction mimetics: an emerging research field. *Aging Cell* 5, 97–108. doi: 10.1111/j.1474-9726.2006.00202.x

Jacobs, D. R. Jr., Gross, M. D., and Tapsell, L. C. (2009). Food synergy: an operational concept for understanding nutrition. *Am. J. Clin. Nutr.* 89, 1543S–1548S. doi: 10.3945/ajcn.2009.26736B

Katayama, Y., Katsumata, T., Muramatsu, H., Usuda, K., Obo, R., and Terashi, A. (1997). Effect of long-term administration of ethyl eicosapentate (EPA-E) on local cerebral blood flow and glucose utilization in stroke-prone spontaneously hypertensive rats (SHRSP). *Brain Res.* 761, 300–305. doi: 10.1016/s0006-8993(97)00350-8

Kaur, N., Chugh, V., and Gupta, A. K. (2014). Essential fatty acids as functional components of foods- a review. *J. Food Sci. Technol.* 51, 2289–2303. doi: 10.1007/s13197-012-0677-0

Kawakita, E., Hashimoto, M., and Shido, O. (2006). Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. *Neuroscience* 139, 991–997. doi: 10.1016/j.neuroscience.2006.01.021

Kennedy, D. O., Wightman, E. L., Reay, J. L., Lietz, G., Okello, E. J., Wilde, A., et al. (2010). Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *Am. J. Clin. Nutr.* 91, 1590–1597. doi: 10.3945/ajcn.2009.28641

Kerti, L., Witte, A. V., Winkler, A., Grittner, U., Rujescu, D., and Flöel, A. (2013). Higher glucose levels associated with lower memory and reduced hippocampal

33

microstructure. *Neurology* 81, 1746–1752. doi: 10.1212/01.wnl.0000435561.00234.ee

Keys, A. (1970). Coronary heart disease in seven countries. *Circulation* 41, 1–198.

Keys, A., Menotti, A., Karvonen, M. J., Aravanis, C., Blackburn, H., Buzina, R., et al. (1986). The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol.* 124, 903–915.

Kim, D., Nguyen, M. D., Dobbin, M. M., Fischer, A., Sananbenesi, F., Rodgers, J. T., et al. (2007). SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. *EMBO J.* 26, 3169–3179. doi: 10.1038/sj.emboj.7601758

Kivipelto, M., Solomon, A., Ahtiluoto, S., Ngandu, T., Lehtisalo, J., Antikainen, R., et al. (2013). The finnish geriatric intervention study to prevent cognitive impairment and disability (FINGER): study design and progress. *Alzheimers Dement.* 9, 657–665. doi: 10.1016/j.jalz.2012.09.012

Kohli, P., and Levy, B. D. (2009). Resolvins and protectins: mediating solutions to inflammation. *Br. J. Pharmacol.* 158, 960–971. doi: 10.1111/j.1476-5381.2009.00290.x

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B., et al. (2010). Blueberry supplementation improves memory in older adults. *J. Agric. Food Chem.* 58, 3996–4000. doi: 10.1021/jf9029332

Kris-Etherton, P. M., Grieger, J. A., and Etherton, T. D. (2009). Dietary reference intakes for DHA and EPA. *Prostaglandins Leukot. Essent. Fatty Acids* 81, 99–104. doi: 10.1016/j.plefa.2009.05.011

Lim, G. P., Calon, F., Morihara, T., Yang, F., Teter, B., Ubeda, O., et al. (2005). A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J. Neurosci.* 25, 3032–3040. doi: 10.1523/jneurosci.4225-04.2005

Liochev, S. I. (2013). Reactive oxygen species and the free radical theory of aging. *Free Radic. Biol. Med.* 60, 1–4. doi: 10.1016/j.freeradbiomed.2013.02.011

Lopez-Garcia, E., Rodriguez-Artalejo, F., Li, T. Y., Fung, T. T., Li, S., Willett, W. C., et al. (2014). The Mediterranean-style dietary pattern and mortality among men and women with cardiovascular disease. *Am. J. Clin. Nutr.* 99, 172–180. doi: 10.3945/ajcn.113.068106

Lourida, I., Soni, M., Thompson-Coon, J., Purandare, N., Lang, I. A., Ukoumunne, O. C., et al. (2013). Mediterranean diet, cognitive function and dementia: a systematic review. *Epidemiology* 24, 479–489. doi: 10.1097/EDE.0b013e3182944410

Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79, 727–747.

Max Rubner-Institut. (2011). "Fisch in der ernährung," in *Max Rubner-Institut*, ed. H. Rehbein (Hamburg: Max-Rubner-Institut), 1–26.

McCann, J. C., and Ames, B. N. (2005). Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am. J. Clin. Nutr.* 82, 281–295.

McNamara, R. K., and Carlson, S. E. (2006). Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot. Essent. Fatty Acids* 75, 329–349. doi: 10.1016/j.plefa.2006.07.010

Molteni, R., Barnard, R. J., Ying, Z., Roberts, C. K., and Gómez-Pinilla, F. (2002). A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity and learning. *Neuroscience* 112, 803–814. doi: 10.1016/s0306-4522(02)00123-9

Moore, S. C., Yoder, E., Murphy, S., Dutton, G. R., and Spector, A. A. (1991). Astrocytes, not neurons, produce Docosahexaenoic Acid (22:60ω–3) and Arachidonic Acid (20:40ω–6). *J. Neurochem.* 56, 518–524. doi: 10.1111/j.1471-4159.1991.tb08180.x

Mori, T. A., and Beilin, L. J. (2004). Omega-3 fatty acids and inflammation. *Curr. Atheroscler. Rep.* 6, 461–467. doi: 10.1007/s11883-004-0087-5

Morris, M. C., Evans, D. A., Tangney, C. C., Bienias, J. L., and Wilson, R. S. (2005). Fish consumption and cognitive decline with age in a large community study. *Arch. Neurol.* 62, 1849–1853. doi: 10.1001/archneur.62.12.noc50161

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., et al. (2015). A 2 year multidomain intervention of diet, exercise, cognitive training and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet* 385, 2255–2263. doi: 10.1016/s0140-6736(15)60461-5

Nurk, E., Refsum, H., Drevon, C. A., Tell, G. S., Nygaard, H. A., Engedal, K., et al. (2009). Intake of flavonoid-rich wine, tea and chocolate by elderly men and women is associated with better cognitive test performance. *J. Nutr.* 139, 120–127. doi: 10.3945/jn.108.095182

Okereke, O. I., Rosner, B. A., Kim, D. H., Kang, J. H., Cook, N. R., Manson, J. E., et al. (2012). Dietary fat types and 4-year cognitive change in community-dwelling older women. *Ann. Neurol.* 72, 124–134. doi: 10.1002/ana.23593

Pottala, J. V., Yaffe, K., Robinson, J. G., Espeland, M. A., Wallace, R., and Harris, W. S. (2014). Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI study. *Neurology* 82, 435–442. doi: 10.1212/wnl.0000000000000080

Quinn, J. F., Raman, R., Thomas, R. G., Yurko-Mauro, K., Nelson, E. B., Van Dyck, C., et al. (2010). Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. *JAMA* 304, 1903–1911. doi: 10.1001/jama.2010.1510

Ramassamy, C., and Belkacémi, A. (2011). Nutrition and alzheimer's disease: is there any connection? *Curr. Alzheimer Res.* 8, 443–444. doi: 10.2174/156720511796391890

Reddy, R., Fleet-Michaliszyn, S., Condray, R., Yao, J. K., Keshavan, M. S., and Reddy, R. (2011). Reduction in perseverative errors with adjunctive ethyl-eicosapentaenoic acid in patients with schizophrenia: preliminary study. *Prostaglandins Leukot. Essent. Fatty Acids* 84, 79–83. doi: 10.1016/j.plefa.2010.12.001

Sala-Vila, A., and Ros, E. (2011). Mounting evidence that increased consumption of a-linolenic acid, the vegetable n-3 fatty acid, may benefit cardiovascular health. *Clin. Lipidol.* 6, 365–369. doi: 10.2217/clp.11.36

Salvati, S., Natali, F., Attorri, L., Di Benedetto, R., Leonardi, F., Di Biase, A., et al. (2008). Eicosapentaenoic acid stimulates the expression of myelin proteins in rat brain. *J. Neurosci. Res.* 86, 776–784. doi: 10.1002/jnr.21537

Scarmeas, N., Stern, Y., Tang, M. X., Mayeux, R., and Luchsinger, J. A. (2006). Mediterranean diet and risk for Alzheimer's disease. *Ann. Neurol.* 59, 912–921. doi: 10.1002/ana.20854

Small, B. J., Rawson, K. S., Martin, C., Eisel, S. L., Sanberg, C. D., McEvoy, C. L., et al. (2014). Nutraceutical intervention improves older adults' cognitive functioning. *Rejuvenation Res.* 17, 27–32. doi: 10.1089/rej.2013.1477

Studzinski, C. M., Li, F., Bruce-Keller, A. J., Fernandez-Kim, S. O., Zhang, L., Weidner, A. M., et al. (2009). Effects of short-term Western diet on cerebral oxidative stress and diabetes related factors in APP x PS1 knock-in mice. *J. Neurochem.* 108, 860–866. doi: 10.1111/j.1471-4159.2008.05798.x

Su, K. P., Huang, S. Y., Chiu, C. C., and Shen, W. W. (2003). Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. *Eur. Neuropsychopharmacol.* 13, 267–271. doi: 10.1016/j.euroneuro.2003.10.001

Sun, A. Y., Wang, Q., Simonyi, A., and Sun, G. Y. (2011). "Botanical Phenols and Neurodegeneration," in *Herbal Medicine: Biomolecular and Clinical Aspects*, 2nd Edn. eds Benzie, I. F. F. and S. Wachtel-Galor (Boca Raton, FL: CRC Press), 315–325.

Sydenham, E., Dangour, A. D., and Lim, W. S. (2012). Omega 3 fatty acid for the prevention of cognitive decline and dementia. *Cochrane Database Syst. Rev.* 6:CD005379. doi: 10.1002/14651858.CD005379.pub3

Takahashi, M., Tsuboyama-Kasaoka, N., Nakatani, T., Ishii, M., Tsutsumi, S., Aburatani, H., et al. (2002). Fish oil feeding alters liver gene expressions to defend against PPARalpha activation and ROS production. *Am. J. Physiol. Gastrointest. Liver Physiol.* 282, G338–G348. doi: 10.1152/ajpgi.00376.2001

Tan, Z. S., Harris, W. S., Beiser, A. S., Au, R., Himali, J. J., Debette, S., et al. (2012). Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging. *Neurology* 78, 658–664. doi: 10.1212/WNL.0b013e318249f6a9

Tangney, C. C., Li, H., Wang, Y., Barnes, L., Schneider, J. A., Bennett, D. A., et al. (2014). Relation of DASH- and Mediterranean-like dietary patterns to cognitive decline in older persons. *Neurology* 83, 1410–1416. doi: 10.1212/wnl.0000000000000884

Trichopoulou, A., Kouris-Blazos, A., Wahlqvist, M. L., Gnardellis, C., Lagiou, P., Polychronopoulos, E., et al. (1995). Diet and overall survival in elderly people. *BMJ* 311, 1457–1460. doi: 10.1136/bmj.311.7018.1457

34

Tsukada, H., Kakiuchi, T., Fukumoto, D., Nishiyama, S., and Koga, K. (2000). Docosahexaenoic acid (DHA) improves the age-related impairment of the coupling mechanism between neuronal activation and functional cerebral blood flow response: a PET study in conscious monkeys. *Brain Res.* 862, 180–186. doi: 10.1016/s0006-8993(00)02115-6

Valls-Pedret, C., and Ros, E. (2013). Commentary: Mediterranean diet and cognitive outcomes: epidemiological evidence suggestive, randomized trials needed. *Epidemiology* 24, 503–506. doi: 10.1097/EDE.0b013e318296bf8e

Valls-Pedret, C., Sala-Vila, A., Serra-Mir, M., Corella, D., de la Torre, R., Martínez-González, M. Á., et al. (2015). Mediterranean diet and age-related cognitive decline: a randomized clinical trial. *JAMA Intern. Med.* doi: 10.1001/jamainternmed.2015.1668 [Epub ahead of print].

van den Berg, E., Kloppenborg, R. P., Kessels, R. P., Kappelle, L. J., and Biessels, G. J. (2009). Type 2 diabetes mellitus, hypertension, dyslipidemia and obesity: a systematic comparison of their impact on cognition. *Biochim. Biophys. Acta* 1792, 470–481. doi: 10.1016/j.bbadis.2008.09.004

van de Rest, O., Berendsen, A. A., Haveman-Nies, A., and de Groot, L. C. (2015). Dietary patterns, cognitive decline and dementia: a systematic review. *Adv. Nutr.* 6, 154–168. doi: 10.3945/an.114.007617

van de Rest, O., Geleijnse, J. M., Kok, F. J., vvan Staveren, W. A., Dullemeijer, C., Olderikkert, M. G., et al. (2008). Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. *Neurology* 71, 430–438. doi: 10.1212/01.wnl.0000324268.45138.86

Wang, L., Negreira, A., LaViolette, P., Bakkour, A., Sperling, R. A., and Dickerson, B. C. (2010). Intrinsic interhemispheric hippocampal functional connectivity predicts individual differences in memory performance ability. *Hippocampus* 20, 345–351. doi: 10.1002/hipo.20771

Willett, W. C., Sacks, F., Trichopoulou, A., Drescher, G., Ferro-Luzzi, A., Helsing, E., et al. (1995). Mediterranean diet pyramid: a cultural model for healthy eating. *Am. J. Clin. Nutr.* 61, 1402S–1406S.

Witte, A. V., Kerti, L., Hermannstadter, H. M., Fiebach, J. B., Schreiber, S. J., Schuchardt, J. P., et al. (2013). Long-chain omega-3 fatty acids improve brain function and structure in older adults. *Cereb. Cortex* 24, 3059–3068. doi: 10.1093/cercor/bht163

Witte, A. V., Kerti, L., Margulies, D. S., and Flöel, A. (2014). Effects of resveratrol on memory performance, hippocampal functional connectivity and glucose metabolism in healthy older adults. *J. Neurosci.* 34, 7862–7870. doi: 10.1523/JNEUROSCI.0385-14.2014

World Health Organization. (2009). Interventions on diet and physical activity: what works: summary report. available at: http://apps.who.int/iris/handle/10665/44140

Wu, A., Ying, Z., and Gomez-Pinilla, F. (2007). Omega-3 fatty acids supplementation restores mechanisms that maintain brain homeostasis in traumatic brain injury. *J. Neurotrauma.* 24, 1587–1595. doi: 10.1089/neu.2007.0313

Yannakoulia, M., Kontogianni, M., and Scarmeas, N. (2015). Cognitive health and Mediterranean diet: just diet or lifestyle pattern? *Ageing Res. Rev.* 20, 74–78. doi: 10.1016/j.arr.2014.10.003

Yates, K. F., Sweat, V., Yau, P. L., Turchiano, M. M., and Convit, A. (2012). Impact of metabolic syndrome on cognition and brain: a selected review of the literature. *Arterioscler. Thromb. Vasc. Biol.* 32, 2060–2067. doi: 10.1161/ATVBAHA.112.252759

Yurko-Mauro, K., McCarthy, D., Rom, D., Nelson, E. B., Ryan, A. S., Blackwell, A., et al. (2010). Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. *Alzheimers Dement.* 6, 456–464. doi: 10.1016/j.jalz.2010.01.013

35

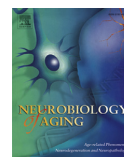## 2.2. Publication2: Original article: Kharabian et al, 2016

Higher body mass index in older adults is associated with lower gray matter volume: Implications for memory performance

Kharabian Masouleh S., Arélin K., Horstmann A., Lampe L., Kipping J.A.,

Luck T., Riedel-Heller S.G., Schroeter M.L., Stumvoll M., Villringer A.,

Witte A.V

Higher body mass index in older adults is associated with lower gray matter volume: implications for memory performance

Shahrzad Kharabian Masouleh [a,f], Katrin Arélin [a,b,c], Annette Horstmann [a,d],
Leonie Lampe [a,b], Judy A. Kipping [a], Tobias Luck [b,e], Steffi G. Riedel-Heller [e,b],
Matthias L. Schroeter [a,b,c], Michael Stumvoll [d,f], Arno Villringer [a,c,f],
Anja Veronica Witte [a,f,*]

[a] Department of Neurology, Max Planck Institute for Cognitive and Brain Sciences, Leipzig, Germany
[b] LIFE—Leipzig Research Center for Civilization Diseases, University of Leipzig, Leipzig, Germany
[c] Clinic for Cognitive Neurology, University of Leipzig, Leipzig, Germany
[d] IFB Adiposity Diseases Faculty of Medicine, University of Leipzig, Leipzig, Germany
[e] Medical Faculty, Institute of Social Medicine, Occupational Health and Public Health (ISAP), University of Leipzig, Leipzig, Germany
[f] Subproject A1, Faculty of Medicine, Collaborative Research Centre 1052 "Obesity Mechanisms", University of Leipzig, Leipzig, Germany

ARTICLE INFO

ABSTRACT

Midlife obesity has been associated with increased dementia risk, yet reports on brain structure and function are mixed. We therefore assessed the effects of body mass index (BMI) on gray matter volume (GMV) and cognition in a well-characterized sample of community-dwelled older adults. GMV was measured using 3T-neuroimaging in 617 participants (258 women, 60—80 years, BMI 17—41 kg/m^2). In addition, cognitive performance and various confounders including hypertension, diabetes, and apolipoprotein E genotype were assessed. A higher BMI correlated significantly with lower GMV in multiple brain regions, including (pre)frontal, temporal, insular and occipital cortex, thalamus, putamen, amygdala, and cerebellum, even after adjusting for confounders. In addition, lower GMV in prefrontal and thalamic areas partially mediated negative effects of (1) higher BMI and (2) higher age on memory performance. We here showed that a higher BMI in older adults is associated with widespread gray matter alterations, irrespective of obesity-related comorbidities and other confounders. Our results further indicate that a higher BMI induces structural alterations that translate into subtle impairments in memory performance in aging.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Numerous epidemiological studies found obesity in midlife to be a risk factor for cognitive impairments later in life, including Alzheimer's Disease (AD; e.g., Anstey et al., 2011; Beydoun et al., 2008; Fitzpatrick et al., 2009; Gustafson, 2006; Kivipelto et al., 2005; Whitmer et al., 2005, 2008; for review, see Emmerzaal et al., 2015; but, see Qizilbash et al., 2015 for recent discussions). However, reports of cognitive performance in nondemented obese compared to lean individuals are equivocal, showing either lower performance in most domains including executive functions and memory (e.g., Benito-León et al., 2013; Walther et al., 2010), or no reliable effect, partly dependent on sex and age range studied

(e.g., Elias et al., 2005; for a review, see Smith et al., 2011). A recent systematic review pointed out that due to methodological limitations, such as incomplete confounder adjustment, the evidence is still not sufficient to draw definite conclusions (Prickett et al., 2015). Potential negative effects on cognition could be a consequence of obesity-associated changes in structural brain properties, due to physiological alterations such as insulin resistance and low-grade inflammation, but also secondary cardiovascular diseases (Biessels et al., 2008; Shefer et al., 2013). For example, obesity has been linked to reduced gray matter volume (GMV) and thickness in frontal, temporal, and subcortical areas, yet the regional patterns often varied between studies (Pannacciulli et al., 2006; Raji et al., 2010; Walther et al., 2010; for a meta-analysis, see Willette and Kapogiannis, 2014). In addition, obesity-related cardiovascular risk factors such as hypertension and type 2 diabetes mellitus have been associated with lower GMV in some, but not all studies, showing again varying regions to be affected (reviewed in

Friedman et al., 2014). Interestingly, even in the absence of manifest obesity, better metabolic profiles have been shown to exert protective effects on AD and related temporal brain areas (Crane et al., 2013; Kerti et al., 2013; Villeneuve et al., 2014), suggesting significant effects of body composition even in the normal-to-overweight range. However, other studies, partly including younger subjects, reported besides negative effects of obesity also positive associations, for example in orbitofrontal and inferior frontal, occipital, temporal, and cerebellar areas, sometimes restricted to males or females (Horstmann et al., 2011; Pannacciulli et al., 2006; Taki et al., 2008; Willette and Kapogiannis, 2014).

In sum, although negative effects of overweight and obesity on the brain even in older ages seem biologically plausible, previous studies could not establish a consistent pattern of regional changes in gray matter structure, and if this would affect cognitive performance. This might be partly due to different sample characteristics such as age, sex, and obesity measures studied (Willette and Kapogiannis, 2014). Moreover, important confounders, such as age- and obesity-associated conditions and related medication intake, for example, hypertension, diabetes, hyperlipidemia, or estrogen supplementation, have not consistently been considered in previous studies (e.g., Brooks et al., 2013; Ho et al., 2011), rendering potential independent effects of higher body mass index (BMI) in older age still debatable (Friedman et al., 2014; Prickett et al., 2015).

To overcome these limitations, the aim of the present study was to comprehensively assess the effect of BMI on regional GMV independent of potential confounders in a well-characterized population-based cohort of otherwise healthy older individuals, using whole-brain GMV analyses and a large sample size. Further, we examined potential interactions with regard to age, sex, and severity of obesity, and aimed to control for manifesting comorbidities including hypertension, diabetes and intake of antihyperlipidemic medication (Biessels et al., 2008; Elias et al., 2005) in both adjusted and sensitivity statistical analyses, as for example, related medical treatment could have confounded the effects of BMI in older age (Beeri et al., 2008; Jennings and Zanstra, 2009; Nadkarni et al., 2015; Patrone et al., 2014). In addition, we sought to additionally control for various factors known to affect brain structure and cognition, including white matter hyperintensities (Wen et al., 2006), depression (Kirwan et al., 2008), smoking and education status (Garibotto et al., 2008; Karama et al., 2015), intake of estrogen supplements (Lord et al., 2008), as well as apolipoprotein E (APOE) e4 genotype (Wishart et al., 2006). We additionally evaluated if BMI indirectly affected cognitive performance through changes in regional GMV using simple mediation analyses, and if this effect was moderated by age. We hypothesized that in our cohort of cognitively healthy older individuals, a higher BMI would on average be associated with lower regional GMV in several brain areas, including frontal, temporal, and parietal areas, independent of confounders. In addition, we speculate that this would correlate with lower performance in higher-order cognitive domains including memory and executive functions, as these processes are known to correlate with regional GMV, for example, in the prefrontal and temporal lobe (Buckner, 2004; Mander et al., 2013; Steffener et al., 2013; Yuan and Raz, 2014). Eventually, this might help to further determine if overweight and obesity have an independent negative impact on the brain in aging populations.

2. Methods

2.1. Participants

All participants were enrolled in the "Health Study of the Leipzig Research Centre for Civilization Diseases" (LIFE). Adult Leipzig inhabitants were randomly invited via the population registry (n = 10,000) and a proportion underwent neuropsychological testing, medical examinations, and magnetic resonance imaging (MRI) of the head at 3T (n = ~2600). Medical history and medication intake was assessed by means of a structured interview by trained staff. Brain pathologies due to stroke, intracranial bleeding, or tumors were defined by a trained neuroradiologist after careful examination of MR images. The study design and complete assessment information have been described in detail in (Loeffler et al., 2015).

All participants signed an informed consent form and received a small financial compensation. The study protocol was in accordance with the declaration of Helsinki and approved by the ethics committee of the University of Leipzig.

Of 985 participants ≥60 years who underwent MRI available at the date of our analyses and were free of stroke, 203 participants were excluded due to intake of medication affecting central nervous system such as antidepressants, and immune suppressive medication (Fig. 1). Confounding parameters comprised "systolic blood pressure" defined as the mean systolic blood pressure of 3 consecutive measurements in a seating position at rest; "smoking status" defined using self-reported information as never smoker, previous smoker, or current smoker; "depression score" assessed using Center for Epidemiologic Studies Depression Scale (Lewinsohn et al., 1997); and "education" defined using International Standard Classification of Education (ISCED, 7 levels, UNESCO, 1997). "Arterial hypertension" (yes or no) was defined as a systolic blood pressure ≥160 mm Hg, a diastolic blood pressure ≥95 mm Hg, or use of antihypertensive medication (Biessels et al., 2006). In addition, based on information assessed from the medical interviews, "diabetes status" was defined as none, diabetes mellitus type 1 (medicated), diabetes mellitus type 2 (medicated), or diabetes mellitus type 2 (unmedicated). Intake of "antihyperlipidemic medication" (any or none) and estrogen supplements (any or none) was also regarded as potential confounders. "Other cardiovascular conditions" were defined as arrhythmia or tachycardia (any or none). Furthermore, "white matter hyperintensities" were assessed according to the Fazekas rating scale by a trained neuroradiologist using 3D fluid-attenuated inversion recovery images (Fazekas et al., 1993). Seventy-four participants were excluded due to missing data in one of these confounders. Furthermore, 78 participants were excluded due to major brain pathologies, severe movement artifacts, or other technical problems. In addition, 13 participants were excluded due to nonintact cognitive performance, defined as a mean cognitive score (i.e., mean of all standardized cognitive subtests, see Section 2.3. for details) of less than 3 standard deviation of the sample mean, leaving 617 participants for GMV analyses. Of these, APOE e4-allele carrier status was identified in 485 participants (see Supplementary Text for details).

2.2. Magnetic resonance imaging

Anatomic T1-weighted images were acquired using a 3-dimensional Magnetization-Prepared Rapid Gradient Echo sequence. Generalized autocalibrating partially parallel acquisition parallel imaging technique (Griswold et al., 2002) was applied on the Alzheimer's Disease Neuroimaging Initiative standard protocol with the following parameters: inversion time, 900 ms; repetition time, 2300 ms; echo time, 2.98 ms; flip angle, 9°; band width, 240 Hz/pixel; image matrix, 256 × 240; 176 partitions; field of view, 256 × 240 × 176 mm^3; sagittal orientation; voxel size, 1 × 1 × 1 mm^3; no interpolation. GMV was assessed using voxel-based morphometry in SPM8 (www.fil.ion.ucl.ac.uk/spm) on T1-weighted MRI scans. Briefly, individual images were segmented into gray matter maps and coregistered to a study-specific cerebral and to a cerebellar-specific template. We performed separate workflows for the cerebrum and the cerebellum to improve tissue
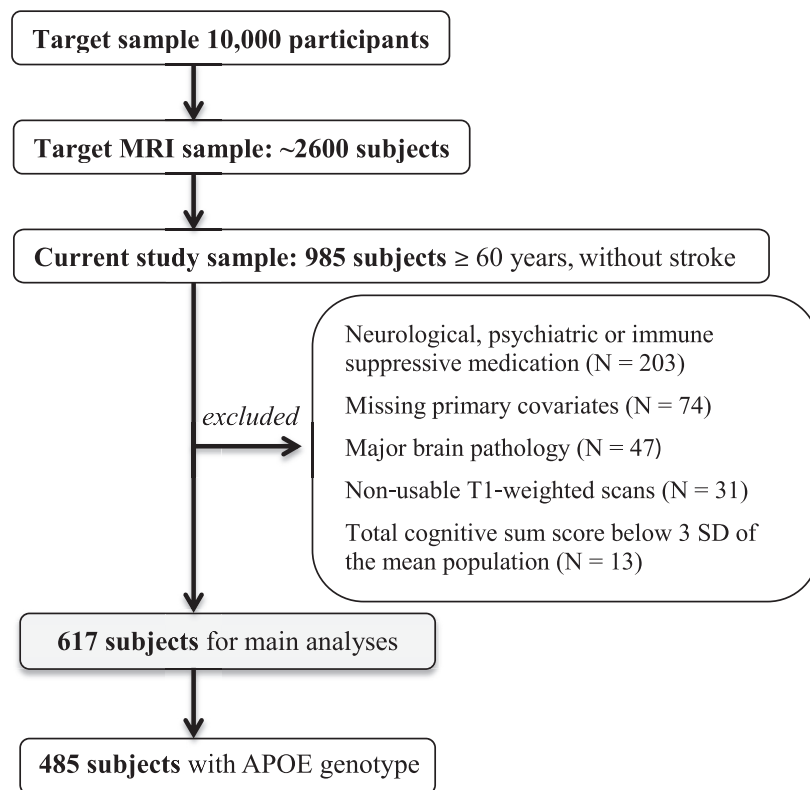
Fig. 1. Flow chart of the study. Of 985 older adults free of stroke, 368 were excluded due to medication intake, missing covariates, nonusable MRI scans, brain pathology, or nonintact cognition, leaving 617 participants for main analyses. Of this sample, APOE-genotype was defined for 485 participants. Abbreviations: APOE, apolipoprotein E; MRI, magnetic resonance imaging.

segmentation and interindividual alignment, see Supplementary Text for details.

2.3. Cognitive testing

Neuropsychological testing comprised trail-making test (TMT) part A and B, semantic, and phonemic verbal fluency and verbal memory, included in the neuropsychological test battery of the Consortium to Establish a Registry for Alzheimer's disease (Morris et al., 1989). Learning was defined as the sum of 3 consecutive learning trials, recall was defined as the sum of correctly recalled words after a delay, in which participants performed a nonverbal task, and recognition was defined as the number of correctly recognized words of a list of 20 mixed words presented afterward. Test scores were z-transformed and combined to create composite scores for executive functions, memory performance, and processing speed (Van de Rest et al., 2008). Composite scores were defined as follows: executive functions = [z_phonemic fluency + z_semantic fluency − z_TMT(part B − part A)/part A]/3; memory = (z_sum_learning + z_recall + z_recognition)/3; processing speed = −z (TMT [part A]). For 39 participants, one of the cognitive test scores was missing, and the respective composite score was calculated based on average performance of the remainder of available tests comprising the composite score (Oosterman et al., 2010; Schaapsmeerders et al., 2013).

2.4. Statistical analysis

2.4.1. BMI and GMV

Voxel-wise associations of BMI and GMV were tested in SPM using multiple regressions in 3 consecutive models. First, in a minimal model, we controlled for the effects of age and sex (model-1). In model-2, we additionally controlled for arterial hypertension, diabetes, other cardiovascular disease, intake of antihyperlipidemic medication, estrogen-supplement intake, education, blood pressure, smoking, depression, and white matter hyperintensities (see Section 1). In model-3, we further adjusted for carrying 1 or 2 APOE e4-alleles (n = 485).

To test if possible effects of BMI were age specific, we tested the interaction term of BMI by age on GMV, controlling for confounders according to models-1, -2, and -3. In addition, independent effects of age on GMV were evaluated using age as main contrast. Furthermore, we tested for interaction effects of BMI by sex, obesity status, or hypertension. In addition, sensitivity analyses were performed in subgroups without diabetes or intake of anti-hyperlipidemic medication to demonstrate that the effects found with respect to obesity are not due to, or biased by, possible effects of comorbidities and related medication intake (see Supplementary Text for details). Correction for multiple testing was performed using the family-wise-error correction based on Gaussian Random Field Theory (Friston et al., 1994) at cluster level ($p < 0.05$)

combined with a primary uncorrected voxel-level threshold of $p < 0.001$ (Woo et al., 2014).

2.4.2. Exploratory analyses

Associations between BMI and cognitive composite scores (executive functions/memory performance/processing speed) were analyzed using bivariate and partial correlations adjusting for confounders according to models-1, -2, and -3. Next, associations between cognitive composite scores and mean GMVs in BMI-associated clusters were evaluated in an exploratory manner using bivariate and partial correlations adjusting for confounders according to models-1, -2, and -3, with a significance threshold of $p < 0.05$ (2 sided). GMV clusters were defined using voxel-level family-wise-error−corrected threshold of $p < 0.05$ and a cluster extent of at least 50 voxels (Bennett et al., 2009; Woo et al., 2014). In the following, we used simple mediation analyses (Hayes, 2013) to investigate if BMI-associated alterations in GMV-mediated effects of BMI on cognitive performance (Fig. 2A), independent of confounders according to models-1, -2, and -3, in line with previous studies (Ferreira et al., 2014; Kerti et al., 2013; Mander et al., 2013). To test if these mediations were modulated by age, we tested a moderating effect of age on the indirect paths (Fig. 2B). In addition, we assessed in simple mediation models if GMV in BMI-associated clusters would mediate effects of age on cognition, in addition to BMI (Fig. 2C). A 99% bias-corrected bootstrap confidence interval (BBCI) excluding zero, based on 10,000 bootstrap samples, was considered to be a robust effect (Hayes, 2013). All mediation models

were tested using standardized values, and results are reported with standardized effects (ab).

We additionally tested for possible alternative models (i.e., causal chain of (1) effects of GMV on cognitive performance and cognitive performance on BMI or (2) effects of GMV on BMI and BMI on cognitive performance) to find the most plausible model as supported by our data (for further discussion, see e.g., Hayes, 2013).

All variables were normally distributed (unimodal, |skewness| ≤ 1) except TMT and memory composite score. These were log transformed to overcome the skewed distribution. Exploratory analyses were performed in SPSS 20 (PASW, SPSS, IBM), significance level was set at $p < 0.05$, corrected for 2-sided nature of the tests.

3. Results

In total, 617 healthy older participants (258 women) were included in main analyses, see Table 1 for demographic characteristics and Table 2 for distribution of raw cognitive test scores. Owing to the nature of our exclusion criteria, participants excluded from main analyses (n = 368) were on average slightly older, more frequently women, exhibited a higher BMI and were less educated compared to those included (all $p < 0.05$, Supplementary Table 1). The subgroup with available APOE genotype tended to be older ($p < 0.001$), yet without differences in age range (60−80 in both groups) or with regard to sex, BMI, and education (all $p > 0.05$; for details see Supplementary Table 1). No significant differences were found between APOE e4-carriers and noncarriers in BMI or other cardiovascular risk factors (all $p > 0.05$; Supplementary Table 2).

3.1. Association between BMI and GMV

Multiple regression model-1 revealed that a higher BMI was associated with lower GMV (Fig. 3, Table 3) within anterior cingulate cortex, paracingulate gyrus, ventral and medial prefrontal cortex (vm-PFC), orbitofrontal cortex (OFC), mid/posterior cingulate gyrus, superior and inferior lateral occipital lobe, occipital and temporal fusiform gyri, middle temporal gyri, insula, thalamus, putamen, caudate nucleus, and left amygdala. Decreased GMV were also found in area V and in the posterior and inferior cerebellum (Fig. 3).
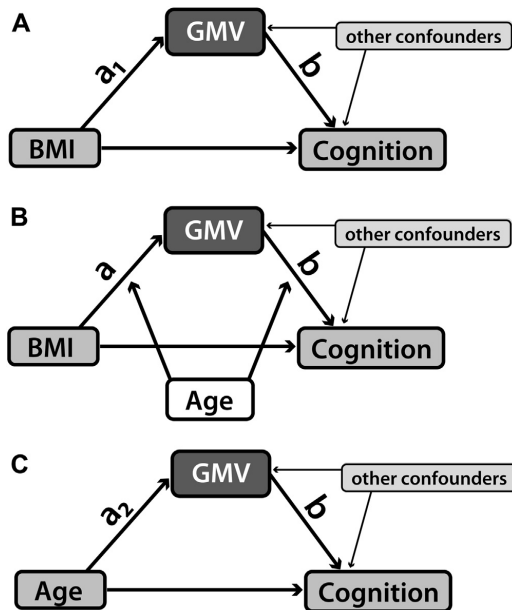

Fig. 2. Conceptual diagrams of main mediation models. (A) Simple mediation models of the indirect path of the effect of body mass index (BMI) on cognitive performance through changes in gray matter volume (GMV) in BMI-associated clusters, controlling for confounding effects of covariates. (B) Conceptual diagram, representing moderation effects of age on the indirect path by which BMI affects cognitive performance through GMV changes in BMI-associated clusters. (C) Simple mediation model of the indirect path of the effect of age on cognitive performance through changes in GMV in BMI-associated clusters, controlling for confounders. a, a1, a2: regression coefficient of association between GMV and BMI or age, respectively. b: regression coefficient of association between GMV and cognitive scores, controlling for confounders.

Table 1
Sample characteristics

	Participants, n = 617 (258 women)
Age (y)	68.7 ± 4.6 (60−79)
BMI (kg/m²)	27.5 ± 4 (16.8−41.4)
Mean systolic BP (mm Hg)	136.1 ± 16.9 (89−197)
Education (%) [without SS-LD/SS-LD/advanced SS-LD/ advanced technical SS-LD/technical college-entrance degree/university-entrance degree]	0.2/10.7/6.3/42.6/5.2/35
Depression scale (CES-D) [score]	9.38 ± 5.1 (0−34)
Smoking (%) [current/previous/never]	7.5/33.2/59.3
APOE status (n) [e4 carrier/non−e4 carrier/missing]	97/388/132
Arterial hypertension (%) [yes]	55.8
Cardiovascular diseases (%) [any]	19.1
Diabetes status (%) [none/type 1 medicated, type 2 medicated, type 2 nonmedicated]	84.4/0.5/12.2/2.9
Antihyperlipidemic medication (%) [yes]	22.2
Estrogen supplement (% females) [yes]	7.4
White matter hyperintensities (%) [Fazekas score 0/1/2/3]	23.5/59.8/16.2/0.5

Data are mean (SD) (minimum−maximum) unless indicated otherwise.
Key: APOE, apolipoprotein E; BMI, body mass index; BP, blood pressure; CES-D, Center for Epidemiologic Studies Depression Scale; SS-LD, secondary school-leaving degree, which represents the end of 9th grade, The university-entrance degree equals to 12−13 years of education, in Germany; SD, standard deviation.

Table 2
Distribution of raw cognitive test scores in the main sample

Semantic fluency (animals)[*] (no. of words)	23.5 ± 5.8 (6−42)
Phonemic fluency (s-words)[**] (no. of words)	13.3 ± 4.4 (1−27)
TMT, part A (s)	39 [32−45] (17−120)
TMT, part B (s)	87 [71−109] (25−300)
Word list learning (no. of words)	21.5 ± 3.6 (6−29)
Word list recall (no. of words)	7.6 ± 1.7 (0−10)
Word list recognition[***] (no. of words)	20 [20−20] (17−20)

Data are mean ± SD (minimum−maximum) or median [IQR] (minimum−maximum).
n = 617, except, [*]n = 616, [**]n = 578, [***]n = 615.
Key: TMT, Trail-making test; SD, standard deviation; IQR, interquartile range.

Controlling for additional confounders included in model-2 did not affect these results (Supplementary Fig. 1). In addition, a similar regional pattern was seen after correcting for APOE status (model-3), yet significance was slightly reduced in frontal and parietal lobes (Supplementary Fig. 2). In addition, APOE e4-carrier status was not associated with significant differences in regional GMV.

We did not observe a significant interaction effect between BMI and age on GMV. Although, multiple regression model-1 revealed a significant negative association of age with most parts of the cerebrum, sparing the cerebellum (data not shown). No significant interaction was found between BMI and sex, BMI and obesity status, or BMI and hypertension on GMV. In addition, constraining the sample to participants without diabetes (n = 518) or to the ones that do not take lipid-lowering medication (n = 480) did not change the pattern of BMI-GMV associations (Supplementary Figs. 3 and 4).

3.2. Associations with cognitive performance

A higher BMI correlated with lower scores on executive function (r = −0.11, p = 0.006). This association persisted after controlling for model-1 (partial-r = −0.108, p = 0.007) but diminished after

adjusting for other confounders in models-2 and -3. Other composite scores did not show significant associations with BMI (all $p > 0.05$). As expected, age was significantly negatively correlated with performance in memory (r = −0.17, $p < 10^{-3}$) and processing speed (r = −0.19, $p < 10^{-3}$). Executive function sum score, however, was not associated with age in our sample ($p > 0.05$).

3.2.1. Executive functions

Bivariate correlations showed that larger GMV in left paracingulate gyrus and right planum temporale were associated with higher scores on executive function (see Supplementary Table 3 for r- and p-values). These associations remained significant after correction for confounders in model-1 but slightly diminished in models-2 and -3. We did not find significant mediations between BMI and executive function via GMV in these clusters ($p > 0.05$).

3.2.2. Memory performance

Considering memory, lower GMVs in most of BMI-associated clusters were associated with lower performance (for r- and p-values, see Supplementary Table 3). These correlations remained significant after adjusting for confounders in the vm-PFC, right medial thalamus, bilateral lateral OFC/Insula, and left paracingulate gyrus.

Furthermore, simple mediation models supported existence of an indirect path by which a higher BMI affected memory performance via alterations in GMV in frontal and thalamic clusters (Fig. 4, vm-PFC: ab = −0.035, BBCI: [−0.075, −0.006]; right medial thalamus: ab = −0.0235, BBCI: [−0.052, −0.003]; right lateral OFC: ab = −0.022, BBCI: [−0.05, −0.0028]; left paracingulate gyrus: ab = −0.022, BBCI: [−0.052, −0.003]). Mediations of the first 2 clusters stayed significant after controlling for both models-2 and -3.

Considering further mediation models, we did not find evidence for a moderating effect of age on the indirect effects of BMI on memory through differences in GMV in the previously described
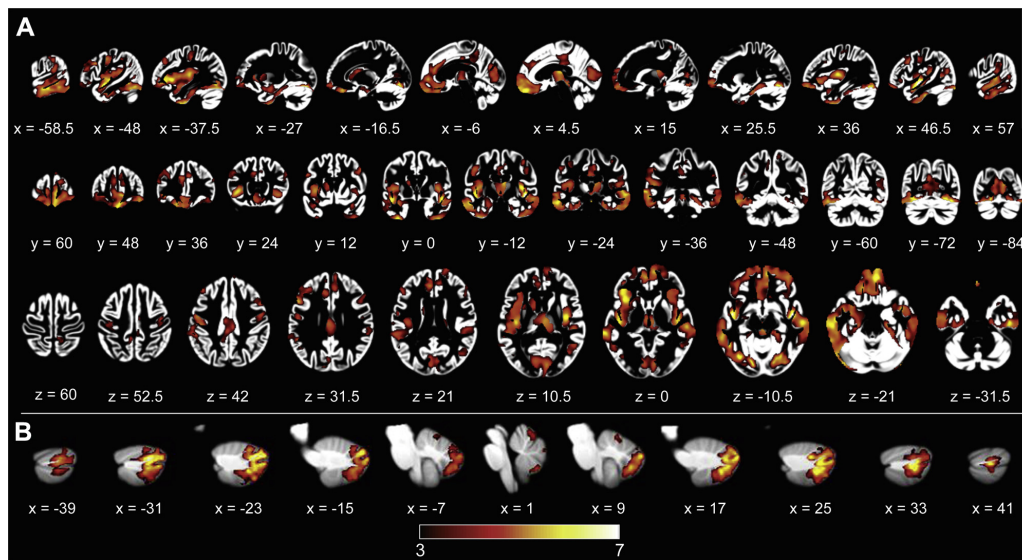

Fig. 3. Body mass index (BMI)-associated gray matter alterations. Significant association between higher BMI and lower gray matter volume (GMV), according to multiple regression analysis adjusted for age and sex (model-1) in a cohort of 617 older individuals. Significant clusters, surviving a voxel-level threshold of $p < 0.001$ (uncorrected) and a cluster level threshold of $p < 0.05$ (FWE-corrected), are displayed in the cerebrum (A) and cerebellum (B), superimposed on a study-specific gray matter template. Color bar shows the *t* value at each significant voxel. Abbreviation: FWE, family-wise-error. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Peak coordinates of significant negative associations between gray matter volume and BMI, adjusted for model-1 (voxel-level FWE-corrected threshold of $p < 0.05$ and a cluster extent of at least 50 voxels)

Regions	MNI coordinates (mm, hot voxel)			z-score	Cluster size (voxels)
	x	y	z		
Cerebral clusters					
Frontal medial cortex(R)	6	52	−23	6.43	4023
Frontal pole (R)	40	54	−15		
Subcallosal cortex (R)	4	30	−23		
Frontal pole (L)	−10	57	−3		
Frontal orbital cortex (R)	33	32	−5	5.08	216
Paracingulate gyrus (L)	−4.5	40.5	18	4.98	109
Precentral gyrus (L)	−48	−7	40	4.96	86
Superior temporal gyrus (R)	46	−9	−9	7.32	4118
Insular cortex (R)	39	−9	9		
Planum polare (L)	−45	−4	−14	7.03	3399
Insular cortex (L)	−39	18	−3		
Planum temporale (R)	50	−30	12	5.02	94
Planum temporale (L)	−52	−36	12	5.06	164
Parahippocampal gyrus (R)	22	−19	−26	5.47	182
Parahippocampal gyrus (L)	−21	−19	−24	4.90	64
Temporal fusiform cortex (L)	−34	0	−50	6.08	461
Occipital fusiform gyrus (R)	36	−72	−18	6.29	862
Occipital fusiform gyrus (L)	−30	−78	−17	6.86	5100
Inferior temporal gyrus (L)	−62	−22	−20		
Intracalcarine cortex (R)	9	−84	13	5.20	278
Thalamus (R)	6	−15	16	5.75	887
Cerebellar clusters					
Right crus I	31	−68	−38	7.03	9939
Left crus I	−25	−73	−32	6.69	9984

Key: BMI, body mass index; FWE, family-wise-error; L, left; MTL, medial temporal lobe; PFC, prefrontal cortex; R, right.

clusters. Yet, we detected independent effects of age, that is, that increasing age resulted in lower memory scores partly through variations in GMV in BMI-associated clusters, according to mediation models controlling for sex and BMI (vm-PFC: ab = −0.036, BBCI: [−0.071, −0.006]; right medial thalamus: ab = −0.027, BBCI: [−0.058, −0.005]; right lateral OFC: ab = −0.017, BBCI: [−0.04, −0.002]; left paracingulate gyrus: ab = −0.023, BBCI: [−0.05, −0.0035]), and other confounders (data not shown).

Testing of alternative mediation models within these clusters did not yield significant results.

Taken together, mediation analyses indicated that both higher BMI and higher age affected memory performance independently through lower GMV in frontal and thalamic BMI-associated regions, yet without showing significant interactive effects in our sample.

3.2.3. Processing speed

A faster processing speed was also associated with higher GMV in almost all BMI-associated clusters. Adjusting for confounders in models-1 and -2 suggested an independent association of processing speed and GMV within the bilateral temporal/insular clusters and in intracalcarine cortex (see Supplementary Table 3 for r- and p-values). However, these effects diminished after correction for parameters in model-3. Furthermore, mediation models did not support existence of a significant causal link between BMI and processing speed through any of these gray matter regions.

4. Discussion

Using a large population-based cohort of 617 healthy older adults, we found that a higher BMI is associated with lower GMV in multiple cortical and subcortical areas and in the cerebellum, even after adjusting for age, sex, obesity status, and other confounders such as arterial hypertension, diabetes, and APOE genotype. In addition, mediation analyses indicated that a higher BMI affected memory through reduced regional GMV in frontal and thalamic brain areas. Further, mediation analyses indicated that higher age contributed to decreases in memory performance through reduced GMV in BMI-associated brain areas, yet not moderating the indirect effects of BMI.

4.1. BMI and GMV

Our finding of an inverse linear association between BMI and GMV in a large population-based cohort of nondemented older adults confirms and extends previous studies that used obesity-related measures such as BMI, waist circumference, and visceral adipose tissue (Raji et al., 2010; Walther et al., 2010; Willette and Kapogiannis, 2014). Several reports described reductions in similar areas of the frontal lobes, cingulate gyrus, amygdala, dorsal striatum, thalamus, and cerebellum with higher weight (Marqués-Iturria et al., 2013; Walhovd et al., 2014), mainly in older age (Brooks et al., 2013; Driscoll et al., 2012; Raji et al., 2010; Walther et al., 2010), for a review see Willette and Kapogiannis (2014). However, some studies yielded contrary results, linking a higher BMI to higher regional GMV (Horstmann et al., 2011; Taki et al., 2008). For example, besides regional decreases, Taki et al. (2008) observed in men, but not in women, increases in frontal GMV with higher BMI, using voxel-based morphometry in a large cohort (n = 1428) of 12- to 80-year-old Japanese. Horstmann et al. (2011) reported positive associations of BMI and GMV in women in reward-related areas, using a cohort of 122 young adults. Several differences between these studies render a direct comparison of results difficult, for example, regarding age range, considered confounders, image acquisition and processing software (Streitbürger et al., 2014), and BMI distribution [i.e., BMI $\geq$ 25 kg/m² in over 70% of our population-based older cohort, compared to ~40% in the young sample (Horstmann et al., 2011) and only 15% males and 12% females in the Japanese sample (Taki et al., 2008)].

4.2. Associations with cognitive performance

Our data suggest that a lower BMI was associated with better executive functions in bivariate correlations, a link that has been reported previously (Davidson and Martin, 2014; Elias et al., 2005; Fergenbaum et al., 2009; Gunstad et al., 2007), and might be explained by working memory advantages that lower the risk to develop obesity later in life (Brooks et al., 2013; Davidson and Martin, 2014). However, we show that this association depended on confounders, and we did not find evidence for correlations with memory and processing speed, indicating that BMI might not directly relate to cognitive outcomes or that the explained variance is rather small in our sample of cognitively intact older adults. In addition, in contrast to our assumptions, our data do not support a causal link between higher BMI, subsequent gray matter alterations, and lower executive functions in aging. This lack of mediation might be insightful given that executive functions are known to depend on information transfer between distant brain regions (Delbeuck et al., 2003; Uhlhaas and Singer, 2006). Thus, future studies that implement structural or functional connectivity measures are needed to clarify possible interactions here.

Turning to memory, lower GMV in frontal, insular, and thalamic clusters were associated with lower performance, independent of confounders. Moreover, mediation results indicated that higher BMI affected memory performance indirectly via its negative effect on GMV in frontal and thalamic regions, which is in line with our hypothesis. The frontal lobe cluster belongs to the Brodmann areas 10 and 11, which contribute to a variety of tasks including strategic
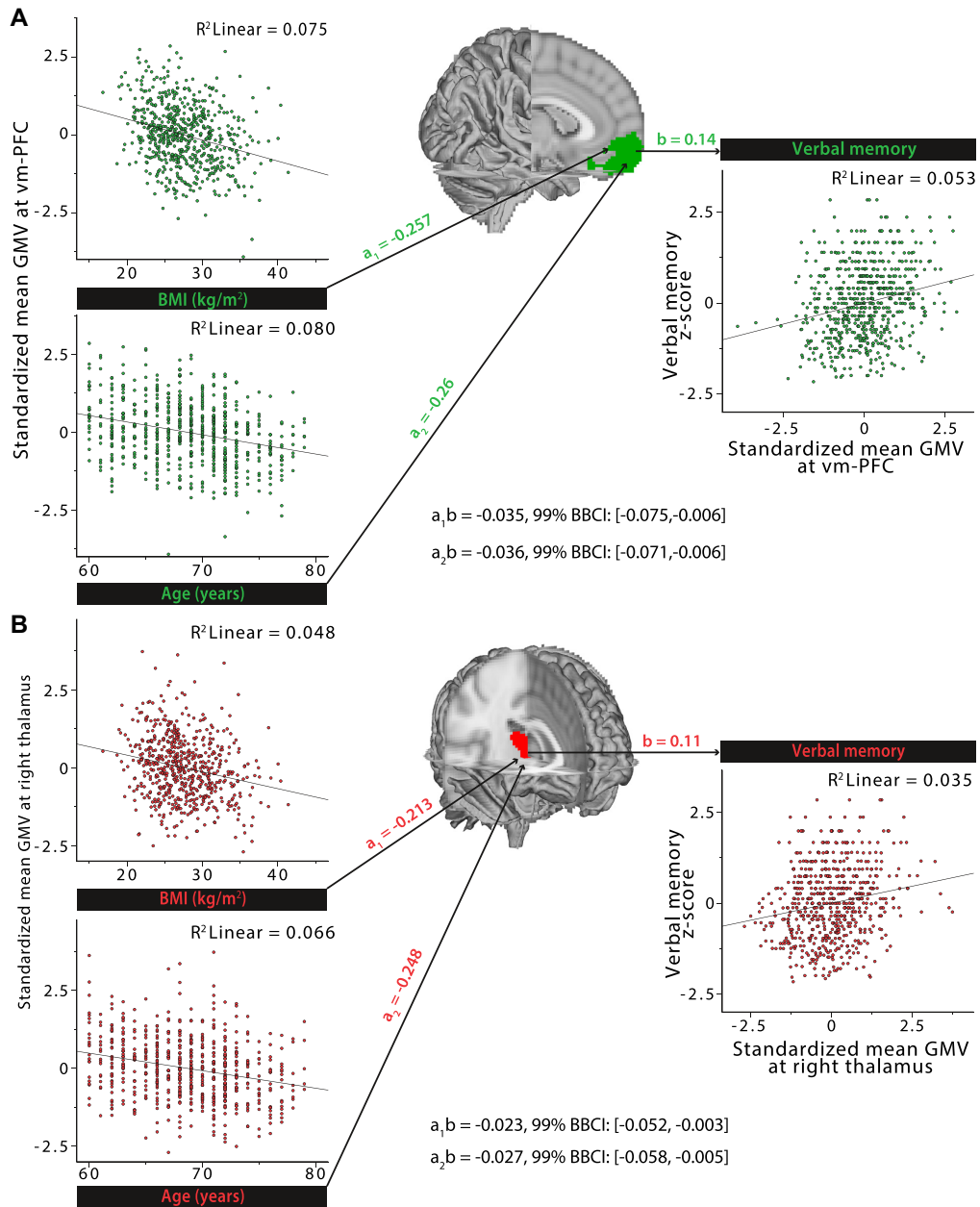
Fig. 4. Mediating pathways between body mass index (BMI), gray matter volume (GMV), and verbal memory performance. According to mediation analysis, a higher BMI and higher age independently affected verbal memory performance through lower GMV in (A) the ventral-medial prefrontal cortex (vm-PFC, green cluster) and (B) in the right medial thalamus (red cluster). Scatterplots show bivariate associations. R^2 is the coefficient of determination. a_1: standardized regression coefficient of the association between mean GMV at each cluster and BMI, in a model controlling for age and sex. a_2: standardized regression coefficient of the association between mean GMV at each cluster and age, in a model controlling for BMI and sex. b: regression coefficient of the association between mean GMV at each cluster and verbal memory z-scores, controlling for age, BMI and sex. Significance of indirect paths were tested using 99% BBCIs of 10,000 bootstrap samples, a_1b and a_2b are standardized indirect effects, n = 617. Abbreviation: BBCI, bias-corrected bootstrap confidence interval. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

processes in memory recall and encoding new information into long-term memory (Euston et al., 2012; Kirwan et al., 2008). The thalamic cluster, on the other hand, involves regions of the intralaminar and medial thalamic nuclei that exhibit long-range connections predominantly with the medial prefrontal cortex (Behrens et al., 2003; Eckert et al., 2012; Klein et al., 2010). Others have shown that focal lesions to the mediodorsal thalamus, similar to those resulting from PFC damage, are associated with deficient memory processing (Staudigl et al., 2012; Zola-Morgan and Squire, 1985). This is in line with our findings indicating that GMV alterations within these areas, suggestive of degenerative processes such as neuronal loss, shrinkage of neurons, reductions in synaptic spines, or other changes in the structural substrates of interneuronal communication (Fjell and Walhovd, 2010; Raz, 2005), provoked alterations in memory performance. Notably, Mander et al. (2013) previously observed that medial PFC atrophy is a key player in mediating the impact of aging on impaired memory consolidation. We were able to replicate and extend these findings, showing that besides BMI, higher age contributed independently to lower memory through frontal and thalamic GMV decreases in our cohort. Yet, mediation analyses did not provide evidence that age moderated the indirect effects of BMI, indicating that the influence of BMI in healthy older adults does not increase or diminish substantially between 60 and 80 years of age. Taken together, our findings indicate that gray matter in frontal and thalamic areas show vulnerability to both age- and BMI-related morphological changes, which, as a consequence, translate into subtle declines in memory functions. The observed effect sizes in our study were relatively small though, which could be due to cognitive reserve or ceiling effects of the used memory task, as we analyzed a cognitively intact sample. However, the described BMI-related structural changes, linked with initial memory impairments, are of considerable importance, as they illustrate a further, yet modifiable load on intact cognition, and put individuals into a more vulnerable condition.

Our results also indicate that lower GMV in temporal/insular and occipital clusters correlated with slower processing speed, mainly independent of confounders. This is in line with previous studies that found negative associations between processing speed and GMV in similar regions (Eckert et al., 2010; Steffener et al., 2013), but see Ferreira et al., 2014 for positive effects of lower regional GMV in certain frontal areas on reaction times. However, also note that slower processing speed has been mainly attributed to alterations in white matter integrity (Madden et al., 2009, 2012; Penke et al., 2010), which might explain a lack of significance of a mediating path between BMI and processing speed in our sample. This hypothesis needs to be tested in upcoming studies that evaluate mediation models involving (frontal) white matter microstructure.

4.3. Potential underlying mechanisms

A higher BMI could have affected GMV via several pathways. For example, excessive weight is associated with conditions that exert negative effects on neuronal tissue, for example, endothelial dysfunction, microbleeds, subclinical strokes, and ischemic events (Ferguson et al., 2003) or production of advanced glycated endproducts and reactive oxygen species (Biessels et al., 2008). In a previous study, it was shown that higher glucose (which is correlated with higher BMI) even in the normal range could exert negative effects on brain structure and function (Kerti et al., 2013). Adipose tissue is also associated with increased production of inflammatory cytokines (Gregor and Hotamisligil, 2011) and altered lipid metabolism, which are associated with detrimental effects on the brain (Haley et al., 2013). In addition, obesity-induced alterations in production and sensitivity to adipose tissue–derived hormones, such as leptin, could play an important role, as these factors can pass the blood-brain barrier and affect neuronal functioning (Davis et al., 2014). Future studies implementing sensitive measures of energy metabolism and brain function should further address these issues.

4.4. Strengths and limitations

Several limitations should be considered when interpreting our findings. First, although mediation analyses are frequently used to identify potential underlying mechanisms (Hayes, 2013; Kerti et al., 2013; Mander et al., 2013), we are unable to infer causal relationships due to the cross-sectional nature of our study. Particularly with regard to obesity, it has been speculated that impairments in executive functions could predispose an individual to gain excessive weight (Reinert et al., 2013), and cognitive impairment, obesity and gray-matter changes might have caused each other reciprocally (Davidson and Martin, 2014), a hypothesis that needs to be tested in carefully designed longitudinal assessments. However, note that mediation analyses did not yield significance for alternative paths, rendering a causal relationship between GMV and cognitive performance (or BMI) via indirect effects of GMV on BMI (or on cognition) in our older cohort unlikely. Second, we cannot exclude residual confounding due to poor sleep quality or sarcopenia, which are frequent in the elderly and have been linked to poorer brain health and risk for AD (Benedict et al., 2015; Burns et al., 2010; Sprecher et al., 2015). Third, in the subgroup analyses including APOE-genotype status, the lower number of participants might have resulted in lower statistical power. However, strength of this study rely on the large community-based sample of healthy older adults randomly chosen via the city registry office, which is considered to be more representative of the general population than sampling via advertising (Martinson et al., 2010). In addition, participants underwent high-resolution neuroimaging at 3T and provided information of a comprehensive set of potential confounders, accompanied by assessment of cognitive tests in major domains that are affected in normal and pathological aging.

4.5. Conclusions

Using a large sample of community-dwelled participants, we were able to show that a higher BMI is linked with lower GMV in the aging population, even after controlling for important confounders. Moreover, mediation analyses indicated that higher BMI and higher age contributed independently to lower memory performance, through reductions in GMV in BMI-associated frontal and thalamic brain areas. Thus, gray matter structural preservations caused by lower weight could eventually be protective for specific cognitive domains, independent from other comorbid, environmental, or genetic factors. Our findings further implicate that reducing overweight and obesity in our societies could help to maintain brain health into late life, a hypothesis that has already led to initiation of promising large-scale longitudinal controlled trials (Ngandu et al., 2015). Future studies focusing on physiological manifestations of a higher BMI and associations with BMI-related gray matter alterations would further help us to walk through better individualized therapies and preventing cognitive decline in aging.

Disclosure statement

The authors declare no competing interest.

microstructure. *Neurology* 81, 1746–1752. doi: 10.1212/01.wnl.0000435561. 00234.ee

Keys, A. (1970). Coronary heart disease in seven countries. *Circulation* 41, 1–198.

Keys, A., Menotti, A., Karvonen, M. J., Aravanis, C., Blackburn, H., Buzina, R., et al. (1986). The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol.* 124, 903–915.

Kim, D., Nguyen, M. D., Dobbin, M. M., Fischer, A., Sananbenesi, F., Rodgers, J. T., et al. (2007). SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. *EMBO J.* 26, 3169–3179. doi: 10.1038/sj.emboj.7601758

Kivipelto, M., Solomon, A., Ahtiluoto, S., Ngandu, T., Lehtisalo, J., Antikainen, R., et al. (2013). The finnish geriatric intervention study to prevent cognitive impairment and disability (FINGER): study design and progress. *Alzheimers Dement.* 9, 657–665. doi: 10.1016/j.jalz.2012.09.012

Kohli, P., and Levy, B. D. (2009). Resolvins and protectins: mediating solutions to inflammation. *Br. J. Pharmacol.* 158, 960–971. doi: 10.1111/j.1476-5381.2009. 00290.x

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B., et al. (2010). Blueberry supplementation improves memory in older adults. *J. Agric. Food Chem.* 58, 3996–4000. doi: 10.1021/jf9029332

Kris-Etherton, P. M., Grieger, J. A., and Etherton, T. D. (2009). Dietary reference intakes for DHA and EPA. *Prostaglandins Leukot. Essent. Fatty Acids* 81, 99–104. doi: 10.1016/j.plefa.2009.05.011

Lim, G. P., Calon, F., Morihara, T., Yang, F., Teter, B., Ubeda, O., et al. (2005). A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J. Neurosci.* 25, 3032–3040. doi: 10.1523/jneurosci.4225-04.2005

Liochev, S. I. (2013). Reactive oxygen species and the free radical theory of aging. *Free Radic. Biol. Med.* 60, 1–4. doi: 10.1016/j.freeradbiomed.2013.02.011

Lopez-Garcia, E., Rodriguez-Artalejo, F., Li, T. Y., Fung, T. T., Li, S., Willett, W. C., et al. (2014). The Mediterranean-style dietary pattern and mortality among men and women with cardiovascular disease. *Am. J. Clin. Nutr.* 99, 172–180. doi: 10.3945/ajcn.113.068106

Lourida, I., Soni, M., Thompson-Coon, J., Purandare, N., Lang, I. A., Ukoumunne, O. C., et al. (2013). Mediterranean diet, cognitive function and dementia: a systematic review. *Epidemiology* 24, 479–489. doi: 10.1097/EDE. 0b013e3182944410

Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79, 727–747.

Max Rubner-Institut. (2011). "Fisch in der ernährung," in *Max Rubner-Institut*, ed. H. Rehbein (Hamburg: Max-Rubner-Institut), 1–26.

McCann, J. C., and Ames, B. N. (2005). Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am. J. Clin. Nutr.* 82, 281–295.

McNamara, R. K., and Carlson, S. E. (2006). Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot. Essent. Fatty Acids* 75, 329–349. doi: 10.1016/j.plefa.2006.07.010

Molteni, R., Barnard, R. J., Ying, Z., Roberts, C. K., and Gómez-Pinilla, F. (2002). A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity and learning. *Neuroscience* 112, 803–814. doi: 10. 1016/s0306-4522(02)00123-9

Moore, S. C., Yoder, E., Murphy, S., Dutton, G. R., and Spector, A. A. (1991). Astrocytes, not neurons, produce Docosahexaenoic Acid (22:60ω−3) and Arachidonic Acid (20:40ω−6). *J. Neurochem.* 56, 518–524. doi: 10.1111/j.1471-4159.1991.tb08180.x

Mori, T. A., and Beilin, L. J. (2004). Omega-3 fatty acids and inflammation. *Curr. Atheroscler. Rep.* 6, 461–467. doi: 10.1007/s11883-004-0087-5

Morris, M. C., Evans, D. A., Tangney, C. C., Bienias, J. L., and Wilson, R. S. (2005). Fish consumption and cognitive decline with age in a large community study. *Arch. Neurol.* 62, 1849–1853. doi: 10.1001/archneur.62.12. noc50161

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., et al. (2015). A 2 year multidomain intervention of diet, exercise, cognitive training and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet* 385, 2255–2263. doi: 10.1016/s0140-6736(15)60461-5

Nurk, E., Refsum, H., Drevon, C. A., Tell, G. S., Nygaard, H. A., Engedal, K., et al. (2009). Intake of flavonoid-rich wine, tea and chocolate by elderly men and women is associated with better cognitive test performance. *J. Nutr.* 139, 120–127. doi: 10.3945/jn.108.095182

Okereke, O. I., Rosner, B. A., Kim, D. H., Kang, J. H., Cook, N. R., Manson, J. E., et al. (2012). Dietary fat types and 4-year cognitive change in community-dwelling older women. *Ann. Neurol.* 72, 124–134. doi: 10.1002/ana. 23593

Pottala, J. V., Yaffe, K., Robinson, J. G., Espeland, M. A., Wallace, R., and Harris, W. S. (2014). Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI study. *Neurology* 82, 435–442. doi: 10. 1212/wnl.0000000000000080

Quinn, J. F., Raman, R., Thomas, R. G., Yurko-Mauro, K., Nelson, E. B., Van Dyck, C., et al. (2010). Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. *JAMA* 304, 1903–1911. doi: 10. 1001/jama.2010.1510

Ramassamy, C., and Belkacémi, A. (2011). Nutrition and alzheimer's disease: is there any connection? *Curr. Alzheimer Res.* 8, 443–444. doi: 10. 2174/156720511796391890

Reddy, R., Fleet-Michaliszyn, S., Condray, R., Yao, J. K., Keshavan, M. S., and Reddy, R. (2011). Reduction in perseverative errors with adjunctive ethyl-eicosapentaenoic acid in patients with schizophrenia: preliminary study. *Prostaglandins Leukot. Essent. Fatty Acids* 84, 79–83. doi: 10.1016/j.plefa.2010. 12.001

Sala-Vila, A., and Ros, E. (2011). Mounting evidence that increased consumption of a-linolenic acid, the vegetable n-3 fatty acid, may benefit cardiovascular health. *Clin. Lipidol.* 6, 365–369. doi: 10.2217/clp.11.36

Salvati, S., Natali, F., Attorri, L., Di Benedetto, R., Leonardi, F., Di Biase, A., et al. (2008). Eicosapentaenoic acid stimulates the expression of myelin proteins in rat brain. *J. Neurosci. Res.* 86, 776–784. doi: 10.1002/jnr. 21537

Scarmeas, N., Stern, Y., Tang, M. X., Mayeux, R., and Luchsinger, J. A. (2006). Mediterranean diet and risk for Alzheimer's disease. *Ann. Neurol.* 59, 912–921. doi: 10.1002/ana.20854

Small, B. J., Rawson, K. S., Martin, C., Eisel, S. L., Sanberg, C. D., McEvoy, C. L., et al. (2014). Nutraceutical intervention improves older adults' cognitive functioning. *Rejuvenation Res.* 17, 27–32. doi: 10.1089/rej.2013.1477

Studzinski, C. M., Li, F., Bruce-Keller, A. J., Fernandez-Kim, S. O., Zhang, L., Weidner, A. M., et al. (2009). Effects of short-term Western diet on cerebral oxidative stress and diabetes related factors in APP x PS1 knock-in mice. *J. Neurochem.* 108, 860–866. doi: 10.1111/j.1471-4159.2008.05798.x

Su, K. P., Huang, S. Y., Chiu, C. C., and Shen, W. W. (2003). Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. *Eur. Neuropsychopharmacol.* 13, 267–271. doi: 10.1016/j.euroneuro.2003. 10.001

Sun, A. Y., Wang, Q., Simonyi, A., and Sun, G. Y. (2011). "Botanical Phenols and Neurodegeneration," in *Herbal Medicine: Biomolecular and Clinical Aspects*, 2nd Edn. eds Benzie, I. F. F. and S. Wachtel-Galor (Boca Raton, FL: CRC Press), 315–325.

Sydenham, E., Dangour, A. D., and Lim, W. S. (2012). Omega 3 fatty acid for the prevention of cognitive decline and dementia. *Cochrane Database Syst. Rev.* 6:CD005379. doi: 10.1002/14651858.CD005379.pub3

Takahashi, M., Tsuboyama-Kasaoka, N., Nakatani, T., Ishii, M., Tsutsumi, S., Aburatani, H., et al. (2002). Fish oil feeding alters liver gene expressions to defend against PPARalpha activation and ROS production. *Am. J. Physiol. Gastrointest. Liver Physiol.* 282, G338–G348. doi: 10.1152/ajpgi. 00376.2001

Tan, Z. S., Harris, W. S., Beiser, A. S., Au, R., Himali, J. J., Debette, S., et al. (2012). Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging. *Neurology* 78, 658–664. doi: 10.1212/WNL.0b013e31 8249f6a9

Tangney, C. C., Li, H., Wang, Y., Barnes, L., Schneider, J. A., Bennett, D. A., et al. (2014). Relation of DASH- and Mediterranean-like dietary patterns to cognitive decline in older persons. *Neurology* 83, 1410–1416. doi: 10.1212/wnl. 0000000000000884

Trichopoulou, A., Kouris-Blazos, A., Wahlqvist, M. L., Gnardellis, C., Lagiou, P., Polychronopoulos, E., et al. (1995). Diet and overall survival in elderly people. *BMJ* 311, 1457–1460. doi: 10.1136/bmj.311.7018.1457

45

Hensch, T., Hinz, A., Holzendorf, V., Husser, D., Kersting, A., Kiel, A., Kirsten, T., Kratzsch, J., Krohn, K., Luck, T., Melzer, S., Netto, J., Nüchter, M., Raschpichler, M., Rauscher, F.G., Riedel-Heller, S.G., Sander, C., Scholz, M., Schönknecht, P., Schroeter, M.L., Simon, J.C., Speer, R., Stäker, J., Stein, R., Stöbel-Richter, Y., Stumvoll, M., Tarnok, A., Teren, A., Teupser, D., Then, F.S., Tönjes, A., Treudler, R., Villringer, A., Weissgerber, A., Wiedemann, P., Zachariae, S., Wirkner, K., Thiery, J., 2015. The LIFE-Adult-Study: objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. BMC Public Health 15, 691.

Lord, C., Buss, C., Lupien, S.J., Pruessner, J.C., 2008. Hippocampal volumes are larger in postmenopausal women using estrogen therapy compared to past users, never users and men: a possible window of opportunity effect. Neurobiol. Aging 29, 95–101.

Madden, D.J., Bennett, I.J., Burzynska, A., Potter, G.G., Chen, N.K., Song, A.W., 2012. Diffusion tensor imaging of cerebral white matter integrity in cognitive aging. Biochim. Biophys. Acta 1822, 386–400.

Madden, D.J., Bennett, I.J., Song, A.W., 2009. Cerebral white matter integrity and cognitive aging: contributions from diffusion tensor imaging. Neuropsychol. Rev. 19, 415–435.

Mander, B.A., Rao, V., Lu, B., Saletin, J.M., Lindquist, J.R., Ancoli-Israel, S., Jagust, W., Walker, M.P., 2013. Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging. Nat. Neurosci. 16, 357–364.

Marqués-Iturria, I., Pueyo, R., Garolera, M., Segura, B., Junqué, C., García-García, I., José Sender-Palacios, M., Vernet-Vernet, M., Narberhaus, A., Ariza, M., Jurado, M.Á., 2013. Frontal cortical thinning and subcortical volume reductions in early adulthood obesity. Psychiatry Res. 214, 109–115.

Martinson, B.C., Crain, A.L., Sherwood, N.E., Hayes, M.G., Pronk, N.P., O'Connor, P.J., 2010. Population reach and recruitment bias in a maintenance RCT in physically active older adults. J. Phys. Act. Health 7, 127–135.

Morris, J.C., Heyman, A., Mohs, R.C., Hughes, J.P., van Belle, G., Fillenbaum, G., Mellits, E.D., Clark, C., 1989. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39, 1159–1165.

Nadkarni, N.K., Perera, S., Hanlon, J.T., Lopez, O., Newman, A.B., Aizenstein, H., Elam, M., Harris, T.B., Kritchevsky, S., Yaffe, K., Rosano, C., 2015. Statins and brain integrity in older adults: secondary analysis of the Health ABC study. Alzheimers Dement. 11, 1202–1211.

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., Bäckman, L., Hänninen, T., Jula, A., Laatikainen, T., Lindström, J., Mangialasche, F., Paajanen, T., Pajala, S., Peltonen, M., Rauramaa, R., Stigsdotter-Neely, A., Strandberg, T., Tuomilehto, J., Soininen, H., Kivipelto, M., 2015. A 2 year multi-domain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet 385, 2255–2263.

Oosterman, J.M., Vogels, R.L.C., van Harten, B., Gouw, A.A., Poggesi, A., Scheltens, P., Kessels, R.P.C., Scherder, E.J.A., 2010. Assessing mental flexibility: neuroanatomical and neuropsychological correlates of the Trail Making test in elderly people. Clin. Neuropsychol. 24, 203–219.

Pannacciulli, N., Del Parigi, A., Chen, K., Le, D.S.N.T., Reiman, E.M., Tataranni, P.A., 2006. Brain abnormalities in human obesity: a voxel-based morphometric study. Neuroimage 31, 1419–1425.

Patrone, C., Eriksson, O., Lindholm, D., 2014. Diabetes drugs and neurological disorders: new views and therapeutic possibilities. Lancet Diabetes Endocrinol. 2, 256–262.

Penke, L., Muñoz Maniega, S., Murray, C., Gow, A.J., Hernández, M.C.V., Clayden, J.D., Starr, J.M., Wardlaw, J.M., Bastin, M.E., Deary, I.J., 2010. A general factor of brain white matter integrity predicts information processing speed in healthy older people. J. Neurosci. 30, 7569–7574.

Prickett, C., Brennan, L., Stolwyk, R., 2015. Examining the relationship between obesity and cognitive function: a systematic literature review. Obes. Res. Clin. Pract. 9, 93–113.

Qizilbash, N., Gregson, J., Johnson, M.E., Pearce, N., Douglas, I., Wing, K., Evans, S.J.W., Pocock, S.J., 2015. BMI and risk of dementia in two million people over two decades: a retrospective cohort study. Lancet Diabetes Endocrinol. 3, 431–436.

Raji, C., Ho, A., Parikshak, N., 2010. Brain structure and obesity. Hum. Brain Mapp. 31, 353–364.

Raz, N., 2005. Ageing and the Brain. Encycl. Life Sci. 1–6. http://dx.doi.org/10.1038/npg.els.0004063.

Reinert, K.R.S., Po'e, E.K., Barkin, S.L., 2013. The relationship between executive function and obesity in children and adolescents: a systematic literature review. J. Obes. 2013, 820956.

Schaapsmeerders, P., Maaijwee, N.A.M., van Dijk, E.J., Rutten-Jacobs, L.C.A., Arntz, R.M., Schoonderwaldt, H.C., Dorresteijn, L.D.A., Kessels, R.P.C., de Leeuw, F.E., 2013. Long-term cognitive impairment after first-ever ischemic stroke in young adults. Stroke 44, 1621–1628.

Shefer, G., Marcus, Y., Stern, N., 2013. Is obesity a brain disease? Neurosci. Biobehav. Rev. 37, 2489–2503.

Smith, E., Hay, P., Campbell, L., Trollor, J.N., 2011. A review of the association between obesity and cognitive function across the lifespan: implications for novel approaches to prevention and treatment. Obes. Rev. 12, 740–755.

Sprecher, K.E., Bendlin, B.B., Racine, A.M., Okonkwo, O.C., Christian, B.T., Koscik, R.L., Sager, M.A., Asthana, S., Johnson, S.C., Benca, R.M., 2015. Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. Neurobiol. Aging 36, 2568–2576.

Staudigl, T., Zaehle, T., Voges, J., Hanslmayr, S., Esslinger, C., Hinrichs, H., Schmitt, F.C., Heinze, H.J., Richardson-Klavehn, A., 2012. Memory signals from the thalamus: early thalamocortical phase synchronization entrains gamma oscillations during long-term memory retrieval. Neuropsychologia 50, 3519–3527.

Steffener, J., Brickman, A.M., Habeck, C.G., Salthouse, T.A., Stern, Y., 2013. Cerebral blood flow and gray matter volume covariance patterns of cognition in aging. Hum. Brain Mapp. 34, 3267–3279.

Streitbürger, D.P., Pampel, A., Krueger, G., Lepsien, J., Schroeter, M.L., Mueller, K., Möller, H.E., 2014. Impact of image acquisition on voxel-based-morphometry investigations of age-related structural brain changes. Neuroimage 87, 170–182.

Taki, Y., Kinomura, S., Sato, K., Inoue, K., Goto, R., Okada, K., Uchida, S., Kawashima, R., Fukuda, H., 2008. Relationship between body mass index and gray matter volume in 1,428 healthy individuals. Obesity (Silver Spring) 16, 119–124.

Uhlhaas, P.J., Singer, W., 2006. Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. Neuron 52, 155–168.

United Nations Educational, Scientific and Cultural Organization (UNESCO), 1997. International Standard Classification of Education. Switzerland, Geneva.

Van de Rest, O., Geleijnse, J.M., Kok, F.J., van Staveren, W.A., Dullemeijer, C., Olderikkert, M.G.M., Beekman, A.T.F., de Groot, C.P.G.M., 2008. Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. Neurology 71, 430–438.

Villeneuve, S., Reed, B.R., Madison, C.M., Wirth, M., Marchant, N.L., Kriger, S., Mack, W.J., Sanossian, N., DeCarli, C., Chui, H.C., Weiner, M.W., Jagust, W.J., 2014. Vascular risk and Aβ interact to reduce cortical thickness in AD vulnerable brain regions. Neurology 83, 40–47.

Walhovd, K.B., Storsve, A.B., Westlye, L.T., Drevon, C.A., Fjell, A.M., 2014. Blood markers of fatty acids and vitamin D, cardiovascular measures, body mass index, and physical activity relate to longitudinal cortical thinning in normal aging. Neurobiol. Aging 35, 1055–1064.

Walther, K., Birdsill, A.C., Glisky, E.L., Ryan, L., 2010. Structural brain differences and cognitive functioning related to body mass index in older females. Hum. Brain Mapp. 31, 1052–1064.

Wen, W., Sachdev, P.S., Chen, X., Anstey, K., 2006. Gray matter reduction is correlated with white matter hyperintensity volume: a voxel-based morphometric study in a large epidemiological sample. Neuroimage 29, 1031–1039.

Whitmer, R.A., Gunderson, E.P., Barrett-Connor, E., Quesenberry, C.P., Yaffe, K., 2005. Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. BMJ 330, 1360.

Whitmer, R.A., Gustafson, D.R., Barrett-Connor, E., Haan, M.N., Gunderson, E.P., Yaffe, K., 2008. Central obesity and increased risk of dementia more than three decades later. Neurology 71, 1057–1064.

Willette, A.A., Kapogiannis, D., 2014. Does the brain shrink as the waist expands? Ageing Res. Rev. 20, 1–12.

Wishart, H.A., Saykin, A.J., McAllister, T.W., Rabin, L.A., McDonald, B.C., Flashman, L.A., Roth, R.M., Mamourian, A.C., Tsongalis, G.J., Rhodes, C.H., 2006. Regional brain atrophy in cognitively intact adults with a single APOE epsilon4 allele. Neurology 67, 1221–1224.

Woo, C.W., Krishnan, A., Wager, T.D., 2014. Cluster-extent based thresholding in fMRI analyses: pitfalls and recommendations. Neuroimage 91, 412–419.

Yuan, P., Raz, N., 2014. Prefrontal cortex and executive functions in healthy adults: a meta-analysis of structural neuroimaging studies. Neurosci. Biobehav. Rev. 42, 180–192.

Zola-Morgan, S., Squire, L.R., 1985. Amnesia in monkeys after lesions of the mediodorsal nucleus of the thalamus. Ann. Neurol. 17, 558–564.

2.3. Publication3: Original article: Beyer et al, 2017

Higher body mass index is associated with reduced default mode

connectivity in older adults

Beyer F., Kharabian Masouleh S., Huntenburg J.M., Lampe L., Luck T.,

Riedel-Heller S.G., Loeffler M., Schroeter M.L., Stumvoll M., Villringer A.,

Witte A.V.

Human Brain Mapping

2017

Higher Body Mass Index is Associated with Reduced Posterior Default Mode Connectivity in Older Adults

Frauke Beyer ⬤,[1,2] **Sharzhad Kharabian Masouleh,**[1] **Julia M. Huntenburg,**[3]
Leonie Lampe,[1,4] **Tobias Luck,**[4,5] **Steffi G Riedel-Heller,**[4,5] **Markus Loeffler,**[4]
Matthias L. Schroeter,[4,6] **Michael Stumvoll,**[2,7] **Arno Villringer,**[1,2,4] **and**
A. Veronica Witte[1,2]*

[1]*Department of Neurology, Max Planck Institute for Cognitive and Brain Sciences, Leipzig,
Germany*
[2]*Subproject A1, Collaborative Research Centre 1052 "Obesity Mechanisms", University of
Leipzig, Leipzig, Germany*
[3]*Max Planck Research Group for Neuroanatomy and Connectivity, Max Planck Institute for
Cognitive and Brain Sciences, Leipzig, Germany*
[4]*LIFE – Leipzig Research Center for Civilization Diseases, University of Leipzig, Leipzig,
Germany*
[5]*Institute of Social Medicine, Occupational Health and Public Health (ISAP), University of
Leipzig, Leipzig, Germany*
[6]*Department of Cognitive Neurology, University of Leipzig, Leipzig, Germany*
[7]*IFB Adiposity Diseases Faculty of Medicine, University of Leipzig, Leipzig, Germany*

◆━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━◆

Abstract: Obesity is a complex neurobehavioral disorder that has been linked to changes in brain structure and function. However, the impact of obesity on functional connectivity and cognition in aging humans is largely unknown. Therefore, the association of body mass index (BMI), resting-state network connectivity, and cognitive performance in 712 healthy, well-characterized older adults of the Leipzig Research Center for Civilization Diseases (LIFE) cohort (60–80 years old, mean BMI 27.6 kg/m^2 ± 4.2 SD, main sample: $n = 521$, replication sample: $n = 191$) was determined. Statistical analyses included a multivariate model selection approach followed by univariate analyses to adjust for possible confounders. Results showed that a higher BMI was significantly associated with lower default mode functional connectivity in the posterior cingulate cortex and precuneus. The effect remained stable after controlling for age, sex, head motion, registration quality, cardiovascular, and genetic factors as well as in replication analyses. Lower functional connectivity in BMI-associated areas correlated with worse executive function. In addition, higher BMI correlated with stronger head motion. Using 3T neuroimaging in a large cohort of healthy

*Correspondence to: Veronica Witte, PhD, Department of Neurology, Max Planck Institute for Cognitive and Brain Sciences, Stephanstraße 1a, 04103 Leipzig. Germany.
E-mail: witte@cbs.mpg.de

older adults, independent negative associations of obesity and functional connectivity in the posterior default mode network were observed. In addition, a subtle link between lower resting-state connectivity in BMI-associated regions and cognitive function was found. The findings might indicate that obesity is associated with patterns of decreased default mode connectivity similar to those seen in populations at risk for Alzheimer's disease. *Hum Brain Mapp 38:3502–3515, 2017.* © 2017 **W**iley Periodicals, Inc.

Key words: brain; neuroimaging; obesity; risk factors; cognition

◆ ═══════════════════════════ ◆

INTRODUCTION

Obesity is a complex neurobehavioral disorder resulting from excessive energy intake and insufficient energy expenditure. It has been associated with abnormal functionality of homeostasis brain networks [Grill et al., 2007] and some studies also reported differences in higher cognitive functions such as reward evaluation [Amlung et al., 2016; Stice et al., 2008], executive functions [Benito-León et al., 2013; Gunstad et al., 2007] and learning and memory [Cheke et al., 2017; Smith et al., 2011], yet underlying mechanisms are far from understood.

Using task-based functional MRI (fMRI), several studies revealed differences between lean and obese participants in regional activation patterns during the processing of rewarding food and non-food stimuli [Rothemund et al., 2007; Stice et al., 2008; Stoeckel et al., 2008]. In addition, using resting-state fMRI, obesity has been linked to selective changes in functional connectivity between brain areas, including attentional and default mode resting state networks (RSN) [Garcia-Garcia et al., 2013; Kullmann et al., 2012]. However, previous findings of obesity-related changes in functional connectivity are mixed [Kullmann et al., 2012] and mostly based on small sample sizes in young participants using non-standardized experimental conditions [Hsu et al., 2015; Tregellas et al., 2011]. Recently, several studies have associated RSN connectivity strength with individual differences in cognitive performance such as executive function [Gordon et al., 2015; Reineberg et al., 2015] and memory [Salami et al., 2016; Wang et al., 2010]. Thus, determining changes in functional connectivity that are attributed to obesity might help to better understand the link between body weight and cognition in humans.

In the present study we therefore aimed to investigate the association of obesity with RSN connectivity in a large population-based cohort of healthy older adults. We hypothesized that a higher BMI would be associated with changes in obesity-related RSN such as frontal, attentional, or default mode networks. As functional connectivity has been linked to differences in cognition we also determined if changes in RSN connectivity would correlate with cognitive performance.

METHODS

Participants

All participants took part in the LIFE-Adult-Study [Loeffler et al., 2015] and were randomly selected, community-dwelling volunteers older than 60 years (see Fig. 1 for details on sample selection). In total, 712 subjects were included, thereof 521 subjects in the main sample (sample 1) and another 191 subjects in the replication sample (sample 2) (see Table I for demographics). Exclusion criteria were stroke, cancer treatment in the last 12 months, neuroradiological findings of brain pathology, intake of centrally active medication and a score below 25 in the Mini Mental State Examination. All subjects underwent medical examination, anthropometric measurements, MRI assessment, and neuropsychological testing.

Standard protocol approvals and patient consents

The study was approved by the institutional ethics board of the Medical Faculty of the University of Leipzig and all participants signed an informed consent form.

Neuroimaging

Brain imaging was performed on a 3T Siemens Verio Scanner with a 32 channel head coil. T1-weighted images were acquired using generalized autocalibrating partially parallel acquisition technique [Griswold et al., 2002] and the Alzheimer's Disease Neuroimaging Initiative standard protocol with the following parameters: inversion time, 900 ms; repetition time, 2.3 ms; echo time, 2.98 ms; flip angle, 9°; band width, 240 Hz/pixel; image matrix, 256 × 240; 176 partitions; field of view, 256 × 240 × 176 mm³; sagittal orientation; voxel size, 1 × 1 × 1 mm³; no interpolation.

T2*-weighted functional images were acquired using an echo-planar-imaging sequence with the following parameters: repetition time, 2 s; echo time, 30 ms; flip angle, 90°; image matrix, 64 × 64; 30 slices; field of view, 192 × 192 × 144 mm³, voxel size of 3 mm × 3 mm, slice thickness of 4 mm, slice gap of 0.8 mm; 300 volumes; total acquisition time, 10:04 minutes. For two participants only 299 volumes and for one participant only 215 volumes were acquired. Preprocessing was implemented in a reproducible pipeline using nipype [Gorgolewski et al., 2011] which is available to the public at https://github.com/fBeyer89/LIFE_rs_ICA_preprocessing.

After removal of the first five volumes in order to allow the magnetization to reach steady-state, rigid body, boundary-based coregistration with 6 degrees of freedom
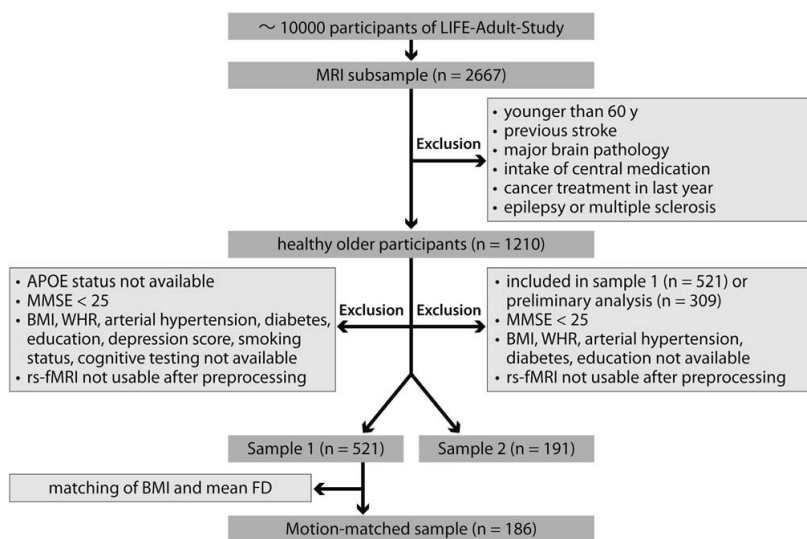
Figure 1.

Flow chart of the study illustrating the exclusion criteria for the selection of sample 1, motion-matched sample, and sample 2.

of the functional scan to the anatomical image, as well as motion and EPI distortion corrections were calculated and jointly applied in a subsequent step to each volume of the functional scan. Scans were slicetime-corrected and non-linearly transformed to MNI space using ANTS Symmetric Normalization (SyN) registration algorithm [Avants et al., 2011], resliced to 3 mm isotropic voxels and smoothed with a gaussian kernel of 6 mm full-width-at-half-maximum. Frame-to-frame head motion was estimated by calculating framewise displacement (FD) [Power et al., 2012]. We excluded 12 participants from sample 1 and 6

participants from sample 2 because of gross motion (maximal FD > 3 mm). Mean FD was calculated across volumes and used as a covariate to correct for head motion in statistical analysis.

All normalized functional images were visually checked and compared with the MNI template which led to the exclusion of 15 and four participants from sample 1 and 2 respectively because of major registration issues (large ventricles, atrophy, or calcified falxes).

A mean functional image was created for the remaining 521 subjects from sample 1 and 191 subjects from sample 2

TABLE I. Demographic characteristics of sample 1 and 2

	Sample 1 $n = 521$ (230 women)	Sample 2 $n = 191$ (96 women)
Age (y)	70.1 ± 3.8 (60–79)	68.8 ± 5.4 (60–82)
BMI (kg/m^2)	27.5 ± 4.1 (16.8–41.4)	28.1 ± 4.5 (18.6–43.9)
Mean FD (mm)	0.27 ± 0.12 (0.05–0.87)	0.28 ± 0.14 (0.06–0.92)
q_r	0.95 ± 0.015 (0.86–0.97)	0.93 ± 0.02 (0.79–0.96)
APOE status (% e4 carriers/non-e4 carriers/missing)	20.7/79.3/–	7.3/27.2/65.4
Arterial hypertension (% yes)	60.7	58.6
Diabetes (% yes)	15.7	15.7
Education (% no SS-LD/SS-LD/advanced SS-LD/university-entrance degree)	0.8/52.6/7.7/39	1.6/65.4/11.5/21.5
CES-D (score)/missing	9.3 ± 5.5 (0–34)/–	11.2 ± 5.6 (0–29)/47
Smoker (% current/previous/never/missing)	6.5/32.8/60.7/–	8.4/29.8/37.7/24.1

Data are mean ± SD (minimum–maximum).
BMI, body mass index; FD, framewise displacement; q_r, registration quality; APOE e4, apolipoprotein E epsilon 4 allele; SS-LD, secondary-school leaving degree; CES-D, Center for Epidemiologic Studies Depression Scale.

using the first volume of each subject's time series. A registration quality index q_r was calculated as the spatial cross correlation of each of the subject's first volumes with the mean image and later used as a covariate describing the accuracy of spatial normalization from functional to anatomical subject and MNI space.

To assess RSN, we applied independent component analysis (ICA) which has been shown to reliably identify RSN across subjects [Damoiseaux et al., 2006] using the GIFT toolbox [Calhoun, 2004]. A high number of $n = 75$ components was chosen because such decompositions have been previously shown to yield detailed and non-overlapping components [Abou-Elseoud et al., 2010; Kivi-niemi et al., 2009]. Independent components were selected as reliable RSN if their spatial cross-correlation with publicly available templates [Allen et al., 2011] was higher than 0.4 and they contained mainly low-frequency fluctuations measured with a power ratio above 3 [Robinson et al., 2009]. Subject-specific component maps were calculated using the GICA-approach implemented in the GIFT toolbox [Erhardt et al., 2011].

Gray matter volume (GMV) probability maps were derived from T1-weighted scans using voxel based morphometry in SPM 8 (www.fil.ion.ucl.ac.uk/spm) and averaged within thresholded ICA component maps to correct for local gray matter volume differences within the resting state networks.

Total intracranial volume, cortical white matter volume as well as cortical and subcortical gray matter volumes were derived using FREESURFER (http://surfer.nm.mgh.harvard.edu/) and used to assess and correct for associations of global brain volume measures with BMI.

Neuropsychological Testing and Confounder Definition

Neuropsychological testing was performed using the CERAD-Plus test battery [Morris et al., 1989] and included the trail-making test (TMT) part A and B, semantic and phonemic verbal fluency and verbal memory. The trail-making test is an indicator of speed of cognitive processing and executive functioning [Sanchez-Cubillo et al., 2009] while phonemic and verbal fluency tests measure executive and verbal reasoning [Van Der Elst et al., 2006]. In the verbal memory test, learning was defined as the sum of 3 consecutive learning trials, recall was defined as the sum of correctly recalled words after a delay, in which participants performed a nonverbal task, and recognition was defined as the number of correctly recognized words of a list of 20 mixed words presented afterwards. Test scores were z-transformed and combined to create composite scores for executive function, memory performance and processing speed [Kerti et al., 2013; Van de Rest et al., 2008]. This allowed us to reduce number of comparisons and investigate specific cognitive domains. Composite scores for executive function, memory performance and processing speed were calculated as follows [Kharabian Masouleh et al., 2016]: executive functions = (z_phonemic fluency + z_semantic fluency + z_TMT(part B + part A)/ part A)/3; memory = (z_sum_learning þ z_recall þ z_recognition)/3; processing speed = −z (TMT [part A]).

Arterial hypertension was defined as systolic blood pressure ≥ 160 mm Hg, diastolic blood pressure ≥ 95 mm Hg or diagnosis of hypertension or use of antihypertensive medication [Biessels et al., 2006]. Diabetes and hyperlipidemia were binarily defined based on self-reported diagnosis or medication intake. Four levels of education were defined: no secondary-school leaving degree (SS-LD), secondary-school leaving degree (corresponding to 8 years of school), advanced secondary-school leaving degree (corresponding to 10 years of school) and university-entrance degree (corresponding to 13 years of school). Depression score was measured using the Center for Epidemiologic Studies Depression Scale (CES-D) [Radloff, 1977]. Smoking status was defined using self-reported information as never smoker, previous smoker or current smoker. Genotyping of the APOE allele status (E2, E3, E4) was performed on a Roche Lightcylcer 480 according to the method of Aslanidis [Aslanidis and Schmitz, 1999]. APOE-e4 carrier status was then defined as carrying none (0) or at least one APOE-e4 allele (1).

Statistical Analysis

Statistical analysis of the association between obesity and RSN functional connectivity was performed using a multi-variate backward model selection approach [Allen et al., 2011] implemented in the MANCOVAN toolbox (http://mialab.mrn.org/software). The primary design matrix contained BMI, age and sex (Model 1). In a second model we additionally added head motion measured by mean FD (log-transformed) and registration quality measured by q_r (Fisher-Z-transformed) as covariates (Model 2). In order to correct for multiple comparison across 18 different RSN that were identified in our sample, the significance level for model selection was set to $0.05/18 = 0.0028$.

After covariate selection, univariate voxelwise testing of multiple regression models was performed as implemented in the MANCOVAN toolbox and results were corrected for multiple comparisons within components using false discovery rate correction (FDR) with $\alpha < 0.05$ [Benjamini and Hochberg, 1995].

In networks significantly associated with BMI we investigated intra-network connectivity using mean cluster connectivity and network eigenvariate (EV) as proposed previously [Glahn et al., 2010]. Statistical analysis on connectivity measures was performed using multiple regression in SPSS 22.0 (IBM). Age, sex, APOE-e4 status, hypertension, diabetes, education, smoking status, and depression score were used as confounding variables.

Associations between BMI, BMI-associated differences in functional connectivity and cognitive performance were
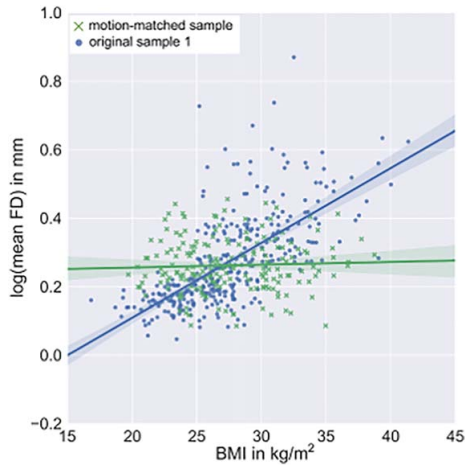
51

Figure 2.

Correlation of body mass index (BMI) and mean framewise displacement (FD) in the motion-matched sample (green) compared with sample 1 (blue): the strong positive correlation between BMI and mean FD has clearly been reduced. [Color figure can be viewed at wileyonlinelibrary.com]

explored without correction for multiple comparisons using bivariate and partial Pearson's correlations.

Confirmatory analysis

In a replication approach we investigated a second sample including 191 participants who had not been used in any prior analysis and had complete information of BMI, arterial hypertension, diabetes and education (sample 2). Subjects included in sample 2 were on average younger (independent samples t-test, $P = 0.005$) while exhibiting a comparable age range, and similar distributions of sex, BMI, mean FD, hypertension and diabetes (independent samples t-tests, Chi-squared test, all $P > 0.1$) (see Table I for details). Using FSL's DUAL REGRESSION we calculated subject-specific spatial maps for sample 2 based on components found in the main analysis of sample 1. We extracted the EV of those components that were significantly associated with BMI and calculated a multiple linear regression using a model containing age, sex, BMI, diabetes, arterial hypertension, and education. APOE-e4 status, depression score and smoking status were not available for all participants in the replication sample. Additionally, we estimated a voxelwise multiple regression model with the same covariates using permutation testing implemented in FSL's RANDOMISE. Results were corrected for multiple comparisons using FDR correction with $\alpha < 0.05$.

To overcome the collinearity between BMI and head motion which was noticed during preprocessing, we separated participants from sample 1 into three BMI-groups

(BMI < 25 kg/m^2, 25 kg/m^2 < BMI < 30 kg/m^2, BMI > 30 kg/m^2) and matched participants from each group for mean FD with an uncertainty of 0.02 mm. This yielded a sample of 186 participants in which BMI and mean FD no longer correlated (motion-matched sample, see Fig. 2). The resulting sample did not significantly differ from the original sample 1 in age, sex, BMI, APOE-e4 status, hypertension, diabetes, education, depression score, and smoking status (independent samples t-tests, Chi-squared test, all $P > 0.1$).

In order to verify that our results were independent of the number of independent components used, we repeated the analysis in sample 1 with 20 instead of 75 components.

RESULTS

RSN Components

Using independent component analysis, we identified 18 RSN components that belong to six commonly described networks, that is, attentional, default mode, frontal, sensorimotor, auditory, and visual network (see Fig. 3 for overview).

Multivariate Results

Multivariate backward model selection analysis of model 1 (including BMI, age, and sex) detected BMI as a significant predictor of functional connectivity strength in the default mode network components 29 and 42, and in the visual network component 25 (see Fig. 4). Backward model selection of model 2 including motion and registration parameters (i.e., FD and q_r) added q_r as a significant predictor for the components 29, 42, and 25.

Univariate Results

Univariate analysis using model 1 showed that higher BMI was significantly associated with decreased functional connectivity within the spatial maps of default mode components 29 and 42 ($P < 0.05$, FDR-corrected, adjusted for sex), more specifically in clusters located in the posterior cingulate cortex (PCC) and precuneus in component 29, and in the precuneus and left parietal cortex in component 42 (see Fig. 5).

We also found a BMI-associated increase of connectivity in visual network component 25. This cluster was located in the right precuneus and left lingual cortex (see Fig. 6).

For model 2 significant BMI effects on voxelwise network connectivity were again found in the PCC and precuneus within the default mode component 29 ($P < 0.05$, FDR-corrected, adjusted for sex and q_r). Effects in component 42 did not survive FDR-correction.

Adding q_r as a covariate into the model for visual component 25 did not change the univariate result showing positive correlations with BMI.
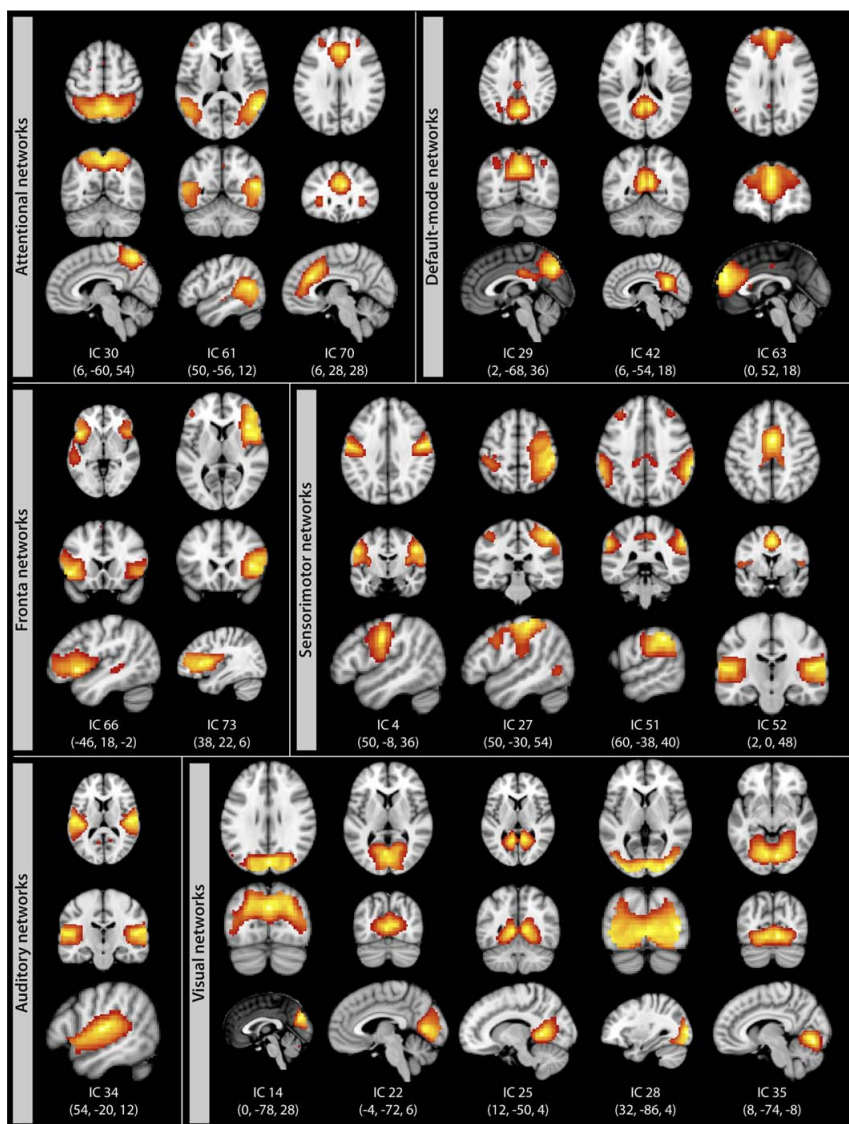
Figure 3.

Spatial maps (SM) of the 18 components identified as resting state networks: SM are plotted as *t*-statistics thresholded at *t* > 12 and displayed at the three most informative slices. Coordinates refer to the maximal *t*-value in MNI-space coordinates. [Color figure can be viewed at wileyonlinelibrary.com]

Analysis of Intra-Network Connectivity

In order to analyze if the association of BMI and posterior default mode network connectivity was independent of further known confounders, we used a multiple linear regression on the intra-network functional connectivity of the spatial maps and corrected for age, sex, APOE-e4 status, diabetes, hypertension, education level, smoking
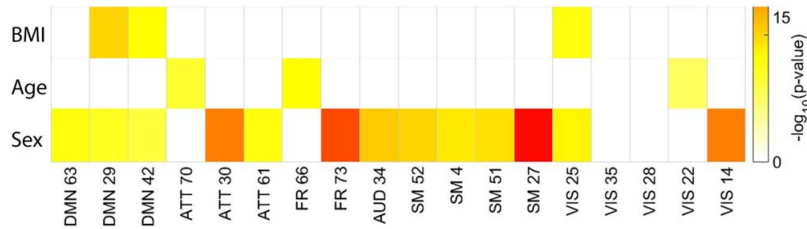
Figure 4.

Results from the multivariate analysis on 18 identified resting state network (RSN) components using Model 1 including BMI, age, and sex ($\alpha < 0.0028$). Colorscale indicates log(P), white cells indicate that covariates were removed from the full model during backward selection. [Color figure can be viewed at wileyonlinelibrary.com]

status, and depression score. Accordingly, BMI was significantly negatively associated with intra-network connectivity of default mode component 29, even after adjusting for confounders ($\beta = -0.148$, $P = 0.001$, $R^2_{adjusted} = 0.075$, see Table II and Fig. 7). Age, smoking and APOE-e4 status were all negatively associated with intra-network connectivity (Age: $\beta = -0.14$, $P = 0.002$, smoking status: $\beta = -0.14$, $P = 0.001$, APOE-e4 status: $\beta = -0.1$, $P = 0.018$), while hypertension, diabetes, education, and depression score did not contribute significantly to the model (see Table II).

This result remained stable when additionally including HbA1c as a covariate ($N = 516$, $\beta = -0.14$, $P = 0.002$, correcting for HbA1c, age, sex, APOE-e4 status, diabetes, hypertension, education level, smoking status, and depression score) and correcting for presence of hyperlipidemia ($N = 521$, $\beta = -0.15$, $P = 0.001$, correcting for age, sex, APOE-e4 status, hyperlipidemia, arterial hypertension, diabetes, BMI, education level, smoking status, and depression score). We also included mean GMV within component 29 and total cortical GMV into the model to correct for possible effects of reduced GMV in the region of interest and globally, which did not attenuate the results ($\beta = -0.145$, $P = 0.001$, linear regression on EV of posterior DMN 29, corrected for age, sex, diabetes, hypertension, APOE-e4-status, depression score, smoking-status, education, mean GMV in DMN 29, and mean global GMV). Total mean GMV was significantly associated with BMI (partial correlation coefficient $\rho = -0.12$, $P = 0.005$, corrected for age and sex) while mean GMV within
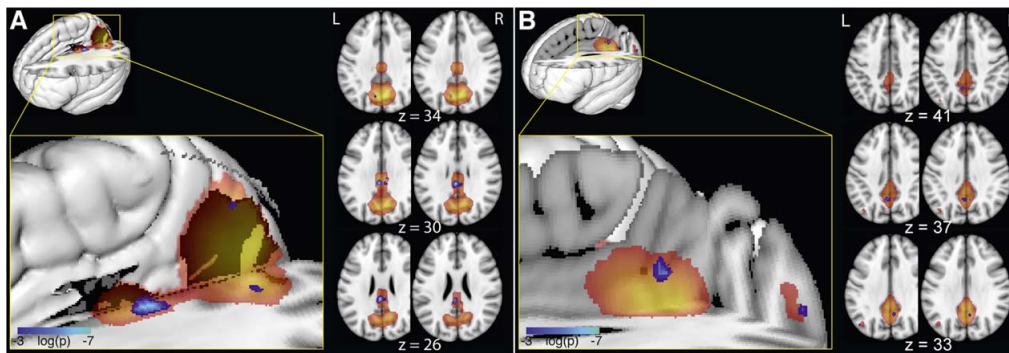

Figure 5.

Higher BMI is associated with decreased posterior default mode network connectivity. A: Decreased functional connectivity in default mode network component 29 is found in clusters in the posterior cingulate cortex (PCC) and precuneus. Blue color map represents log(P)-values of significant voxels ($P < 0.05$, FDR corrected, using model 1: main BMI effect correcting for sex). MNI coordinates of peak voxel in component 29 in the PCC is (-3, -33, 27). Red color map represents the spatial map of the component. B: Decreased functional connectivity in default mode network component 42 is found in clusters in the precuneus and parietal cortex. Blue color map represents log(p)-values of significant voxels ($P < 0.05$, FDR corrected, using model 1: main BMI effect correcting for sex). MNI coordinates of peak voxel in the precuneus: (-3, -54, 26). Red color map represents the spatial map of the component. [Color figure can be viewed at wileyonlinelibrary.com]
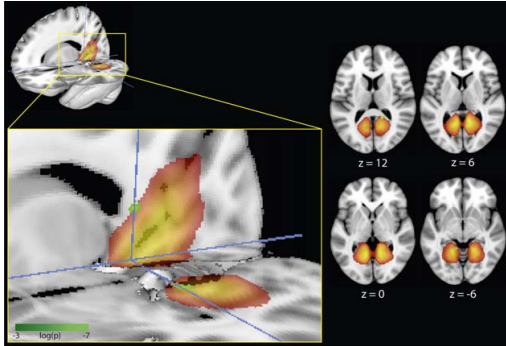
Figure 6.

Association of higher BMI with increased connectivity: In visual network component 25 higher BMI is associated with increased functional connectivity. Green color map represents log(P)-values of significant voxels ($P < 0.05$, FDR corrected, using model 1: main BMI effect correcting for sex). MNI coordinates of peak voxel is (15, −45, 22). Red color map represents the spatial map of the component. [Color figure can be viewed at wileyonlinelibrary.com]
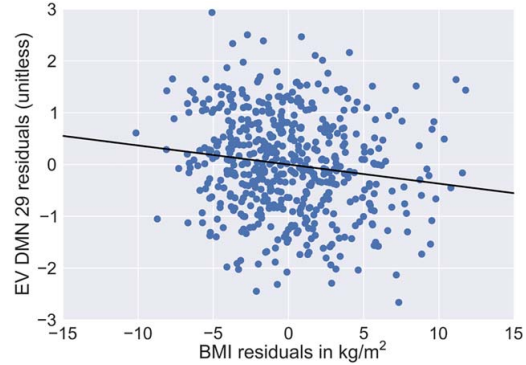

Figure 7.

Association of higher BMI and reduced connectivity after controlling for genetic and environmental confounders: Linear regression of BMI and intra-network functional connectivity of default mode network component 29, controlling for age, sex, APOE-e4 status, hypertension, diabetes, education, smoking status, and depression score. [Color figure can be viewed at wileyonlinelibrary.com]

component 29 was not ($\rho = -0.023$, $P = 0.6$, corrected for age and sex). BMI was not significantly associated with intra-network connectivity of the visual component 25 when correcting for age, sex, APOE-e4 status, diabetes, hypertension, education level, smoking status, and depression score ($\beta = 0.056$, $P = 0.22$, $R^2_{adjusted} = 0.027$).

Associations with Cognitive Performance

Higher BMI was significantly correlated with lower executive performance ($r = -0.11$, $P = 0.015$), even when adjusting for age and sex (partial correlation coefficient $\rho = -0.10$, $P = 0.02$). In addition, higher mean cluster connectivity in the PCC of component 29 was associated with higher executive function ($\rho = 0.10$, $P = 0.03$, corrected for age and sex) although the association became non-

TABLE II. Results of multiple regression performed on EV of DMN component 29 (standardized regression coefficient β, t-value t, and P-value) ($R^2_{adjusted} = 0.074$)

	β	t	P
BMI	−0.15	−3.35	0.001
Age	−0.14	−3.16	0.002
Sex	−0.04	−0.78	0.44
APOE−e4 status	−0.1	−2.37	0.02
Arterial hypertension	0.04	0.81	0.42
Diabetes	−0.034	−0.78	0.44
Education	0.009	0.19	0.84
Smoking status	−0.14	−3.21	0.001
Depression score	−0.06	−1.23	0.21

significant when additionally controlling for BMI ($\rho = 0.075$, $P = 0.09$). We also observed lower memory performance to be associated with lower PCC cluster connectivity ($r = 0.11$, $P = 0.009$); however, without reaching statistical significance when correcting for age and sex (PCC-ROI: $\rho = 0.06$, $P = 0.17$).

Confirmatory Analyses

In the replication sample, we found a significant association of higher BMI and lower intra-network connectivity of DMN 29$_{sample2}$ (BMI: $\beta = -0.29$, $P < 0.001$, with age, sex, diabetes, arterial hypertension, and education as covariates). In an additional voxelwise analysis we found BMI-associated connectivity reductions to be located mainly in precuneus (significant at $P < 0.05$, whole brain FDR corrected, see Fig. 8) correcting for age, sex, diabetes, arterial hypertension, and education.

We observed in part strong effects of the head motion parameter mean FD on RSN connectivity in the multivariate analysis and found BMI and mean FD to be highly collinear. We, therefore, conducted a sensitivity analysis in a motion-matched sub-sample. Here again, according to linear regression, higher BMI correlated significantly with lower mean connectivity in the cluster previously identified in the PCC ($\beta = -0.18$, $t = -2.48$, $P = 0.014$, correcting for age, sex, APOE-e4 status, diabetes, hypertension, education level, smoking status, depression score, and mean FD). Mean FD was also negatively correlated with reduced connectivity ($\beta = -0.16$, $t = -2.1$, $P = 0.03$).
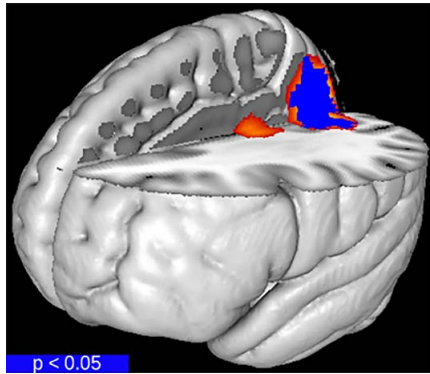
Figure 8.
In the replication sample 2, higher BMI is associated with decreased connectivity of dual-regression derived DMN 29. Blue color map represents p-values of significantly associated voxels ($P < 0.05$, FDR corrected, adjusted for age, sex, hypertension, diabetes, and education). RSN spatial map is shown in red. [Color figure can be viewed at wileyonlinelibrary.com]

We also repeated the ICA with a model order of 20 and found higher BMI to be associated with reduced precuneus and PCC connectivity in a default mode network component (data not shown), which is in line with our initial finding and shows that the result does not depend on the number of extracted independent components.

DISCUSSION

In this study, we detected significant negative associations of BMI and DMN connectivity in the PCC and precuneus using 3T resting-state fMRI in a large cohort of healthy older adults. These findings were independent of age, sex, obesity-associated co-morbidities and other confounders, and remained stable in replication analyses. In addition, posterior default mode connectivity correlated with executive function.

Functional Connectivity and Obesity in Aging

Our main finding is a reduction of posterior default mode connectivity with higher BMI. This effect was found in the main sample ($n = 521$), in a ROI-based analysis of a motion-matched subgroup ($n = 186$), as well as in an independent replication sample in the same age range ($n = 191$), underlining the robustness of the association.

Our finding is in line with and extends a recent report in which lower DMN connectivity was associated with higher BMI in a young sample but no differences of DMN functional connectivity in siblings with differing obesity status were found, indicating the connectivity differences

to be subsequent, not prior to the development of obesity [Doucet et al., 2017]. In addition, previous studies on cardiovascular risk factors in middle-aged samples have linked insulin resistance and type 2 diabetes to alterations in default mode connectivity [Buckner et al., 2008; Kenna et al., 2013; Musen et al., 2012]. Notably, decreased default mode connectivity has also been reported in young individuals at risk for Alzheimer's disease (AD) such as APOE e4-carriers, and in older MCI patients [Sheline et al., 2010; Sorg et al., 2007]; moreover several studies suggest that modifiable AD risk factors are linked to alterations in DMN connectivity [Buckner et al., 2008; Kenna et al., 2013; Musen et al., 2012]. Thus, our results suggest an association of obesity and connectivity changes similar to those seen in populations at risk for AD, and support the view of obesity being a risk factor for dementia [Beydoun et al., 2008; Kivipelto et al., 2005].

This view, however, is controversially discussed. While a recent meta-analysis reported that being obese below the age of 65 increased the risk of dementia and being obese above this age lowered dementia risk [Pedditizi et al., 2016], it was also reported that the incidence of dementia decreased with increasing BMI [Qizilbash et al., 2015] and that weight loss in mid-age independent of weight status was associated with increased risk of dementia three to four decades later [Strand et al., 2017]. Selection bias and reverse causation have been proposed to contribute to these contradictory results: obesity is strongly associated with cardiovascular risk factors which are themselves risk factors for dementia [Skoog et al., 1996] as well as overall mortality risk [Stevens et al., 1998] and weight loss 10–20 years before onset of dementia is well known [Knopman et al., 2007]. Our sample solely comprised healthy, cognitively intact older adults with a narrow age range between 67 (1. quartile) and 72 (3. quartile). Half of the sample was younger than the postulated reverse point of 70 years [Gustafson et al., 2009] and only very few were considerably older. This leads us to believe that our sample represents subjects vulnerable to the adverse effects of obesity on cognition who have not yet experienced prodromal dementia-related weight loss. Other studies reporting BMI to be associated with gray matter volume decline and cognitive deficits in old-age [Kharabian Masouleh et al., 2016; Walther et al., 2010] support this association of obesity and brain damage in older subjects.

In line with the literature, we found APOE-4 genotype to be independently associated with precuneus DMN connectivity [Sheline et al., 2010]. Opposed to a previous finding in individuals above the age of 70 years [Bäckman et al., 2015], there was no significant interaction of BMI and APOE-4 status. The modifiable risk factor obesity and the genetic risk factor APOE-4 might thus be associated with similar patterns of decreased posterior DMN connectivity, hinting to a common mechanism such as dysregulated lipid metabolism [Chouinard-Watkins et al., 2015; Romas et al., 1999; Sheline et al., 2010].

Our results remained significant when correcting for age, sex, obesity-associated co-morbidities arterial hypertension and diabetes, and other confounders. This indicates that the association is not primarily due to conditions frequently associated with obesity and known to affect brain structure and function [Jennings and Zanstra, 2009; Moheet et al., 2015].

We found the association of BMI and reduced posterior default mode connectivity to be independent of GMV reductions in the context of pathological aging and did not observe an association of DMN GMV with BMI. In the literature, mixed associations for BMI and precuneus/posterior cingulate cortex gray matter volume have been reported [Willette and Kapogiannis, 2015], leaving the interplay of gray matter volume and functional connectivity strength a matter of debate. Functional connectivity within the DMN is thought to be based on white matter connections between its anterior and posterior regions [Greicius et al., 2008] and decreased functional connectivity could thus be a result of decreased white matter fiber integrity. Obesity has been shown to be associated with reduced indices of white matter microstructure within the limbic system and in other regions [Kullmann et al., 2015] and recently higher BMI was associated with decreased white matter volume in a stereotactic white matter mask of the DMN [Figley et al., 2016]. Upcoming longitudinal studies thus need to further disentangle if obesity-associated white matter microstructural changes within the DMN precede or follow observed obesity-associated decreases in functional connectivity.

Concerning further associations of BMI and functional connectivity, only the visual network was found to be associated with higher BMI, but the extent of increased connectivity was very limited. In our large cohort, we did not observe previously reported increased putamen and insula connectivity [Hogenkamp et al., 2016], decreased insula–anterior cingulate cortex (ACC) connectivity [Moreno-Lopez et al., 2016], increased salience network connectivity [Figley et al., 2016; Garcia-Garcia et al., 2013], reduced temporal lobe network connectivity [Kullmann et al., 2012] or increased DMN connectivity [Kullmann et al., 2012; Legget et al., 2016; Tregellas et al., 2011] with higher BMI. Similar to our results, one study reported reduced precuneus connectivity for obese compared with lean participants, although the results might have been confounded by the significant age difference between groups [Geha et al., 2016]. In a recent study with 496 participants, DMN cohesiveness has been shown to be reduced in young, obese compared with lean individuals, with highest effect size found for the posterior DMN component which is in line with our results. A siblings analysis suggested this to be a consequence rather than a driving factor of obesity [Doucet et al., 2017].

Taken together, our results only partly replicate these findings obtained in young participants (age <40 years); this might be due to an interaction of obesity and aging in

the brain potentially involving changes in eating behavior [Elsner, 2002] and levels of circulating hormones such as leptin [Isidori et al., 2000; Moller et al., 1998]. Also, the negative effects of obesity on the brain are probably not detectable at young age but accumulate proportionally to "obesity pack-years" [Abdullah et al., 2011].

The only study investigating obesity and resting state connectivity in aged individuals showed that lower DMN activity during a finger-tapping task in older obese compared with lean participants predicted better working memory performance 12 months later [Hsu et al., 2015]. The authors argued that functional connectivity of the DMN might be a neuroprotective mechanism of higher BMI. Considering the mean sample age of 75 years and the steeper decline in cognitive scores within the normal weight group, we would rather consider this to be an effect of reverse causation. Interestingly, baseline cognitive scores were significantly lower for the overweight and obese groups compared with the lean group which fits to the notion of higher BMI exerting negative effects on the brain in mid-to-late-life.

Cognitive Performance

We observed BMI-associated connectivity changes in a region which is considered to be affected early during cognitive decline [Sorg et al., 2007]. Our results show that both higher BMI and lower mean connectivity in the BMI-associated cluster within the PCC of DMN 29 correlated with slightly worse performance in the memory and more so in the executive domain. Several studies indicate that the DMN plays an important role not only in episodic memory, but also in executive function, as its successful deactivation is predictive of performance in attention and working memory tasks [Daselaar et al., 2004; Wang et al., 2007; Weissman et al., 2006]. Thus, we speculate that a higher BMI in older age might exert negative effects on posterior DMN connectivity, which eventually translate into subtle cognitive impairments. Future longitudinal studies are needed to further test this hypothesis.

Effects of Head Motion

As motion has been shown to exert massive and widespread effects on connectivity [Power et al., 2015] we aimed to account for motion by (1) adding mean FD as a covariate into the multivariate backward model selection and by (2) selecting a sub-sample in which motion and BMI were not correlated. Notably, BMI was retained in the backward model selection process even after including mean FD as a covariate and it remained a significant predictor of reduced PCC intra-network connectivity in the motion-matched sample. This leads us to conclude that there is an association of BMI with posterior default mode connectivity independent of confounding motion effects.

It has also been suggested that by using the common approach of strictly correcting for the effects of motion one might remove information related to the phenotype under study. Along this line, inter-individual differences in motion have been explained by a neurobiological trait of long-range default mode connectivity [Zeng et al., 2014] and head motion has been shown to positively correlate with impulsivity [Kong et al., 2014]. As elevated BMI has been linked to increased impulsivity [Braet et al., 2007], the BMI-related motion increase found in our cohort might not be simply due to increased discomfort during the scan (thereby confounding BMI-related analyses), but reflect an obesity-related trait. This is further supported by recent findings of common genetic factors associated with head motion and BMI that have been reported in two large cohorts [Hodgson et al., 2016]. Thus, disentangling the effects of BMI and motion remains difficult and merits careful investigation in future studies.

Limitations

Several limitations should be considered when interpreting our results. First, our cross-sectional data does not allow us to draw conclusions on the causal relationship between BMI and posterior default mode connectivity and the underlying mechanisms should be carefully studied in longitudinal designs. We demonstrated that the described association of BMI and connectivity was not solely driven by head motion differences, however head motion was a major confounder in this study and it remains unclear whether it is inherently associated with obesity. Physiological parameters [Glover et al., 2000] were not measured and related noise could thus not be controlled for.

In addition, spatial normalization accuracy might be limited in large samples like ours which might have biased our results. However, besides controlling for registration quality as a confounder, we generally achieved a high registration quality through state-of-the-art registration tools [Klein et al., 2009] and careful visual inspection that led to exclusion of subjects with morphological alterations such as calcifications or atrophies/large ventricles as well as brain extraction failures. Another limitation is the definition of obesity by BMI, as this does not reflect age-related changes in body composition, such as conversion of lean body mass to fat [Zamboni et al., 2005]. A more precise measure of body fat (such as MRI-assessment of abdominal fat) would have allowed us to characterize the relationship between obesity and resting-state connectivity more specifically. Our analysis of the associations between BMI, connectivity and cognitive performance was exploratory and should thus be expanded to gain more insight into the cognitive implications of our result. An important strength of this study is that it relies on a large sample size of community-based well-characterized healthy older adults, supplemented by a homogenous replication sample. Also, various potential confounders were comprehensively assessed and controlled for. Our results remained significant when correcting for age, sex, hypertension, diabetes and other confounders, but the high covariance of BMI and obesity-associated comorbidities make it difficult to disentangle their contributions to functional connectivity differences in our cross-sectional design.

CONCLUSION

In the current study we showed that higher BMI is associated with reduced connectivity of the default mode network in the PCC and in the precuneus in a large sample of healthy older adults. This finding was independent of obesity-related comorbidities, changes in regional gray matter volume and APOE-e4 genotype. Moreover, our results indicate that regional changes in default mode connectivity translate into subtle differences in cognitive performance.

Thus, our results support the view that obesity might independently contribute to accelerated brain aging in older individuals without incident dementia, as lower default mode connectivity has been detected in populations at risk for AD, and it has been proposed as an early biomarker for emerging AD [Sorg et al., 2007]. The modifiable risk factor obesity might thus share the pattern of decreased posterior default mode connectivity with the unmodifiable risk factor APOE-e4 allele [Sheline et al., 2010]. Future studies should further investigate potential mechanisms underlying the association of obesity and resting state connectivity and infer obesity-preventing strategies to maintain cognitive function in aging.

ACKNOWLEDGMENTS

REFERENCES

Abdullah A, Wolfe R, Stoelwinder JU, de Courten M, Stevenson C, Walls HL, Peeters A (2011): The number of years lived with obesity and the risk of all-cause and cause-specific mortality. Int J Epidemiol 40:985–996.

Abou-Elseoud A, Starck T, Remes J, Nikkinen J, Tervonen O, Kiviniemi V (2010): The effect of model order selection in group PICA. Hum Brain Mapp 31:1207–1216.

Allen EA, Erhardt EB, Damaraju E, Gruner W, Segall JM, Silva RF, Havlicek M, Rachakonda S, Fries J, Kalyanam R, Michael AM, Caprihan A, Turner JA, Eichele T, Adelsheim S, Bryan AD, Bustillo J, Clark VP, Feldstein Ewing SW, Filbey F, Ford CC, Hutchison K, Jung RE, Kiehl KA, Kodituwakku P, Komesu YM, Mayer AR, Pearlson GD, Phillips JP, Sadek JR, Stevens M, Teuscher U, Thoma RJ, Calhoun VD (2011): A baseline for the multivariate comparison of resting-state networks. Front Syst Neurosci 5:2.

Amlung M, Petker T, Jackson J, Balodis I, MacKillop J (2016): Steep discounting of delayed monetary and food rewards in obesity: A meta-analysis. Psychol Med 46:2423–2434.

Aslanidis C, Schmitz G (1999): High-speed apolipoprotein E genotyping and apolipoprotein B3500 mutation detection using real-time fluorescence PCR and melting curves. Clin Chem 45: 1094–1097.

Avants BB, Tustison NJ, Song G, Cook PA, Klein A, Gee JC (2011): A reproducible evaluation of ANTs similarity metric performance in brain image registration. Neuroimage 54: 2033–2044.

Bäckman K, Joas E, Waern M, Östling S, Guo X, Blennow K, Skoog I, Gustafson DR (2015): 37 years of body mass index and dementia: Effect modification by the APOE genotype: Observations from the prospective population study of women in Gothenburg, Sweden. J Alzheimers Dis 48:1119–1127.

Benito-León J, Mitchell AJ, Hernández-Gallego J, Bermejo-Pareja F (2013): Obesity and impaired cognitive functioning in the elderly: A population-based cross-sectional study (NEDICES). Eur J Neurol 20:899.

Benjamini Y, Hochberg Y (1995): Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodological) 57:289–300.

Beydoun MA, Beydoun HA, Wang Y (2008): Obesity and central obesity as risk factors for incident dementia and its subtypes: A systematic review and meta-analysis. Obes Rev 9:204–218.

Biessels GJ, De Leeuw FE, Lindeboom J, Barkhof F, Scheltens P (2006): Increased cortical atrophy in patients with Alzheimer's disease and type 2 diabetes mellitus. J Neurol Neurosurg Psychiatry 77:304–307.

Braet C, Claus L, Verbeken S, Van Vlierberghe L (2007): Impulsivity in overweight children. Eur Child Adolesc Psychiatry 16: 473–483.

Buckner RL, Andrews-Hanna JR, Schacter DL (2008): The brain's default network: Anatomy, function, and relevance to disease. Ann NY Acad Sci 1124:1–38.

Calhoun VD (2004): Group ICA of fMRI toolbox (GIFT).

Cheke LG, Bonnici HM, Clayton NS, Simons JS (2017): Obesity and insulin resistance are associated with reduced activity in core memory regions of the brain. Neuropsychologia 96:137–149.

Chouinard-Watkins R, Conway V, Minihane AM, Jackson KG, Lovegrove JA, Plourde M (2015): Interaction between BMI and APOE genotype is associated with changes in the plasma long-chain–PUFA response to a fish-oil supplement in healthy participants. Am J Clin Nutr 102:505–513.

Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006): Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci USA 103: 13848–13853.

Daselaar S, Prince S, Cabeza R (2004): When less means more: Deactivations during encoding that predict subsequent memory. Neuroimage 23:921–927.

Doucet, GE, Rasgon, N, McEwen, BS, Micali, N, Frangou, S. (2017) Elevated body mass index is associated with increased integration and reduced cohesion of sensory-driven and internally guided resting-state functional brain networks. Cereb Cortex doi:10.1093/cercor/bhx008. (in press).

Elsner RJF (2002): Changes in eating behavior during the aging process. Eat Behav 3:15–43.

Erhardt EB, Rachakonda S, Bedrick EJ, Allen EA, Adali T, Calhoun VD (2011): Comparison of multi-subject ICA methods for analysis of fMRI data. Hum Brain Mapp 32:2075–2095.

Figley CR, Asem JSA, Levenbaum EL, Courtney SM (2016): Effects of body mass index and body fat percent on default mode, executive control, and salience network structure and function. Front Neurosci 10:234.

Garcia-Garcia I, Jurado MA, Garolera M, Segura B, Sala-Llonch R, Marques-Iturria I, Pueyo R, Sender-Palacios MJ, Vernet-Vernet M, Narberhaus A, Ariza M, Junque C (2013): Alterations of the salience network in obesity: A resting-state fMRI study. Hum Brain Mapp 34:2786–2797.

Geha P, Cecchi G, Todd Constable R, Abdallah C, Small DM (2016): Reorganization of brain connectivity in obesity. Hum Brain Mapp 38:1403–1420.

Glahn DC, Winkler AM, Kochunov P, Almasy L, Duggirala R, Carless MA, Curran JC, Olvera RL, Laird AR, Smith SM, Beckmann CF, Fox PT, Blangero J (2010): Genetic control over the resting brain. Proc Natl Acad Sci USA 107:1223–1228.

Glover GH, Li T-Q, Ress D (2000): Image-based method for retrospective correction of physiological motion effects in fMRI: Retroicor. Magn Reson Med 44:162–167.

Gordon EM, Devaney JM, Bean S, Vaidya CJ (2015): Resting-state striato-frontal functional connectivity is sensitive to dat1 genotype and predicts executive function. Cereb Cortex 25:336–345.

Gorgolewski K, Burns CD, Madison C, Clark D, Halchenko YO, Waskom ML, Ghosh SS (2011): Nipype: A flexible, lightweight and extensible neuroimaging data processing framework in python. Front Neuroinform 5:13.

Greicius MD, Supekar K, Menon V, Dougherty RF (2008): Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb Cortex 19:72–78.

Grill HJ, Skibicka KP, Hayes MR (2007): Imaging obesity: Fmri, food reward, and feeding. Cell Metab 6:423–425.

Griswold MA, Jakob PM, Heidemann RM, Nittka M, Jellus V, Wang J, Kiefer B, Haase A (2002): Generalized autocalibrating partially parallel acquisitions (GRAPPA). Magn Reson Med 47: 1202–1210.

Gunstad J, Paul RH, Cohen RA, Tate DF, Spitznagel MB, Gordon E (2007): Elevated body mass index is associated with executive dysfunction in otherwise healthy adults. Compr Psychiatry 48:57–61.

Gustafson D, Bäckman K, Waern M, Östling S, Guo X, Zandi P, Mielke M, Bengtsson C, Skoog I (2009): Adiposity indicators and dementia over 32 years in Sweden. Neurology 73: 1559–1566.

Hodgson K, Poldrack RA, Curran JE, Knowles EE, Mathias S, Göring HHH, Yao N, Olvera RL, Fox PT, Almasy L (2016): Shared genetic factors influence head motion during mri and body mass index. Cereb Cortex doi:10.1093/cercor/bhw321. (in press).

Hogenkamp PS, Zhou W, Dahlberg LS, Stark J, Larsen AL, Olivo G, Wiemerslage L, Larsson EM, Sundbom M, Benedict C, Schioth HB (2016): Higher resting-state activity in reward-related brain circuits in obese versus normal-weight females independent of food intake. Int J Obes 40:1687–1692.

Hsu CL, Voss MW, Best JR, Handy TC, Madden K, Bolandzadeh N, Liu-Ambrose T (2015): Elevated body mass index and maintenance of cognitive function in late life: Exploring underlying neural mechanisms. Front Aging Neurosci 7:155.

Isidori AM, Strollo F, Morè M, Caprio M, Aversa A, Moretti C, Frajese G, Riondino G, Fabbri A (2000): Leptin and aging: Correlation with endocrine changes in male and female healthy adult populations of different body weights. J Clin Endocrinol Metab 85:1954–1962.

59

Jennings JR, Zanstra Y (2009): Is the brain the essential in hypertension?. Neuroimage 47:914–921.

Kenna H, Hoeft F, Kelley R, Wroolie T, DeMuth B, Reiss A, Rasgon N (2013): Fasting plasma insulin and the default mode network in women at risk for Alzheimer's disease. Neurobiol Aging 34:641–649.

Kerti L, Witte AV, Winkler A, Grittner U, Rujescu D, Flöel A (2013): Higher glucose levels associated with lower memory and reduced hippocampal microstructure. Neurology 81:1746–1752.

Kharabian Masouleh S, Arélin K, Horstmann A, Lampe L, Kipping JA, Luck T, Riedel-Heller SG, Schroeter ML, Stumvoll M, Villringer A, Witte AV (2016): Higher body mass index in older adults is associated with lower gray matter volume: Implications for memory performance. Neurobiol Aging 40:1–10.

Kiviniemi V, Starck T, Remes J, Long X, Nikkinen J, Haapea M, Veijola J, Moilanen I, Isohanni M, Zang YF, Tervonen O (2009): Functional segmentation of the brain cortex using high model order group PICA. Hum Brain Mapp 30:3865–3886.

Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, Winblad B, Helkala EL, Tuomilehto J, Soininen H, Nissinen A (2005): Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Arch Neurol 62:1556–1560.

Klein A, Andersson J, Ardekani BA, Ashburner J, Avants B, Chiang M-C, Christensen GE, Collins DL, Gee J, Hellier P, Song JH, Jenkinson M, Lepage C, Rueckert D, Thompson P, Vercauteren T, Woods RP, Mann JJ, Parsey RV (2009): Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. Neuroimage 46:786–802.

Knopman DS, Edland SD, Cha RH, Petersen RC, Rocca WA (2007): Incident dementia in women is preceded by weight loss by at least a decade. Neurology 69:739–746.

Kong XZ, Zhen Z, Li X, Lu HH, Wang R, Liu L, He Y, Zang Y, Liu J (2014): Individual differences in impulsivity predict head motion during magnetic resonance imaging. PLoS One 9:e4989.

Kullmann S, Heni M, Veit R, Ketterer C, Schick F, Haring HU, Fritsche A, Preissl H (2012): The obese brain: Association of body mass index and insulin sensitivity with resting state network functional connectivity. Hum Brain Mapp 33:1052–1061.

Kullmann S, Schweizer F, Veit R, Fritsche A, Preissl H (2015): Compromised white matter integrity in obesity. Obes Rev 16:273–281.

Legget KT, Wylie KP, Cornier M-A, Melanson EL, Paschall CJ, Tregellas JR (2016): Exercise-related changes in between-network connectivity in overweight/obese adults. Physiol Behav 158:60–67.

Loeffler M, Engel C, Ahnert P, Alfermann D, Arelin K, Baber R, Beutner F, Binder H, Brahler E, Burkhardt R, Ceglarek U, Enzenbach C, Fuchs M, Glaesmer H, Girlich F, Hagendorff A, Hantzsch M, Hegerl U, Henger S, Hensch T, Hinz A, Holzendorf V, Husser D, Kersting A, Kiel A, Kirsten T, Kratzsch J, Krohn K, Luck T, Melzer S, Netto J, Nuchter M, Raschpichler M, Rauscher FG, Riedel-Heller SG, Sander C, Scholz M, Schonknecht P, Schroeter ML, Simon JC, Speer R, Staker J, Stein R, Stobel-Richter Y, Stumvoll M, Tarnok A, Teren A, Teupser D, Then FS, Tonjes A, Treudler R, Villringer A, Weissgerber A, Wiedemann P, Zachariae S, Wirkner K, Thiery J (2015): The LIFE-Adult-Study: Objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. BMC Publ Health 15:691.

Moheet A, Mangia S, Seaquist ER (2015): Impact of diabetes on cognitive function and brain structure. Ann NY Acad Sci 1353:60–71.

Moller N, O'Brien P, Nair KS (1998): Disruption of the relationship between fat content and leptin levels with aging in humans1. J Clin Endocrinol Metab 83:931–934.

Moreno-Lopez L, Contreras-Rodriguez O, Soriano-Mas C, Stamatakis EA, Verdejo-Garcia A (2016): Disrupted functional connectivity in adolescent obesity. NeuroImage: Clin 12:262–268.

Morris J, Heyman A, Mohs R, Hughes J, Van Belle G, Fillenbaum G, Mellits E, Clark C (1989): The consortium to establish a registry for Alzheimer's disease (CERAD): I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39:1159–1165.

Musen G, Jacobson AM, Bolo NR, Simonson DC, Shenton ME, McCartney RL, Flores VL, Hoogenboom WS (2012): Resting-state brain functional connectivity is altered in type 2 diabetes. Diabetes 61:2375–2379.

Pedditizi E, Peters R, Beckett N (2016): The risk of overweight/obesity in mid-life and late life for the development of dementia: A systematic review and meta-analysis of longitudinal studies. Age Ageing 45:14–21.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012): Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 59:2142–2154.

Power J, Schlaggar B, Petersen S (2015): Recent progress and outstanding issues in motion correction in resting state fMRI. Neuroimage 105:536–551.

Qizilbash N, Gregson J, Johnson ME, Pearce N, Douglas I, Wing K, Evans SJ, Pocock SJ (2015): BMI and risk of dementia in two million people over two decades: A retrospective cohort study. Lancet Diabetes Endocrinol 3:431–436.

Radloff L (1977): The CES-D scale a self-report depression scale for research in the general population. Appl Psychol Meas 1:385–401.

Reineberg AE, Andrews-Hanna JR, Depue BE, Friedman NP, Banich MT (2015): Resting-state networks predict individual differences in common and specific aspects of executive function. Neuroimage 104:69–78.

Robinson S, Basso G, Soldati N, Sailer U, Jovicich J, Bruzzone L, Kryspin-Exner I, Bauer H, Moser E (2009): A resting state network in the motor control circuit of the basal ganglia. BMC Neurosci 10:137.

Romas SN, Tang M-X, Berglund L, Mayeux R (1999): APOE genotype, plasma lipids, lipoproteins, and AD in community elderly. Neurology 53:517-517

Rothemund Y, Preuschhof C, Bohner G, Bauknecht H-C, Klingebiel R, Flor H, Klapp BF (2007): Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals. Neuroimage 37:410–421.

Salami A, Wåhlin A, Kaboodvand N, Lundquist A, Nyberg L (2016): Longitudinal evidence for dissociation of anterior and posterior mtl resting-state connectivity in aging: Links to perfusion and memory. Cereb Cortex 26:3953–3963.

Sanchez-Cubillo I, Perianez JA, Adrover-Roig D, Rodriguez-Sanchez JM, Rios-Lago M, Tirapu J, Barcelo F (2009): Construct validity of the Trail Making Test: Role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. J Int Neuropsychol Soc 15:438.

Sheline Y, Morris J, Snyder A, Price J, Yan Z, D'Angelo G, Liu C, Dixit S, Benzinger T, Fagan A, Goate A, Mintun M (2010):

APOE-4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Aper. J Neurosci 30:17035–17040.

Skoog I, Nilsson L, Persson G, Lernfelt B, Landahl S, Palmertz B, Andreasson LA, Odén A, Svanborg A (1996): 15-year longitudinal study of blood pressure and dementia. Lancet 347:1141–1145.

Smith E, Hay P, Campbell L, Trollor J (2011): A review of the association between obesity and cognitive function across the lifespan: Implications for novel approaches to prevention and treatment. Obes Rev 12:740–755.

Sorg C, Riedl V, Muhlau M, Calhoun VD, Eichele T, Laer L, Drzezga A, Forstl H, Kurz A, Zimmer C, Wohlschlager AM (2007): Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. Proc Natl Acad Sci USA 104:18760–18765.

Stevens J, Cai J, Pamuk ER, Williamson DF, Thun MJ, Wood JL (1998): The effect of age on the association between body-mass index and mortality. N Engl J Med 338:1–7.

Stice E, Spoor S, Bohon C, Veldhuizen MG, Small DM (2008): Relation of reward from food intake and anticipated food intake to obesity: A functional magnetic resonance imaging study. J Abnorm Psychol 117:924.

Stoeckel LE, Weller RE, Cook Iii EW, Twieg DB, Knowlton RC, Cox JE (2008): Widespread reward-system activation in obese women in response to pictures of high-calorie foods. Neuroimage 41:636–647.

Strand BH, Wills AK, Langballe EM, Rosness TA, Engedal K, Bjertness E (2017): Weight change in midlife and risk of mortality from dementia up to 35 years later. J Gerontol Ser A: Biol Sci Medl Sci 72:855–860.

Tregellas JR, Wylie KP, Rojas DC, Tanabe J, Martin J, Kronberg E, Cordes D, Cornier MA (2011): Altered default network activity in obesity. Obesity (Silver Spring) 19:2316–2321.

Van de Rest O, Geleijnse JM, Kok FJ, van Staveren WA, Dullemeijer C, OldeRikkert MGM, Beekman ATF, de Groot CPGM (2008): Effect of fish oil on cognitive performance in older subjects: A randomized, controlled trial. Neurology 71: 430–438.

Van Der Elst WIM, Van Boxtel MPJ, Van Breukelen GJP, Jolles J (2006): Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. J Int Neuropsychol Soc 12:80–89.

Walther K, Birdsill AC, Glisky EL, Ryan L (2010): Structural brain differences and cognitive functioning related to body mass index in older females. Hum Brain Mapp 31:1052–1064.

Wang K, Liang M, Wang L, Tian L, Zhang X, Li K, Jiang T (2007): Altered functional connectivity in early Alzheimer's disease: A resting-state fMRI study. Hum Brain Mapp 28:967–978.

Wang L, LaViolette P, O'Keefe K, Putcha D, Bakkour A, Van Dijk KRA, Pihlajamaki M, Dickerson BC, Sperling RA (2010): Intrinsic connectivity between the hippocampus and posteromedial cortex predicts memory performance in cognitively intact older individuals. Neuroimage 51:910–917.

Weissman DH, Roberts KC, Visscher KM, Woldorff MG (2006): The neural bases of momentary lapses in attention. Nat Neurosci 9:971–978.

Willette AA, Kapogiannis D (2015): Does the brain shrink as the waist expands?. Ageing Res Rev 20:86–97.

Zamboni M, Mazzali G, Zoico E, Harris TB, Meigs JB, Di Francesco V, Fantin F, Bissoli L, Bosello O (2005): Health consequences of obesity in the elderly: A review of four unresolved questions. Int J Obes Relat Metab Disord 29:1011–1029.

Zeng LL, Wang D, Fox MD, Sabuncu M, Hu D, Ge M, Buckner RL, Liu H (2014): Neurobiological basis of head motion in brain imaging. Proc Natl Acad Sci USA 111:6058–6062.

2.4. Publication4: Original article: Kharabian et al, 2017

62

Cardiovascular risk factors are associated with grey matter structural networks in ageing

Kharabian Masouleh S., Beyer F., Lampe L., Loeffler M., Luck T., Riedel-Heller S.G., Schroeter M.L., Stumvoll M., Villringer A., Witte A.V

journal of cerebral blood flow and metabolism

2017

Gray matter structural networks are associated with cardiovascular risk factors in healthy older adults

Shahrzad Kharabian Masouleh[1], Frauke Beyer[1],
Leonie Lampe[1,2], Markus Loeffler[2,3], Tobias Luck[2,4], Steffi
G Riedel-Heller[4], Matthias L Schroeter[1,2,5], Michael Stumvoll[6],
Arno Villringer[1,5] and A Veronica Witte[1]

Abstract

While recent 'big data' analyses discovered structural brain networks that alter with age and relate to cognitive decline, identifying modifiable factors that prevent these changes remains a major challenge. We therefore aimed to determine the effects of common cardiovascular risk factors on vulnerable gray matter (GM) networks in a large and well-characterized population-based cohort. In 616 healthy elderly (258 women, 60–80 years) of the LIFE-Adult-Study, we assessed the effects of obesity, smoking, blood pressure, markers of glucose and lipid metabolism as well as physical activity on major GM-networks derived using linked independent component analysis. Age, sex, hypertension, diabetes, white matter hyperintensities, education and depression were considered as confounders. Results showed that smoking, higher blood pressure, and higher glycated hemoglobin (HbA1c) were independently associated with lower GM volume and thickness in GM-networks that covered most areas of the neocortex. Higher waist-to-hip ratio was independently associated with lower GM volume in a network of multimodal regions that correlated negatively with age and memory performance. In this large cross-sectional study, we found selective negative associations of smoking, higher blood pressure, higher glucose, and visceral obesity with structural covariance networks, suggesting that reducing these factors could help to delay late-life trajectories of GM aging.

Introduction

Recent 'big data' analyses of structural co-variance between brain regions revealed large-scale gray matter (GM) networks that are linked to developmental changes and inter-individual behavioral differences.[1–4] Douaud et al.[3] described a network of transmodal cortical and limbic GM regions that showed correlated shrinkage in healthy aging and links to memory performance. That GM network also mirrored brain regions that exhibit accelerated atrophy in patients with Alzheimer's disease (AD).[3]

The observed network-based effects could hint towards a shared susceptibility of connected regions, indicative of unique morphological properties, to selective pathological processes.[5,6] They also strengthen

[1]Department of Neurology, Max Planck Institute for Cognitive and Brain Sciences, Leipzig, Germany
[2]LIFE – Leipzig Research Center for Civilization Diseases, University of Leipzig, Leipzig, Germany
[3]Institute for Medical Informatics, Statistics, and Epidemiology (IMISE), University of Leipzig, Leipzig, Germany
[4]Institute of Social Medicine, Occupational Health and Public Health (ISAP), Medical Faculty, University of Leipzig, Leipzig, Germany
[5]Clinic for Cognitive Neurology, University of Leipzig, Leipzig, Germany
[6]IFB Adiposity Diseases Faculty of Medicine, University of Leipzig, Leipzig, Germany

Corresponding author:
A Veronica Witte, Department of Neurology, Max Planck Institute for Cognitive and Brain Sciences, Stephanstrasse 1a, Leipzig 04103, Germany.
Email: witte@cbs.mpg.de

the hypothesis that fundamental mechanisms of aging may contribute to (or result from) neurodegenerative pathologies.[7–11] A better understanding of possible modulators of GM networks that are vulnerable to aging would thus open a novel window towards targets for intervention of disease progression.

Using conventional analyses, global and regional decreases in GM volume and cortical thickness have been linked, though not unequivocally,[12] to common cardiovascular risk factors comprising cigarette smoking, hypertension, obesity and metabolic changes.[13–16] However, addressing potential impact of these factors at the network- and population-level remains a major challenge.[17]

We therefore aimed to systematically assess the effects of obesity, smoking, blood pressure, as well as markers of glucose and lipid metabolism and physical activity on major GM networks using linked independent component analysis of cortical volume, thickness, and surface area estimated from T1-weighted MRIs in a large cohort of community-dwelled healthy older individuals. We determined the unique contribution of each risk factor (selected according to the Framingham study[18] and additionally physical activity[19]) to variations in these GM networks using multivariable statistics that were adjusted for confounders. Possible associations between GM networks and cognition were explored using a sumscore of verbal memory performance, known to be highly affected by age.[20] We hypothesized negative effects of cardiovascular risk factors on major GM components that are prone to undergo age-associated changes and linked with cognition.

Materials and methods
Participants

Data were drawn from the baseline examination of the "Health Study of the Leipzig Research Centre for Civilization Diseases" (LIFE), a population-based cohort study of adult Leipzig inhabitants, randomly invited via the population registry. All subjects signed an informed consent form and received a small financial compensation. The study protocol was in accordance with the declaration of Helsinki and approved by the ethics committee of the University of Leipzig.

Participants underwent neuropsychological testing, medical examinations, and a randomly selected subset underwent magnetic resonance imaging (MRI) of the head at 3T ($n \sim 2600$). For details on the study design, see Loeffler et al.[21] Out of a sample of 985 older participants (≥ 60 years) available at the date of analyses, we excluded participants with dementia, neurological, psychiatric or immune suppressive medication

($n = 203$), as well as major brain pathology (e.g. tumors and stroke) ($n = 47$). Also, subjects with missing information on confounding factors ($n = 74$), severe movement artifacts on the MRI or other technical problems ($n = 32$), as well as non-intact cognitive performance ($n = 13$, defined as showing a total cognitive sumscore of < 3 SD of the mean population) were excluded, resulting in a sample of 616 subjects (Figure 1). Due to the nature of our exclusion criteria, participants excluded ($n = 369$) were on average slightly older (mean age: 69.5 ± 6 (SD) years, $p = 0.021$), more frequently women ($p = 0.028$), exhibited a higher BMI (mean BMI: 28.4 ± 4.25 (SD) kg/m^2, $p = 0.001$) and were less educated compared to those included ($p < 0.001$).

MRI acquisition

Anatomic T1-weighted images were acquired in a 3-Tesla Magnetom Verio scanner (Siemens, Erlangen, Germany) equipped with a 32-channel head array coil, using a three-dimensional Magnetization-Prepared Rapid Gradient Echo (MPRAGE) sequence. GRAPPA parallel imaging technique[22] was applied on the Alzheimer's Disease Neuroimaging Initiative (ADNI) standard protocol with the following parameters: TI 900 ms, TR 2300 ms, TE 2.98 ms, flip angle 9°, band width 240 Hz/pixel, image matrix 256×240, 176 partitions, FOV $256 \times 240 \times 176$ mm^3, sagittal orientation, voxel size $1 \times 1 \times 1$ mm^3, no interpolation.

Image processing

T1-weighted images were processed using FSL-VBM,[23] an optimized voxel-based morphometry (VBM)[24] protocol using FMRIB Software Library (FSL) tools (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM; FSL 4.1),[25] in which a symmetric study-specific GM template was built from the images of a sub-group of 260 participants equally matched for males and females, which were not significantly different from the whole sample with regard to age and BMI range and frequency of hypertension and diabetes. Prior to the FSL-VBM processing, the volumes were masked by the full brain-segmented volume output from FreeSurfer (http://surfer.nmr.mgh.harvard.edu/; FreeSurfer 5.0.0),[26] effectively excluding non-brain compartments. After nonlinearly registering all of the brain-extracted, GM-segmented images onto the symmetric study-specific GM template, the optimized FSL-VBM protocol involved a compensation (or "modulation") for the local contraction/ enlargement caused by the nonlinear component of the transformation. In addition, brain structural information was derived from vertex-wise cortical thickness and surface area calculated in FreeSurfer by means of
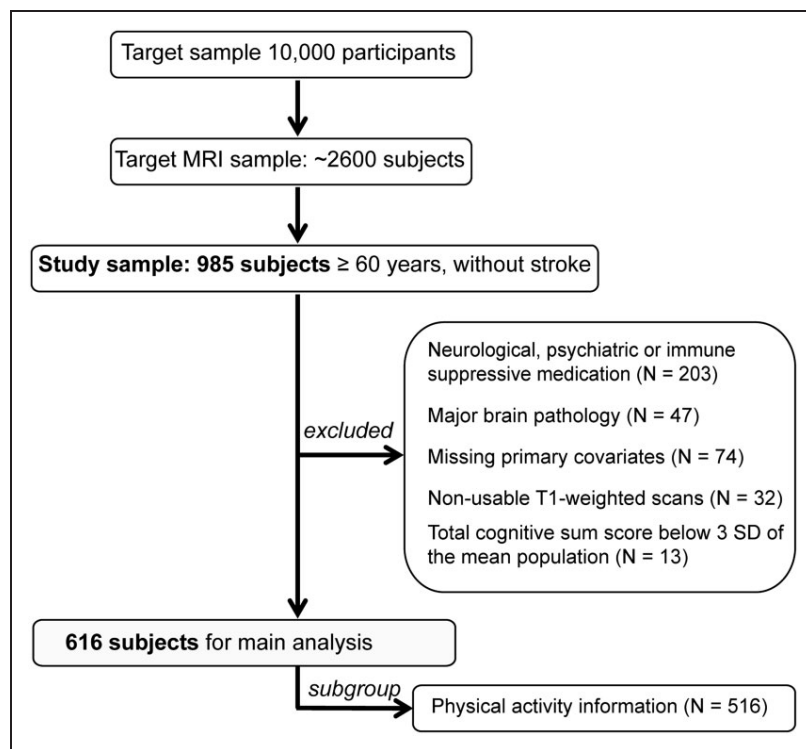
Figure 1. Flow chart of the study. Out of 985 older adults free of stroke, 369 were excluded due to medication intake, brain pathology, missing covariates, non-usable MRI scans, or non-intact cognition, leaving 616 participants for main analyses. Out of this sample, 516 participants had physical activity information.

an automated surface reconstruction scheme.[26] All surface reconstructions were visually inspected in Freeview and manually edited in 31 cases. For computational reasons, we reduced the number of data points in each modality by lowering the resolution of the pre-processed images, while not losing any information about global patterns of structural covariance, due to the smoothness of the pre-processed images. The modulated registered GM-segmented images were first down-sampled to 4 mm isotropic and then were smoothed with an isotropic Gaussian kernel with a σ of 4 mm ($\approx$9.4 mm full width at half maximum (FWHM)). Cortical thickness and surface area maps were sampled from subject space to the fsaverage5 template (10,242 vertices) and then smoothed with a surface FWHM of 10 mm.

Then linked-independent component analysis (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLICA) was applied to measures of GM volume, cortical thickness and surface area, decomposing the data into 70 components, see Douaud et al.[3] and Groves et al.[27,28] for further descriptions. Briefly, here the aim is to model the

group data as a set of interpretable features (the independent components (ICs)), each one characterizing a single mode of variability. Each feature consists of a shared subject loading, which indicates which subjects have more or less of this feature, and the corresponding spatial pattern that is learned for each modality. We selected 12 global networks based on the elbow in the scree plot of the relative amount of total variance explained by each component (similar to Groves et al.[27]) and further denoted them as IC 1–12 (Table 1). Two components (IC1 and IC8) were considered of no further interest, as their respective variance was nearly fully explained by differences in head size (IC1) and image artifacts (IC8). See Supplementary Figure, for illustration of spatial maps of the remaining ten components.

For illustration purposes, we up-sampled the linked-ICA results to the high-resolution versions of the smoothed input data, similar to Groves et al.[27] (GM volume images on 2 mm isotropic and surface measures sampled on fsaverage space, i.e. 163,842 cortical vertices per hemisphere).

Table 1. Relative amount of explained variance by independent components (IC), according to linked-IC analysis of gray matter volume (GMV), cortical thickness, and surface area; and correlation with age and memory performance.

# Component	Explained variance (%)			Correlation (r, p-value)	
	GMV	Thickness	Area	Age	Memory
IC1	1	2	47	**−0.136, 10^{-3}**	−0.075, 0.062
IC2	0	32	0	**−0.115, 0.004**	0.036, 0.376
IC3	9	4	14	**−0.581, 10^{-3}**	**0.192, 10^{-3}**
IC4	8	0	4	0.003, 0.95	−0.078, 0.053
IC5	0	12	0	**0.177, 10^{-3}**	−0.082, 0.042
IC6	4	0	0	**−0.165, 10^{-3}**	0.023, 0.57
IC7	3	1	4	**−0.150, 10^{-3}**	**0.144, 10^{-3}**
IC8	3	2	0	−0.034, 0.4	−0.061, 0.13
IC9	2	1	1	−0.086, 0.034	0.027, 0.5
IC10	3	0	1	0.001, 0.97	**0.184, 10^{-3}**
IC11	3	0	0	**−0.178, 10^{-3}**	−0.018, 0.65
IC12	0	8	0	−0.044, 0.28	0.047, 0.24

Note: r- and p-values are according to Spearman's rank correlations. Significant associations are indicated by bolding the numbers ($p < 0.005$).

Cardiovascular risk factors

All subjects underwent anthropometric assessments, donated blood after fasting overnight and were asked to fill in questionnaires about their lifestyle habits. Cardiovascular risk factors comprised obesity, assessed using "waist-to-hip ratio" (WHR, measured using an ergonomic circumference measuring tape (SECA 201) to the nearest 0.1 cm) and "BMI" (in kg/m[2,] measured) as continuous variables, "smoking status" (current, past or never smokers), systolic "blood pressure" (in mmHg, mean of three consecutive measurements in a seated position at rest), fasting serum concentrations of glucose and lipid metabolism, i.e. "glycated hemoglobin (HbA1c)" (in mmol/mol), "high-density lipoprotein (HDL)" (in mU/mL), and total "cholesterol" (in mmol/L). By using blood pressure and markers of glucose control (HbA1c) as continuous variables of interest related to hypertension and diabetes, we were able to increase statistical power and sensitivity in a dose–response relationship. Six subjects had missing blood values due to technical problems (HbA1c, $n = 5$, HDL, $n = 1$), these values were replaced by the sample's medians. "Physical activity" (self-reported according to the German short version of the international physical activity questionnaire, IPAQ,[29] in MET-minutes/week) was analyzed in a subgroup of 516 subjects due to unreturned questionnaires in 100 subjects.

Memory performance and assessment of confounders

"Memory performance" was assessed using the CERAD verbal learning task.[30] Briefly, subjects were asked to remember and recall immediately and after a delay as many words as possible out of a list of 10 words. The memory performance sumscore was defined as the standardized mean performance in the sum of (1) immediate learning (no. of correct words across the three learning trials), (2) delayed recall (no. of correct words in the recall trial), and (3) recognition (no. of correctly recognized words in the recognition trial, minus false positives).[31,32]

"Depression" was measured using the Center for Epidemiologic Studies Depression Scale (CES-D) questionnaire,[33] "arterial hypertension" was defined as a systolic blood pressure ≥ 160 mmHg, a diastolic blood pressure ≥ 95 mmHg or use of antihypertensive medication,[14] "diabetes" was defined as none, type 1 medicated, type 2 medicated, type 2 unmedicated, and "other cardiovascular conditions" were defined as arrhythmia or tachycardia. "Education" was measured according to the International Standard Classification of Education (7 levels).[34] In addition, "APOE4 carrier status" was defined as carrying one or two E4 alleles of the apolipoproeotein E (APOE) gene ($n = 32$ missings due to lack of DNA samples). Genotyping was performed on a Roche Lightcylcer 480 using genomic

DNA that was isolated from peripheral leukocytes using an automate protocol on the Qiagen Autopure LS (Qiagen, Hilden, Germany).

Statistical analysis

To determine unique associations between the GM networks and cardiovascular risk factors, we conducted partial correlations between subjects' loading on each of the 10 ICs and BMI, WHR, smoking, blood pressure, HbA1c, HDL and total cholesterol, respectively, in line with previous studies.[35,36] When meeting the criteria of bivariate correlation with the respective IC (Figure 2), the remainder of the cardiovascular risk factors as well as the following confounder variables was considered in the partial correlation models: age, sex, depression, hypertension (except for blood pressure analyses), diabetes (except for HbA1c analyses), other

cardiovascular conditions, white matter hyperintensities (WMH, assessed according to the Fazekas rating scale by means of 3D-FLAIR images[37]), education, total intracranial volume (TIV), and APOE4 carrier. We repeated analyses for physical activity in the physical activity subgroup ($n = 516$).

To assess if the relation between blood pressure (or HbA1c) and GM networks would change with medication, we repeated the analysis in those with anti-hypertensive medication (or anti-diabetic medication, respectively), and in those without. In addition, given the link between estrogen replacement therapy and brain aging,[38,39] we excluded women with current estrogen medication in confirmatory analyses. All variables were normally distributed (unimodal, $|\text{skewness}| \leq 1$), except IC7, HbA1c, HDL, and physical activity, therefore non-parametric statistics were used. For partial correlations, missing APOE4 values ($n = 32$) were
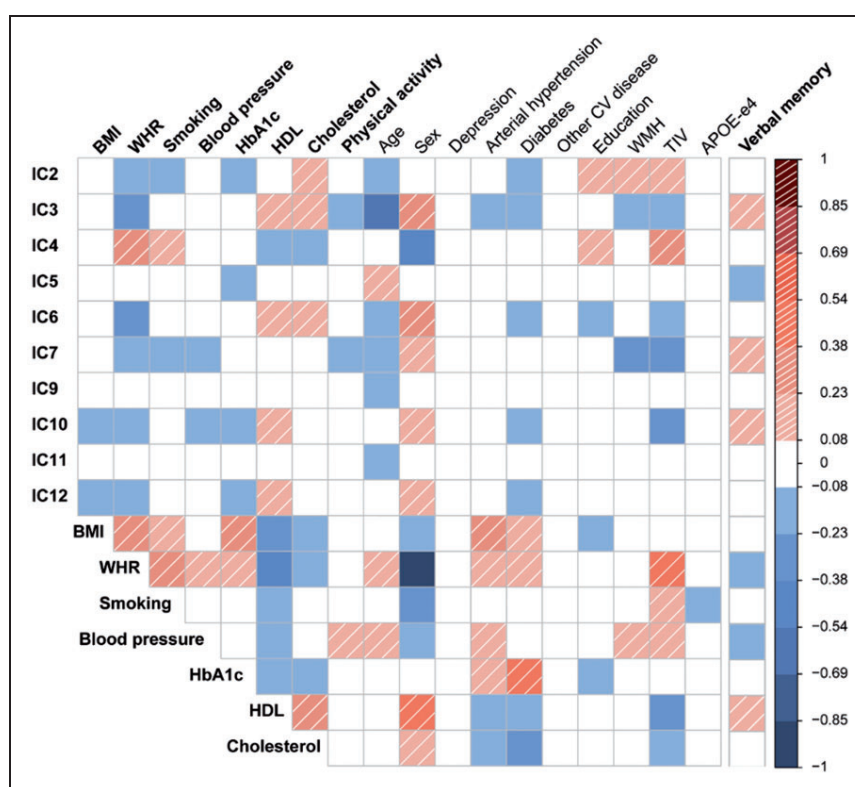

Figure 2. Bivariate correlations among independent components (IC), cardiovascular risk factors, confounders, and verbal memory score. Significant associations (Spearman's correlations, $p < 0.05$) are color-coded in red-shaded (positive) and blue (negative). CV: cardiovascular; WMH: white matter hyperintensities; TIV: total intracranial volume; APOE-e4: apolipoprotein E epsilon-4 carrier status; BMI: body mass index; WHR: waist-to-hip ratio; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein.

substituted by the sample mean. The significance threshold of partial correlations was set to $p < 0.05$ (two-sided) and corrected for the number of ICs tested ($n = 10$), resulting in $p_\alpha < 0.005$. All statistical analyses were performed in SPSS 20 (PASW, SPSS, IBM).

Results

In total, 616 older participants (258 women) were included in the analyses, see Table 2 for demographic characteristics. Subjects without physical activity questionnaires ($n = 100$) were slightly older (mean age: 70 ± 5 (SD) years; $p < 0.001$) and less educated ($p = 0.005$) in comparison to those with complete information ($n = 516$).

Structural networks and cardiovascular risk factors

Out of 10 independent GM components, global networks IC2 and IC7 showed an overall decrease in cortical thickness and volume, respectively, with age (IC2: $r = -0.115$, $p = 0.004$; IC7: $r = -0.150$, $p < 10^{-3}$; Table 1). IC7 was also associated with memory performance ($r = 0.144$, $p < 10^{-3}$).

Independent of further associated risk factors and confounders, cigarette smoking was significantly linked to lower thickness and volume throughout the neocortex in these two global networks (Figure 3(a) and (b), IC2: partial-$r = 0.120$, $p = 0.003$; IC7: partial-$r = -0.143$, $p < 0.001$).

Also, blood pressure was independently associated with IC7, showing an overall cortical volume decrease in association with increased blood pressure (Figure 3(b), partial-$r = -0.122$, $p = 0.003$).

Considering glucose metabolism, we observed that higher serum concentrations of HbA1c were associated with decreased cortical thickness in IC2 after controlling for other risk factors and confounders (partial-$r = -0.158$, $p < 10^{-3}$) (Figure 4(a)). A more regionally specific effect was noted for HbA1c in network IC5, showing lower thickness in medial frontal, insular, cingulate and inferior temporal areas and higher thickness in the postcentral gyrus and in the intraparietal sulcus (partial-$r = -0.206$, $p < 10^{-3}$) (Figure 4(b)). This network exhibited a positive correlation with age ($r = 0.177$, $p < 10^{-3}$).

A strong negative association with age was present in IC3 ($r = -0.58$, $p < 10^{-3}$), which was characterized by changes in GM volume predominantly within the

Table 2. Sample characteristics.

	Participants $n = 616$ (258 women)
Age [y]	69 ± 5 (60–79)
Waist-to-hip ratio	0.96 ± 0.085 (0.73–1.14)
BMI [kg/m^2]	27.5 ± 4 (17–41)
Smoking [%] (current/previous/never)	7.5/33.1/59.4
Mean Systolic BP [mmHg]	136 ± 17 (89–197)
HbA1c [mmol/mol] ($n = 611$)	5.4 [5.16–5.68] (3.84–12.38)
HDL [mU/mL] ($n = 615$)	1.6 [1.32–1.92] (0.45–4.17)
Total cholesterol [mmol/L]	5.9 ± 1.1 (2.3–10.8)
Physical activity [MET-minutes/week] ($n = 516$)	4159 [2374.5–6919.5] (33.0–16398.0)
APOE status [% e4-carrier] ($n = 584$)	20.9
Depression scale (CES-D) [score]	9.4 ± 5.1 (0–34)
Arterial hypertension [%] (yes)	55.7
Diabetes status [%] (none / type 1-medicated, type 2-medicated, type 2-non-medicated)	84.4/0.5/12.3/2.8
Current estrogen supplement [% females] (yes)	7.3
Cardiovascular diseases [%] (any)	19.2
Education [%] (without SS-LD/SS-LD/advanced SS-LD / advanced technical SS-LD / technical college ED / university ED)	0/10.7/6.3/42.7/5.2/35.1
White matter hyperintensities [%] (Fazekas score 0/1/2/3)	23.5/59.8/16.2/0.5

Note: Data are mean $\pm$ SD (minimum-maximum) or median [Interquartile range] (minimum-maximum), unless indicated otherwise. BMI: body mass index; BP: Blood pressure; MET: multiples of the resting metabolic rate; HbA1c: glycated hemoglobin A1c; HDL: high-density lipoproteins; APOE: Apolipoprotein E; CES-D: center for epidemiologic studies depression scale; SS-LD: secondary school-leaving degree; ED: entrance degree.
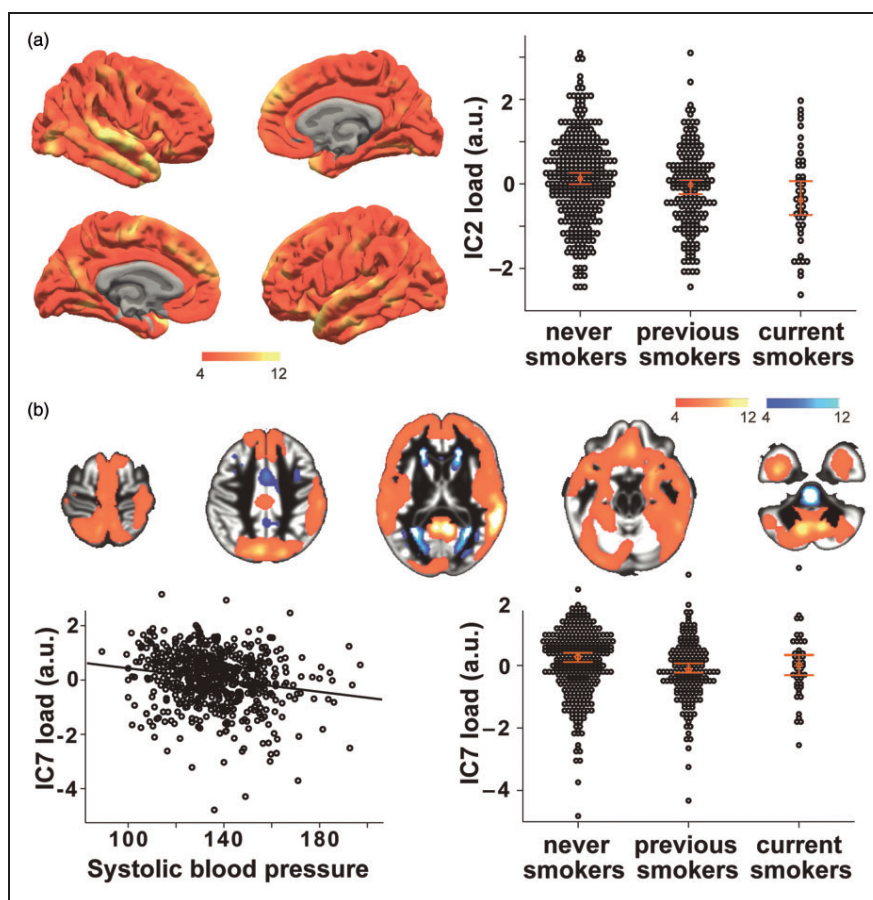
Figure 3. In two global networks, lower gray matter thickness (IC2, a) and volume (IC7, b) were associated with smoking (a) and higher blood pressure (b). Scatter plots show the individual's loading (black dots) and the group's median with 95% SE or linear fit. Colors indicate positive (red/yellow) or negative (blue/light-blue) co-variations within the network ($z > 4$), maps are drawn on a standard brain.

fundus of the sulci in prefrontal, temporal and parietal regions, as well as in limbic and paralimbic areas and in the cerebellum (Figure 5(a) and (b)). Notably, this network had a good spatial agreement with the GM component in Douaud et al.[3] (http://www.fmrib.ox.ac.uk/analysis/LIFO+AD+AOS/), showing late development and accelerated decline in aging, and with GM atrophy seen in AD (spatial correlation of Z-maps: $r = 0.82$, $p < 10^{-3}$; $r = 0.67$ $p < 10^{-3}$, respectively). Lower GM volume in this network correlated with worse memory performance in our sample ($r = 0.192$, $p < 10^{-3}$ Figure 5(b)). In addition, lower GM was independently associated with higher WHR in this component (partial-$r = -0.149$, $p < 10^{-3}$) (Figure 5(c)).

Considering components covering parts of the cerebellum, GM volume in IC10 correlated with memory performance ($r = 0.184$, $p < 10^{-3}$). In addition, IC10 was independently associated with lower blood pressure (partial-$r = -0.129$, $p = 0.001$), showing decreased GMV in the lateral cerebellum, including bilateral Crus I, part of Crus II, area VI, VII-b, and VIII-a.

Subsample analysis

Considering physical activity in the subgroup of 516 subjects, no independent significant associations with the GM networks were found. Additionally, controlling for physical activity did not change the pattern of above-described effects of cardiovascular risk factors and GM networks, as well as when excluding women on estrogen replacement therapy ($n = 19$) (data not shown).

In participants with anti-hypertensive medication ($n = 325$), higher blood pressure was associated with
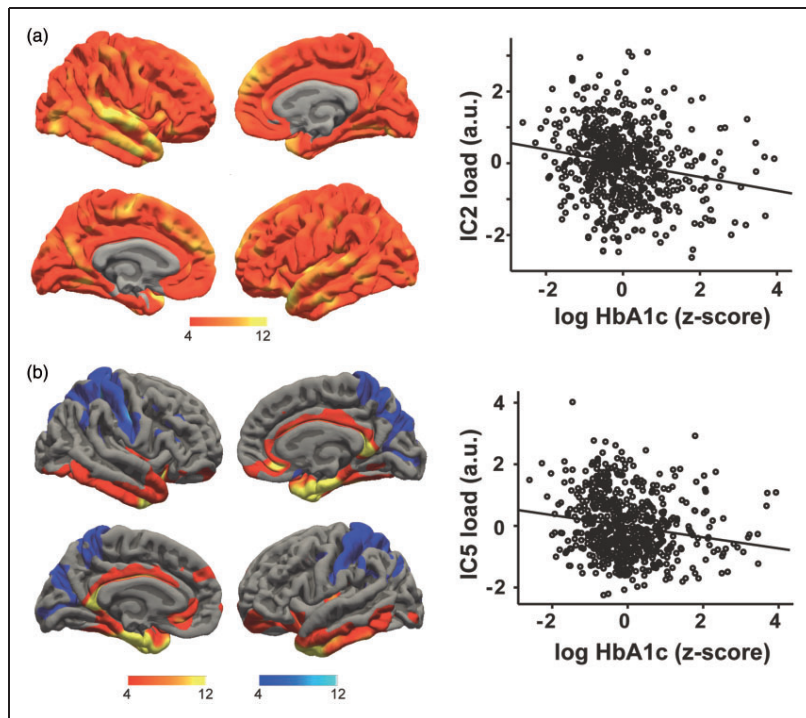
Figure 4. Higher fasting serum levels of HbA1c were associated with lower cortical thickness of IC2 (a) and IC5 (b). Scatter plots show the individual's loading on each network (black dots) and linear fit. Colors indicate positive (red/yellow) or negative (blue/light-blue) co-variations within the network (z > 4), maps are drawn on a standard brain.

lower GM volume in both IC7 (partial-$r = -0.176$, $p = 0.002$) and IC10 (partial-$r = -0.139$, $p = 0.013$). In those without anti-hypertensive medication ($n = 291$), however, associations did not reach significance. In participants without anti-diabetic medication ($n = 534$), but not in those with ($n = 82$), higher HbA1c, similar to the whole sample analysis, was negatively associated with IC2 (partial-$r = -0.159$, $p < 10^{-3}$) and IC5 (partial-$r = -0.230$, $p < 10^{-3}$)

Discussion

In this large cross-sectional study, we identified unique associations of cardiovascular risk factors, independent from confounders, on major structural covariance brain networks in a well-characterized cohort of 616 healthy older adults. In two age-associated networks that covered most cortical areas, we detected lower GM volume and thickness in smokers, in participants with higher blood pressure, and in those with higher long-term glucose. Also, WHR was associated with lower GM volume in a multimodal, age- and memory-sensitive network, known to be affected in both normal aging and AD.[3,40]

Smoking

We observed a significant negative association between smoking and global networks IC2 and IC7, pointing to a negative impact of smoking throughout all areas of the neocortex. Our findings are in line with recent results of Karama,[15] which linked smoking to widespread cortical thinning in a similarly large sample of older individuals ($n = 504$). Considering the pattern of GM covariance, these networks could be indicative of ubiquitous neuronal properties that are affected by smoking.[41] This could be due to direct and indirect toxic effects of cigarette smoking, for example as shown in rodents after prenatal exposure to nicotine,[42] or in humans with regard to chronic effects of cigarette smoking on cerebral perfusion.[43]

Blood pressure

Our results indicate that higher blood pressure exerts negative effects on GM volume in networks that covered most parts of the neocortex and cerebellum, with stronger associations in subjects taking anti-hypertensive medication. There is consistent evidence that
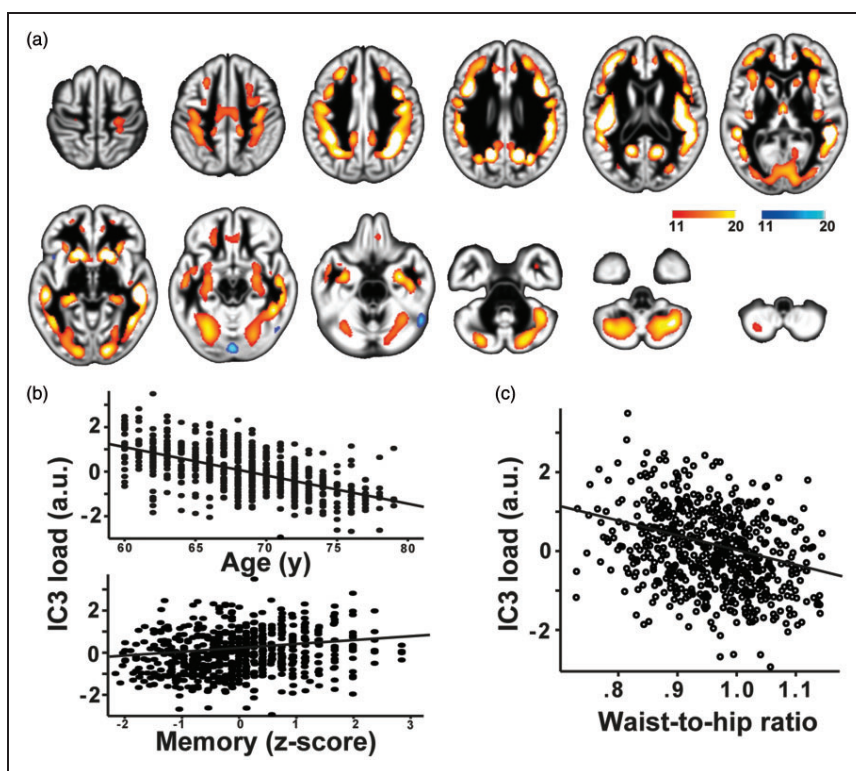
Figure 5. Higher waist-to-hip ratio was associated with lower gray matter volume in a network of multimodal regions (IC3, a, c) that also correlated negatively with age and memory performance (b). Scatter plot shows the individual's loading on the network and linear fit. Colors indicate positive (red/yellow) or negative (blue/light-blue) co-variations within the network ($z > 11$), maps are drawn on a standard brain.

higher blood pressure in mid-life is a risk factor for cognitive decline and AD,[44] and medication intake could indicate a prolonged period of elevated blood pressure, leading to stronger effects of high blood pressure in this group. A meta-analysis of neuroimaging findings concluded that high blood pressure leads to lower GM volume, particularly in frontal and temporal lobes.[13] Possible underlying mechanisms include cerebrovascular lesions due to chronic high blood pressure and GM loss.[45]

Glucose metabolism

Higher concentrations of the long-term marker of glucose metabolism, HbA1c, were associated with two networks of covariance mainly in cortical thickness. Considering the additive nature of components in our analysis, this indicates a negative impact of higher glucose on most parts of the neocortex with stronger effects in medial frontal, cingulate and temporal areas, in line with previous reports in young.[46] Due to

neurotoxic effects of glucose and accumulation of advanced glycation end products (AGEs),[47] persistent episodes of hyperglycemia might have led to GM damage in subjects with higher long-term glucose. It could be speculated that regions of higher metabolism in young and higher Aß accumulation in older cognitively normal subjects[48] would show stronger correlations; however, future studies that for example implement AGE-receptor-PET[49] are needed to expand on these speculations.

Visceral obesity

We found an independent association of higher WHR and lower GM volume in IC3, covering multimodal cortices at the gray-to-white matter border as well as limbic regions. This finding is in line with previous studies that showed negative effects of obesity on regional and total GM volume in older cohorts,[31,50] and extends previous reports that observed more severe changes when looking at visceral obesity measures in

comparison to "crude" BMI.[51] This might be due to the more severe negative effects of visceral adipose tissue compared to (gluetal-) subcutaneous fat, including a higher expression of proinflammatory cytokines.[52] Chronic low-grade inflammation has been speculated to particularly harm myelination, thus affecting white matter tracts and intra-cortical axon collaterals.[53] The network depicted by IC3 in our cohort has been previously linked to AD and described to display a "last-in-first-out" trajectory.[3,40] These effects had been further ascribed to the myelination process of intracortical fibers depicted by the network.[3] Therefore, our data-driven analysis supports the hypothesis that higher visceral fat, possibly through higher inflammatory activity, exerts detrimental effects on the late-myelinated GM. However, future studies combining imaging techniques capable of quantifying myelin content and AD pathology as well as more detailed measures of visceral fat-related inflammation are needed to test this hypothesis.

Lipid markers and physical activity

We did not observe robust association between markers of lipid metabolism or physical activity and GM networks. In our sample of healthy elderly, we observed higher HDL levels and far less individuals on anti-hyperlipidemic medication compared to others,[16,54,55] rendering a low sensitivity to detect HDL-effects on GM structure in our cohort likely. Considering physical activity, longitudinal studies including older adults observed protective effects on GM volume and thickness,[55–57] raising the possibility that our cross-sectional questionnaire (IPAQ short version) might not have been sensitive enough to capture these effects.[58] Furthermore, accumulated evidence suggests a positive impact of leisure activities on cognitive function and lowered risk of AD.[59,60] Specifically, in an elderly population, such as ours, use of standardized leisure activity questionnaire might better depict factors with possible beneficial effect on brain aging.

Limitations

We are unable to infer causal relationships due to the cross-sectional nature of our study, thus we cannot exclude that changes in GM structure were prior to the differences in cardiovascular risk factors. However, it has been suggested that modifiable risk factors at middle-age are a better predictor of structural decline and cognitive outcomes in later life,[61] which potentially imply even stronger associations of risk factors on GM networks than seen here. The effects of smoking in our cohort could have been underestimated as result of cortical recovery after quitting smoking[15]

that might have led to a higher variance within our "previous smoker" group. Despite possible effects of further factors linked with higher cardiovascular risk, such as low "cognitive reserve" or depressive illness, on GM measures,[62–65] we did not evaluate these conditions in detail. Strengths of the study include the large population-based sample and the data-driven multi-modal analysis of GM networks, instead of focusing on traditional voxel-wise associations in one modality. This systems-view could increase the interpretability of the effects in older populations on the brain, especially with regard to underlying mechanisms and potential preventive options.[1,7,8]

Conclusions

Using a large cohort of healthy older adults and a data-driven approach, we were able to replicate and further characterize large-scale, age-sensitive GM networks that inversely correlated with major cardiovascular risk factors, i.e. smoking, blood pressure, long-term glucose, and visceral obesity. The spatial extent and composition of covarying GM measures within the different networks indicated that smoking and, to a lesser degree, higher blood pressure affected GM throughout the brain, which might be attributed to direct and indirect damage of neuronal tissue. Higher HbA1c was found to predominantly affect areas that are known to have high glucose metabolism and early Abeta deposition. In addition, we detected negative effects of visceral obesity on a structural network covering areas rich in intracortical myelinated fibres, possibly pointing to detrimental effects of visceral fat-induced low-grade inflammation on myelin. This proposed mechanism might also help to better understand how a cardiovascular risk factor, in this case WHR, could be a trigger or booster of cognitive decline and regional AD pathology, as this network has specifically been linked to accelerated aging and vulnerability to AD in previous studies.[3] Our additional observation of a negative correlation of both age and memory performance with IC3 further underlines the congruency and the functional relevance of this specific network. Future longitudinal studies including the LIFE follow-up data (starting in August 2017) or those that incorporate more detailed microstructural assessments are now needed to prove our hypotheses and to test if improving cardiovascular risk, specifically visceral obesity, would help to maintain the integrity of GM networks sensitive to aging throughout old age.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

SKM conducted data analysis, statistical analysis, and was involved in the interpretation of results and writing of the manuscript. ML, MLS, SGR-H, MS and TL were involved in study design, data collection and revision of the manuscript. FB and LL contributed to data analysis and manuscript revision. AV was involved in study design and revision of the manuscript. AVW contributed to analysis and interpretation of the data and writing of the manuscript.

Supplementary material

Supplementary material for this paper can be found at the journal website: http://journals.sagepub.com/home/jcb

References

1. Alexander-Bloch A, Giedd JN and Bullmore E. Imaging structural co-variance between human brain regions. *Nat Rev Neurosci* 2013; 14: 322–336.
2. Brickman AM, Habeck C, Ramos MA, et al. A forward application of age associated gray and white matter networks. *Hum Brain Mapp* 2008; 29: 1139–1146.
3. Douaud G, Groves AR, Tamnes CK, et al. A common brain network links development, aging, and vulnerability to disease. *Proc Natl Acad Sci U S A* 2014; 111: 201410378.
4. Evans AC. Networks of anatomical covariance. *Neuroimage* 2013; 80: 489–504.
5. Raji CA, Lopez OL, Kuller LH, et al. White matter lesions and brain gray matter volume in cognitively normal elders. *Neurobiol Aging* 2012; 33: 834.e7–834.e16.
6. Zhou J, Gennatas ED, Kramer JH, et al. Predicting regional neurodegeneration from the healthy brain functional connectome. *Neuron* 2012; 73: 1216–1227.
7. Bartzokis G. Alzheimer's disease as homeostatic responses to age-related myelin breakdown. *Neurobiol Aging* 2011; 32: 1341–1371.
8. Jagust W. Vulnerable neural systems and the borderland of brain aging and neurodegeneration. *Neuron* 2013; 77: 219–234.
9. Raz N. Ageing and the Brain. *Encycl Life Sci* 2005; 1–6. DOI: 10.1038/npg.els.0004063.
10. Seeley WW, Crawford RK, Zhou J, et al. Neurodegenerative diseases target large-scale human brain networks. *Neuron* 2009; 62: 42–52.
11. Wyss-Coray T. Ageing, neurodegeneration and brain rejuvenation. *Nature* 2016; 539: 180–186.
12. Friedman JI, Tang CY, de Haas HJ, et al. Brain imaging changes associated with risk factors for cardiovascular and cerebrovascular disease in asymptomatic patients. *JACC Cardiovasc Imaging* 2014; 7: 1039–1053.
13. Beauchet O, Celle S, Roche F, et al. Blood pressure levels and brain volume reduction: a systematic review and meta-analysis. *J Hypertens* 2013; 31: 1502–1516.
14. Biessels GJ, De Leeuw F-E, Lindeboom J, et al. Increased cortical atrophy in patients with Alzheimer's disease and type 2 diabetes mellitus. *J Neurol Neurosurg Psychiatry* 2006; 77: 304–307.
15. Karama S, Ducharme S, Corley J, et al. Cigarette smoking and thinning of the brain's cortex. *Mol Psychiatry* 2015; 20: 778–785.
16. Villeneuve S, Reed BR, Madison CM, et al. Vascular risk and Aβ interact to reduce cortical thickness in AD vulnerable brain regions. *Neurology* 2014; 83: 40–47.
17. Miller KL, Alfaro-Almagro F, Bangerter NK, et al. Multimodal population brain imaging in the UK Biobank prospective epidemiological study. *Nat Neurosci* 2016; 19: 1523–1536.
18. Pencina MJ, D'Agostino RB, Larson MG, et al. Predicting the 30-year risk of cardiovascular disease: the framingham heart study. *Circulation* 2009; 119: 3078–3084.
19. Walhovd KB, Storsve AB, Westlye LT, et al. Blood markers of fatty acids and vitamin D, cardiovascular measures, body mass index, and physical activity relate to longitudinal cortical thinning in normal aging. *Neurobiol Aging* 2014; 35: 1055–1064.
20. Harada CN, Natelson Love MC and Triebel KL. Normal cognitive aging. *Clin Geriatr Med* 2013; 29: 737–752.
21. Loeffler M, Engel C, Ahnert P, et al. The LIFE-Adult-Study: objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. *BMC Public Health* 2015; 15: 691.
22. Griswold MA, Jakob PM, Heidemann RM, et al. Generalized autocalibrating partially parallel acquisitions (GRAPPA). *Magn Reson Med* 2002; 47: 1202–1210.
23. Douaud G, Smith S, Jenkinson M, et al. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain* 2007; 130: 2375–2386.
24. Good CD, Johnsrude IS, Ashburner J, et al. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 2001; 14: 21–36.
25. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 2004; 23(Suppl 1): S208–S219.

26. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002; 33: 341–355.

27. Groves AR, Smith SM, Fjell AM, et al. Benefits of multimodal fusion analysis on a large-scale dataset: life-span patterns of inter-subject variability in cortical morphometry and white matter microstructure. *Neuroimage* 2012; 63: 365–380.

28. Groves AR, Beckmann CF, Smith SM, et al. Linked independent component analysis for multimodal data fusion. *Neuroimage* 2011; 54: 2198–2217.

29. Hagströmer M, Oja P and Sjöström M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutr* 2007; 9: 755–762.

30. Morris JC, Heyman A, Mohs RC, et al. The consortium to establish a registry for Alzheimer's disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology* 1989; 39: 1159–1165.

31. Kharabian Masouleh S, Arélin K, Horstmann A, et al. Higher body mass index in older adults is associated with lower gray matter volume: implications for memory performance. *Neurobiol Aging* 2016; 40: 1–10.

32. van de Rest O, Geleijnse JM, Kok FJ, et al. Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. *Neurology* 2008; 71: 430–438.

33. Lewinsohn PM, Seeley JR, Roberts RE, et al. Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. *Psychol Aging* 1997; 12: 277–87.

34. UNESCO. *International standard classification of education ISCED 1997*. Geneva,Switzerland: Author, 1997.

35. Allen EA, Erhardt EB, Damaraju E, et al. A baseline for the multivariate comparison of resting-state networks. *Front Syst Neurosci* 2011; 5: 2.

36. Chuang Y-F, Eldreth D, Erickson KI, et al. Cardiovascular risks and brain function: a functional magnetic resonance imaging study of executive function in older adults. *Neurobiol Aging* 2014; 35: 1396–1403.

37. Fazekas F, Kleinert R, Offenbacher H, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* 1993; 43: 1683–1689.

38. Erickson KI, Colcombe SJ, Elavsky S, et al. Interactive effects of fitness and hormone treatment on brain health in postmenopausal women. *Neurobiol Aging* 2007; 28: 179–185.

39. Henderson VW, Paganini-Hill A, Emanuel CK, et al. Estrogen replacement therapy in older women. *Arch Neurol* 1994; 51: 896.

40. Fjell AM, Westlye LT, Grydeland H, et al. Accelerating cortical thinning: unique to dementia or universal in aging? *Cereb Cortex* 2014; 24: 919–934.

41. Durazzo TC, Meyerhoff DJ, Mon A, et al. Chronic cigarette smoking in healthy middle-aged individuals is associated with decreased regional brain N-acetylaspartate and glutamate levels. *Biol Psychiatry* 2016; 79: 481–488.

42. Mychasiuk R, Muhammad A, Gibb R, et al. Long-term alterations to dendritic morphology and spine density associated with prenatal exposure to nicotine. *Brain Res* 2013; 1499: 53–60.

43. Durazzo T, Meyerhoff D and Murray D. Comparison of regional brain perfusion levels in chronically smoking and non-smoking adults. *Int J Environ Res Public Health* 2015; 12: 8198–8213.

44. Norton S, Matthews FE, Barnes DE, et al. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol* 2014; 13: 788–794.

45. Veglio F, Paglieri C, Rabbia F, et al. Hypertension and cerebrovascular damage. *Atherosclerosis* 2009; 205: 331–341.

46. Weinstein G, Maillard P, Himali JJ, et al. Glucose indices are associated with cognitive and structural brain measures in young adults. *Neurology* 2015; 84: 2329–2337.

47. Pugazhenthi S, Qin L and Reddy PH. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta* 2017; 1863: 1037–1045.

48. Oh H, Madison C, Baker S, et al. Dynamic relationships between age, amyloid-β deposition, and glucose metabolism link to the regional vulnerability to Alzheimer's disease. *Brain* 2016; 139: 2275–2289.

49. Cary BP, Brooks AF, Fawaz MV, et al. Synthesis and evaluation of [18F]RAGER: a first generation small-molecule PET radioligand targeting the receptor for advanced glycation endproducts. *ACS Chem Neurosci* 2016; 7: 391–398.

50. Willette AA and Kapogiannis D. Does the brain shrink as the waist expands? *Ageing Res Rev* 2015; 20: 86–97.

51. Debette S, Beiser A, Hoffmann U, et al. Visceral fat is associated with lower brain volume in healthy middle-aged adults. *Ann Neurol* 2010; 68: 136–144.

52. Swarbrick MM. A lifetime on the hips: programming lower-body fat to protect against metabolic disease. *Diabetes* 2014; 63: 3575–3577.

53. Cardoso FL, Herz J, Fernandes A, et al. Systemic inflammation in early neonatal mice induces transient and lasting neurodegenerative effects. *J Neuroinflammation* 2015; 12: 82.

54. Leritz EC, Salat DH, Williams VJ, et al. Thickness of the human cerebral cortex is associated with metrics of cerebrovascular health in a normative sample of community dwelling older adults. *Neuroimage* 2011; 54: 2659–2671.

55. Walhovd KB, Storsve AB, Westlye LT, et al. Blood markers of fatty acids and vitamin D, cardiovascular measures, body mass index, and physical activity relate to longitudinal cortical thinning in normal aging. *Neurobiol Aging* 2014; 35: 1055–1064.

56. Benedict C, Brooks SJ, Kullberg J, et al. Association between physical activity and brain health in older adults. *Neurobiol Aging* 2013; 34: 83–90.

57. Flöel A, Ruscheweyh R, Krüger K, et al. Physical activity and memory functions: are neurotrophins and cerebral gray matter volume the missing link? *Neuroimage* 2010; 49: 2756–2763.

58. Heesch KC, van Uffelen JG, Hill RL, et al. What do IPAQ questions mean to older adults? Lessons from cognitive interviews. *Int J Behav Nutr Phys Act* 2010; 7: 35.

59. Wang HX, Xu W and Pei JJ. Leisure activities, cognition and dementia. *Biochim Biophys Acta* 2012; 1822: 482–491.
60. Verghese J, Lipton RB, Katz MJ, et al. Leisure activities and the risk of dementia in the elderly. *N Engl J Med* 2003; 348: 2508–2516.
61. Debette S, Seshadri S, Beiser A, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology* 2011; 77: 461–468.
62. Stern Y. Cognitive reserve in ageing and Alzheimer's disease. *Lancet Neurol* 2012; 11: 1006–1012.
63. Grieve SM, Korgaonkar MS, Koslow SH, et al. Widespread reductions in gray matter volume in depression. *Neuroimage Clin* 2013; 3: 332–339.
64. Benedict C, Byberg L, Cedernaes J, et al. Self-reported sleep disturbance is associated with Alzheimer's disease risk in men. *Alzheimers Dement* 2015; 11: 1090–1097.
65. Mander BA, Rao V, Lu B, et al. Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging. *Nat Neurosci* 2013; 16: 357–364.

3. Summary

Dissertation zur Erlangung des akademischen Grades Dr. rer. med.

Cardiovascular risk factors in ageing brains: Functional and structural correlates of modifiable risk factors of brain ageing and Alzheimer's disease among older individuals

Eingereicht von: Shahrzad Kharabian Masouleh

Angefertigt am: Max-Planck-Institut für Kognitions- und
                    Neurowissenschaften, Abteilung für Neurologie, Leipzig

Betreut von: Prof. Dr. med. Arno Villringer

                Dr. A. Veronica Witte

March 2018

Due to a world-wide demographic change ageing-associated complications including cognitive impairments and neurodegenerative diseases such as dementia are becoming increasingly prevalent. In 2015, almost 47 million people worldwide were estimated to be affected by dementia, and the numbers are expected to reach 75 million by 2030, and 131 million by 2050, with the greatest increase expected in low-income and middle-income countries (Prince, M.; Wimo, A.; Guerchet, M.; Ali, G.; Wu, Y.; Prina, 2015). As no cure or substantial symptom-relieving treatment is yet available for these ever growing pathologic conditions, identifying modifiable factors that causally impact the risk of these diseases has become an important mission (Barnes and Yaffe, 2011).

Although age is known to be the most important risk factor for these conditions, not all older individuals develop these pathologic states and pathologic neurodegenerative changes are not considered as part of a normal aging process. However, observations

show that almost all aged brains show characteristic changes that are linked to neurodegeneration (Wyss-Coray, 2016). These observations raise the possibility that fundamental mechanisms of ageing may display early disease changes or contribute to the pathogenesis of neurodegenerative disorders (Bartzokis, 2011; Bishop et al., 2010; Raz, 2005). A better understanding of possible modulators of function and structure of brain in regions that are known to be vulnerable in aging would thus open a novel window towards targets for intervention of disease progression.

Epidemiological studies have begun to identify many environmental and genetic risk factors that influence prevalence of neurodegenerative diseases in older ages. Importantly, with respect to Alzheimer's disease (AD), conditions such as depression, obesity and hypertension, specifically in midlife and diabetes are shown to independently affect increased prevalence of AD worldwide. In 2010, fifteen thousand AD-cases world-wide were attributed to cigarette smoking and low physical or mental activity (Norton et al., 2014). Moreover, disadvantageous metabolic profiles such as higher blood glucose levels or lower high-density lipoprotein (HDL) levels have also been associated with worse cognition, brain alterations in AD-vulnerable regions and ultimately increased likelihood of developing AD in older ages (Crane et al., 2013; Villeneuve et al., 2014).

In the first study of this thesis, we reviewed the epidemiological evidence regarding the impact of a "Mediterranean style diet" (MeDi) on brain health in aging (Huhn et al., 2015). MeDi, which is based on high consumption of fruits, vegetables, grains as well as sea-fish and low intake of sweets, convenient food, meat and dairy products, is shown to reduce cardio-vascular risk factors and benefit lipid and glucose metabolism while reducing risk of AD and cognitive dysfunction in aging.

Despite extensive epidemiological evidence, little is known about neurobiological

mechanisms, linking these life-style and health related factors to alterations in cognitive performance and incidence of AD.

In the recent years whole brain magnetic resonance (MR) measurements have immensely increased our knowledge about the brain in health and disease. Novel MR protocols and analysis routines have been invented to assess different aspects of structure of the brain regions and their function within the living individuals.

Studies in elderly AD patients have linked deposition of amyloid plaques, assessed using positron emission tomography (PET), in vulnerable structures such as frontal lobe, medial temporal structures and posterior cingulate area to atrophy and lower metabolic rate of glucose within these brain regions in association with accelerated cognitive decline (Buckner et al., 2005).

Also, within healthy ageing population it has been shown that these AD-prone structures create a network, in which grey matter (GM) volume follow a different ageing trajectory compared to the rest of the brain, with a late development during adolescence and accelerated decline in older ages (Douaud et al., 2014; Fjell et al., 2014). Such coordinated change, specifically in older ages, might be a result of shared susceptibility of regions within this network to selective pathologies or a network-based spread of toxic agents (Zhou et al., 2012).

Consequently, the above-mentioned AD-risk factors could through similar mechanisms impact brain structures within vulnerable regions, resulting in accelerated ageing, possibly reducing resilience of these regions towards AD-related pathology and thus increasing risk of developing AD in older ages. Based on this working hypothesis, in the rest of this doctoral research we investigate cerebral correlates of these risk factors and their impact on cognitive performance in healthy older adults.

We initially focused on obesity as a major epidemic of the twentieth century, a major component of metabolic syndrome and an important AD-risk factor. Here we used

conventional techniques to identify effects of Body-mass index (BMI) on regional GM volume (n = 617) as well as resting-state network connectivity (n = 712) and relations to cognitive performance in well-characterized samples of community-dwelled older adults (60-80 years) from Leipzig Research Centre for Civilization Diseases (LIFE) adult-study. The LIFE-Adult-Study is a population-based cohort study, which has already completed the baseline examination of 10,000 randomly selected participants from Leipzig, out of which ~2600 underwent a 3 Tesla MRI brain scan, structured interviews, neuropsychological tests, and an extensive set of medical assessments (Loeffler et al., 2015).

Our results showed that independent of age and a wide range of other confounding factors such as diabetes, hypertension, smoking status and APOE-genotype, there is a robust linear association between a higher BMI and lower GM volume in multiple brain regions, including (pre)frontal, temporal, insular and occipital cortex, thalamus, putamen, amygdala and cerebellum, which partially mediated negative effects of higher BMI on memory performance in our sample of older adults (Kharabian Masouleh et al., 2016).

Furthermore, in the follow-up study, we found reproducible association between higher BMI and lower functional connectivity of the posterior cingulate cortex with other nodes of the default mode network (Beyer et al., 2017). This network that consists of AD-prone regions within frontal, temporal and parietal lobes, exhibits similar alterations in normal ageing and among patients with AD (Damoiseaux et al., 2012; Tomasi and Volkow, 2012).

Inspired by our results on network-based functional connectivity alterations and in-line with the hypothesis of network-based spread of toxic agents in neurodegenerative diseases, in our third MRI-study, we extended the number of risk factors to cover major "modifiable" risk factors of AD and identified the potential impact of these factors on

morphological properties of large-scale structural covariance networks (Kharabian Masouleh et al., 2017). We therefore systematically assessed independent effects of obesity, smoking, blood pressure, as well as markers of glucose and lipid metabolism and physical activity on major GM networks in the same cohort as our first MR study. Furthermore, we detailed our analysis by adding both BMI as well as waist-to-hip ratio as measures of obesity and identified the structural networks based on information on area, thickness and volume of cortical structures.

The spatial extent and composition of the co-varying GM measures within the different networks indicated that smoking and, to a lesser degree, higher blood pressure affected GM throughout the brain, which might be attributed to direct and indirect damage of neuronal tissue. Higher glycosylated hemoglobin, as a long-term marker of glucose metabolism, was found to predominantly affect areas that are known to have high glucose metabolism and early A-beta deposition. In addition, we detected negative effects of visceral obesity on a structural network consisting of multimodal regions, covering areas rich in intracortical myelinated fibres. This network spatially recapitulated the pattern of brain atrophy observed in Alzheimer's disease and has been previously shown to develop relatively slowly during adolescence but present "accelerated" age-related degeneration at an old age. Accordingly, our findings possibly point towards detrimental effects of visceral fat-induced low-grade inflammation on myelin. This is a hypothesis that we are going to test in our future studies in LIFE (by direct assessment of visceral fat (VAT) on abdominal MRI and inflammatory markers).

Future longitudinal studies that incorporate more detailed microstructural assessments are now needed to prove our proposed neurobiological hypotheses on the underlying mechanisms of the observed effects and to test if improving cardiovascular risk,

specifically visceral obesity, would help to maintain the integrity of GM networks throughout old age and reduce the risk of AD.

Articles included in this thesis:

"Components of a Mediterranean diet and their impact on cognitive functions in aging", by Sebastian Huhn, Shahrzad Kharabian Masouleh, Michael Stumvoll, Arno Villringer, A. Veronica Witte, (Frontiers in Aging Neuroscience, 2015).

"Higher body mass index in older adults is associated with lower gray matter volume: Implications for memory performance", by Shahrzad Kharabian Masouleh, Katrin Arélin, Annette Horstmann, Leonie Lampe, Judy A. Kipping, Tobias Luck, Steffi G. Riedel-Heller, Matthias L. Schroeter, Michael Stumvoll, Arno Villringer, A. Veronica Witte, (neurobiology of ageing, April 2016).

"Higher body mass index is associated with reduced default mode connectivity in older adults", Frauke Beyer, Shahrzad Kharabian Masouleh, Julia M. Huntenburg, Leonie Lampe, Tobias Luck, Steffi G. Riedel-Heller, Markus Loeffler, Matthias L. Schroeter, Michael Stumvoll, Arno Villringer, A. Veronica Witte, (Human Brain Mapping, 2017).

"Cardiovascular risk factors are associated with grey matter structural networks in ageing", by Shahrzad Kharabian Masouleh, Frauke Beyer, Leonie Lampe, Markus Loeffler, Tobias Luck, Steffi G. Riedel-Heller, Matthias L. Schroeter, Michael Stumvoll, Arno Villringer, A. Veronica Witte, (journal of cerebral blood flow and metabolism, 2017).

4. References

Alexander-Bloch, A., Giedd, J.N., Bullmore, E., 2013. Imaging structural co-variance between human brain regions. Nat. Rev. Neurosci. 14, 322–36. doi:10.1038/nrn3465

Andrews-Hanna, J.R., Snyder, A.Z., Vincent, J.L., Lustig, C., Head, D., Raichle, M.E., Buckner, R.L., 2007. Disruption of Large-Scale Brain Systems in Advanced Aging. Neuron 56, 924–935. doi:10.1016/j.neuron.2007.10.038

Anstey, K.J., Cherbuin, N., Budge, M., Young, J., 2011. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obes. Rev. 12, e426-37. doi:10.1111/j.1467-789X.2010.00825.x

Barnes, D.E., Yaffe, K., 2011. The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol. 10, 819–28. doi:10.1016/S1474-4422(11)70072-2

Bartzokis, G., 2011. Alzheimer's disease as homeostatic responses to age-related myelin breakdown. Neurobiol. Aging 32, 1341–71. doi:10.1016/j.neurobiolaging.2009.08.007

Bell, A.J., Sejnowski, T.J., 1995. An information-maximization approach to blind separation and blind deconvolution. Neural Comput. 7, 1129–1159. doi:doi:10.1162/neco.1995.7.6.1129

Benito-León, J., Mitchell, A.J., Hernández-Gallego, J., Bermejo-Pareja, F., 2013. Obesity and impaired cognitive functioning in the elderly: a population-based cross-sectional study (NEDICES). Eur. J. Neurol. 20, 899–906, e76-7. doi:10.1111/ene.12083

Beydoun, M.A., Beydoun, H.A., Wang, Y., 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obes. Rev. 9, 204–18. doi:10.1111/j.1467-789X.2008.00473.x

Beyer, F., Kharabian Masouleh, S., Huntenburg, J.M., Lampe, L., Luck, T., Riedel-Heller, S.G., Loeffler, M., Schroeter, M.L., Stumvoll, M., Villringer, A., Witte, A.V., 2017. Higher body mass index is associated with reduced posterior default mode connectivity in older adults. Hum. Brain Mapp.

doi:10.1002/hbm.23605

Bishop, N.A., Lu, T., Yankner, B.A., 2010. Neural mechanisms of ageing and cognitive decline. Nature 464, 529–535. doi:10.1038/nature08983

Biswal, B., Yetkin, F.Z., Haughton, V.M., Hyde, J.S., 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn. Reson. Med. 34, 537–41.

Brickman, A.M., Habeck, C., Ramos, M.A., Scarmeas, N., Stern, Y., 2008. A forward application of age associated gray and white matter networks. Hum. Brain Mapp. 29, 1139–1146. doi:10.1002/hbm.20452

Brickman, A.M., Habeck, C., Zarahn, E., Flynn, J., Stern, Y., 2007. Structural MRI covariance patterns associated with normal aging and neuropsychological functioning. Neurobiol. Aging 28, 284–95. doi:10.1016/j.neurobiolaging.2005.12.016

Buchman, A., Boyle, P., Yu, L., Shah, R., 2012. Total daily physical activity and the risk of AD and cognitive decline in older adults. Neurology 1323–1329.

Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L., 2008. The brain's default network: Anatomy, function, and relevance to disease. Ann. N. Y. Acad. Sci. doi:10.1196/annals.1440.011

Buckner, R.L., Snyder, A.Z., Shannon, B.J., LaRossa, G., Sachs, R., Fotenos, A.F., Sheline, Y.I., Klunk, W.E., Mathis, C.A., Morris, J.C., Mintun, M.A., 2005. Molecular, Structural, and Functional Characterization of Alzheimer's Disease: Evidence for a Relationship between Default Activity, Amyloid, and Memory. J. Neurosci. 25, 7709–7717. doi:10.1523/JNEUROSCI.2177-05.2005

Calhoun, V.D., Adali, T., Pearlson, G.D., Pekar, J.J., 2001. A method for making group inferences from functional MRI data using independent component analysis. Hum. Brain Mapp. 14, 140–151. doi:10.1002/hbm.1048

Colcombe, S.J., Erickson, K.I., Scalf, P.E., Kim, J.S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D.X., Hu, L., Kramer, A.F., 2006. Aerobic Exercise Training Increases Brain Volume in Aging Humans. Journals Gerontol. Ser. A Biol. Sci. Med. Sci. 61, 1166–1170. doi:10.1093/gerona/61.11.1166

Crane, P.K., Walker, R., Hubbard, R. a, Li, G., Nathan, D.M., Zheng, H., Haneuse, S., Craft, S., Montine, T.J., Kahn, S.E., McCormick, W., McCurry, S.M., Bowen, J.D., Larson, E.B., 2013. Glucose levels and risk of dementia. N. Engl. J. Med. 369, 540–8. doi:10.1056/NEJMoa1215740

Damoiseaux, J.S., Beckmann, C.F., Arigita, E.J.S., Barkhof, F., Scheltens, P., Stam, C.J., Smith, S.M., Rombouts, S.A.R.B., 2007. Reduced resting-state brain activity in the "default network" in normal aging. Cereb. Cortex 18, 1856–1864.

Damoiseaux, J.S., Prater, K.E., Miller, B.L., Greicius, M.D., 2012. Functional connectivity tracks clinical deterioration in Alzheimer's disease. Neurobiol. Aging 33, 828.e19-30. doi:10.1016/j.neurobiolaging.2011.06.024

de Bruijn, R.F.A.G., Schrijvers, E.M.C., de Groot, K.A., Witteman, J.C.M., Hofman, A., Franco, O.H., Koudstaal, P.J., Ikram, M.A., 2013. The association between physical activity and dementia in an elderly population: the Rotterdam Study. Eur. J. Epidemiol. 28, 277–83. doi:10.1007/s10654-013-9773-3

Douaud, G., Groves, A.R., Tamnes, C.K., Westlye, L.T., Duff, E.P., Engvig, A., Walhovd, K.B., James, A., Gass, A., Monsch, A.U., Matthews, P.M., Fjell, A.M., Smith, S.M., Johansen-Berg, H., 2014. A common brain network links development, aging, and vulnerability to disease. Proc. Natl. Acad. Sci. 111, 201410378. doi:10.1073/pnas.1410378111

Elias, M.F., Elias, P.K., Sullivan, L.M., Wolf, P. a, D'Agostino, R.B., 2005. Obesity, diabetes and cognitive deficit: The Framingham Heart Study. Neurobiol. Aging 26 Suppl 1, 11–6. doi:10.1016/j.neurobiolaging.2005.08.019

Emmerzaal, T.L., Kiliaan, A.J., Gustafson, D.R., 2015. 2003-2013: a decade of body mass index, Alzheimer's disease, and dementia. J. Alzheimers. Dis. 43, 739–55. doi:10.3233/JAD-141086

Erhardt, E.B., Rachakonda, S., Bedrick, E.J., Allen, E.A., Adali, T., Calhoun, V.D., 2011. Comparison of multi-subject ICA methods for analysis of fMRI data. Hum. Brain Mapp. 32, 2075–2095. doi:10.1002/hbm.21170

Erickson, K.I., Leckie, R.L., Weinstein, A.M., 2014. Physical activity, fitness, and gray matter volume. Neurobiol. Aging 35, S20–S28.

doi:10.1016/j.neurobiolaging.2014.03.034

Fischl, B., Dale, A.M., 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc. Natl. Acad. Sci. 97, 11050–11055. doi:10.1073/pnas.200033797

Fitzpatrick, A.L., Kuller, L.H., Lopez, O.L., Diehr, P., O'Meara, E.S., Longstreth, W.T., Luchsinger, J.A., 2009. Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Arch. Neurol. 66, 336–42. doi:10.1001/archneurol.2008.582

Fjell, A.M., Amlien, I.K., Sneve, M.H., Grydeland, H., Tamnes, C.K., Chaplin, T.A., Rosa, M.G.P., Walhovd, K.B., 2015. The roots of Alzheimer's disease: Are high-expanding cortical areas preferentially targeted? Cereb. Cortex 25, 2556–2565. doi:10.1093/cercor/bhu055

Fjell, A.M., Walhovd, K.B., 2010. Structural brain changes in aging: courses, causes and cognitive consequences. Rev. Neurosci. 21, 187–221. doi:10.1515/REVNEURO.2010.21.3.187

Fjell, A.M., Westlye, L.T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Dale, A.M., Walhovd, K.B., 2014. Accelerating cortical thinning: unique to dementia or universal in aging? Cereb. Cortex 24, 919–34. doi:10.1093/cercor/bhs379

Flöel, a., Ruscheweyh, R., Krüger, K., Willemer, C., Winter, B., Völker, K., Lohmann, H., Zitzmann, M., Mooren, F., Breitenstein, C., Knecht, S., 2010. Physical activity and memory functions: Are neurotrophins and cerebral gray matter volume the missing link? Neuroimage 49, 2756–2763. doi:10.1016/j.neuroimage.2009.10.043

Foster-Dingley, J.C., Moonen, J.E.F., van den Berg-Huijsmans, A.A., de Craen, A.J.M., de Ruijter, W., van der Grond, J., van der Mast, R.C., 2015. Lower Blood Pressure and Gray Matter Integrity Loss in Older Persons. J. Clin. Hypertens. (Greenwich). 17, 630–637. doi:10.1111/jch.12550

Fotuhi, M., Do, D., Jack, C., 2012. Modifiable factors that alter the size of the hippocampus with ageing. Nat. Rev. Neurol. 8, 189–202. doi:10.1038/nrneurol.2012.27

Friedman, J.I., Tang, C.Y., de Haas, H.J., Changchien, L., Goliasch, G., Dabas, P., Wang, V., Fayad, Z.A., Fuster, V., Narula, J., 2014. Brain

imaging changes associated with risk factors for cardiovascular and cerebrovascular disease in asymptomatic patients. JACC. Cardiovasc. Imaging 7, 1039–53. doi:10.1016/j.jcmg.2014.06.014

Grady, C., 2012. The cognitive neuroscience of ageing. Nat. Rev. Neurosci. 13, 491–505. doi:10.1038/nrn3256

Groves, A.R., Beckmann, C.F., Smith, S.M., Woolrich, M.W., 2011. Linked independent component analysis for multimodal data fusion. Neuroimage 54, 2198–217. doi:10.1016/j.neuroimage.2010.09.073

Gustafson, D., 2006. Adiposity indices and dementia. Lancet. Neurol. 5, 713–20. doi:10.1016/S1474-4422(06)70526-9

Harada, C.N., Natelson Love, M.C., Triebel, K.L., 2013. Normal cognitive aging. Clin. Geriatr. Med. 29, 737–52. doi:10.1016/j.cger.2013.07.002

Huhn, S., Kharabian Masouleh, S., Stumvoll, M., Villringer, A., Witte, a. V., 2015. Components of a Mediterranean diet and their impact on cognitive functions in aging. Front. Aging Neurosci. 7, 1–10. doi:10.3389/fnagi.2015.00132

Karama, S., Ducharme, S., Corley, J., Chouinard-Decorte, F., Starr, J.M., Wardlaw, J.M., Bastin, M.E., Deary, I.J., 2015. Cigarette smoking and thinning of the brain's cortex. Mol. Psychiatry 20, 778–85. doi:10.1038/mp.2014.187

Kerti, L., Witte, a V., Winkler, A., Grittner, U., Rujescu, D., Flöel, A., 2013. Higher glucose levels associated with lower memory and reduced hippocampal microstructure. Neurology 81, 1746–52. doi:10.1212/01.wnl.0000435561.00234.ee

Kharabian Masouleh, S., Arélin, K., Horstmann, A., Lampe, L., Kipping, J.A., Luck, T., Riedel-Heller, S.G., Schroeter, M.L., Stumvoll, M., Villringer, A., Witte, A.V., 2016. Higher body mass index in older adults is associated with lower gray matter volume: implications for memory performance. Neurobiol. Aging. doi:10.1016/j.neurobiolaging.2015.12.020

Kharabian Masouleh, S., Beyer, F., Lampe, L., Loeffler, M., Luck, T., Riedel-Heller, S.G., Schroeter, M.L., Stumvoll, M., Villringer, A., Witte, A.V., 2017. Gray matter structural networks are associated with cardiovascular risk factors in healthy older adults. J. Cereb. Blood Flow Metab. 0271678X1772911. doi:10.1177/0271678X17729111

Kiliaan, A.J., Arnoldussen, I. a C., Gustafson, D.R., 2014. Adipokines: a link between obesity and dementia? Lancet Neurol. 13, 913–923. doi:10.1016/S1474-4422(14)70085-7

Kivimäki, M., Luukkonen, R., Batty, G.D., Ferrie, J.E., Pentti, J., Nyberg, S.T., Shipley, M.J., Alfredsson, L., Fransson, E.I., Goldberg, M., Knutsson, A., Koskenvuo, M., Kuosma, E., Nordin, M., Suominen, S.B., Theorell, T., Vuoksimaa, E., Westerholm, P., Westerlund, H., Zins, M., Kivipelto, M., Vahtera, J., Kaprio, J., Singh-Manoux, A., Jokela, M., 2017. Body mass index and risk of dementia: Analysis of individual-level data from 1.3 million individuals. Alzheimer's Dement. doi:10.1016/J.JALZ.2017.09.016

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., Helkala, E.-L., Tuomilehto, J., Soininen, H., Nissinen, A., 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Arch. Neurol. 62, 1556–60. doi:10.1001/archneur.62.10.1556

Loeffler, M., Engel, C., Ahnert, P., Alfermann, D., Arelin, K., Baber, R., Beutner, F., Binder, H., Brähler, E., Burkhardt, R., Ceglarek, U., Enzenbach, C., Fuchs, M., Glaesmer, H., Girlich, F., Hagendorff, A., Häntzsch, M., Hegerl, U., Henger, S., Hensch, T., Hinz, A., Holzendorf, V., Husser, D., Kersting, A., Kiel, A., Kirsten, T., Kratzsch, J., Krohn, K., Luck, T., Melzer, S., Netto, J., Nüchter, M., Raschpichler, M., Rauscher, F.G., Riedel-Heller, S.G., Sander, C., Scholz, M., Schönknecht, P., Schroeter, M.L., Simon, J.-C., Speer, R., Stäker, J., Stein, R., Stöbel-Richter, Y., Stumvoll, M., Tarnok, A., Teren, A., Teupser, D., Then, F.S., Tönjes, A., Treudler, R., Villringer, A., Weissgerber, A., Wiedemann, P., Zachariae, S., Wirkner, K., Thiery, J., 2015. The LIFE-Adult-Study: objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. BMC Public Health 15, 691. doi:10.1186/s12889-015-1983-z

Logothetis, N.K., 2002. The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. Philos. Trans. R. Soc. B Biol. Sci. 357, 1003–1037. doi:10.1098/rstb.2002.1114

MacDonald, D., Kabani, N., Evans, A.C., Avis, D., 2000. Automated 3-D extraction of inner and outer surfaces of cerebral cortex from MRI.

Neuroimage 12, 340–56. doi:10.1006/nimg.1999.0534

Mccabe, D.P., Henry L. Roediger III, Mcdaniel, M. a, Balota, D.A., Hambrick, D.Z., 2010. The Relationship Between Working Memory Capacity and Executive Functioning: Evidence for a Common Executive Attention Construct. Neuropsychology 24, 222–243. doi:10.1037/a0017619

Miller, M.I., Massie, A.B., Ratnanather, J.T., Botteron, K.N., Csernansky, J.G., 2000. Bayesian construction of geometrically based cortical thickness metrics. Neuroimage 12, 676–87. doi:10.1006/nimg.2000.0666

Moll van Charante, E.P., Richard, E., Eurelings, L.S., van Dalen, J.-W., Ligthart, S.A., van Bussel, E.F., Hoevenaar-Blom, M.P., Vermeulen, M., van Gool, W.A., 2016. Effectiveness of a 6-year multidomain vascular care intervention to prevent dementia (preDIVA): a cluster-randomised controlled trial. Lancet (London, England) 388, 797–805. doi:10.1016/S0140-6736(16)30950-3

Moreno-Gonzalez, I., Estrada, L.D., Sanchez-Mejias, E., Soto, C., 2013. Smoking exacerbates amyloid pathology in a mouse model of Alzheimer's disease. Nat. Commun. 4, 1495. doi:10.1038/ncomms2494

NCD Risk Factor Collaboration, 2016. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet 387, 1377–1396. doi:10.1016/S0140-6736(16)30054-X

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., Bäckman, L., Hänninen, T., Jula, A., Laatikainen, T., Lindström, J., Mangialasche, F., Paajanen, T., Pajala, S., Peltonen, M., Rauramaa, R., Stigsdotter-Neely, A., Strandberg, T., Tuomilehto, J., Soininen, H., Kivipelto, M., 2015. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet. doi:10.1016/S0140-6736(15)60461-5

Norton, S., Matthews, F.E., Barnes, D.E., Yaffe, K., Brayne, C., 2014. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 13, 788–794. doi:10.1016/S1474-4422(14)70136-X

Ogawa, S., Lee, T.M., Nayak, A.S., Glynn, P., 1990. Oxygenation-sensitive

contrast in magnetic resonance image of rodent brain at high magnetic fields. Magn. Reson. Med. 14, 68–78. doi:10.1002/mrm.1910140108

Petrella, J.R., Sheldon, F.C., Prince, S.E., Calhoun, V.D., Doraiswamy, P.M., 2011. Default mode network connectivity in stable vs progressive mild cognitive impairment. Neurology 76, 511–7. doi:10.1212/WNL.0b013e31820af94e

Prickett, C., Brennan, L., Stolwyk, R., 2015. Examining the relationship between obesity and cognitive function: a systematic literature review. Obes. Res. Clin. Pract. 9, 93–113. doi:10.1016/j.orcp.2014.05.001

Prince, M.; Wimo, A.; Guerchet, M.; Ali, G.; Wu, Y.; Prina, M., 2015. Alzheimer's Disease International. World Alzheimer Report 2015 The Global Impact of Dementia. Alzheimer's Disease International (ADI), London.

Qizilbash, N., Gregson, J., Johnson, M.E., Pearce, N., Douglas, I., Wing, K., Evans, S.J.W., Pocock, S.J., 2015. BMI and risk of dementia in two million people over two decades: a retrospective cohort study. Lancet Diabetes Endocrinol. 3, 431–6. doi:10.1016/S2213-8587(15)00033-9

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc. Natl. Acad. Sci. U. S. A. 98, 676–82. doi:10.1073/pnas.98.2.676

Raz, N., 2005. Ageing and the Brain. Encycl. Life Sci. 1–6. doi:10.1038/npg.els.0004063

Raz, N., Gunning-Dixon, F.M., Head, D., Dupuis, J.H., Acker, J.D., 1998. Neuroanatomical correlates of cognitive aging: Evidence from structural magnetic resonance imaging. Neuropsychology 12, 95–114. doi:10.1037//0894-4105.12.1.95

Ruscheweyh, R., Willemer, C., Krüger, K., Duning, T., Warnecke, T., Sommer, J., Völker, K., Ho, H.V., Mooren, F., Knecht, S., Flöel, A., 2011. Physical activity and memory functions: An interventional study. Neurobiol. Aging 32, 1304–1319. doi:10.1016/j.neurobiolaging.2009.08.001

Salat, D.H., Buckner, R.L., Snyder, A.Z., Greve, D.N., Desikan, R.S.R., Busa, E., Morris, J.C., Dale, A.M., Fischl, B., 2004. Thinning of the cerebral cortex in aging. Cereb. Cortex 14, 721–30. doi:10.1093/cercor/bhh032

Salthouse, T.A., Madden, D.J., 2013. Information processing speed and aging, in: Information Processing Speed in Clinical Populations. pp. 221–242. doi:10.4324/9780203783054

Smith, E., Hay, P., Campbell, L., Trollor, J.N., 2011. A review of the association between obesity and cognitive function across the lifespan: implications for novel approaches to prevention and treatment. Obes. Rev. 12, 740–55.

Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., Mackay, C.E., Filippini, N., Watkins, K.E., Toro, R., Laird, A.R., Beckmann, C.F., 2009. Correspondence of the brain's functional architecture during activation and rest. Proc. Natl. Acad. Sci. U. S. A. 106, 13040–5. doi:10.1073/pnas.0905267106

Sorg, C., Riedl, V., Mühlau, M., Calhoun, V.D., Eichele, T., Läer, L., Drzezga, A., Förstl, H., Kurz, A., Zimmer, C., Wohlschläger, A.M., 2007. Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 104, 18760–5. doi:10.1073/pnas.0708803104

Sowell, E.R., Peterson, B.S., Thompson, P.M., Welcome, S.E., Henkenius, A.L., Toga, A.W., 2003. Mapping cortical change across the human life span. Nat. Neurosci. 6, 309–15. doi:10.1038/nn1008

Steffener, J., Brickman, A.M., Habeck, C.G., Salthouse, T.A., Stern, Y., 2013. Cerebral blood flow and gray matter volume covariance patterns of cognition in aging. Hum. Brain Mapp. 34, 3267–79. doi:10.1002/hbm.22142

Tomasi, D., Volkow, N.D., 2012. Aging and functional brain networks. Mol. Psychiatry 17, 549–558. doi:10.1038/mp.2011.81

Vellas, B., Carrie, I., Gillette-Guyonnet, S., Touchon, J., Dantoine, T., Dartigues, J.F., Cuffi, M.N., Bordes, S., Gasnier, Y., Robert, P., Bories, L., Rouaud, O., Desclaux, F., Sudres, K., Bonnefoy, M., Pesce, A., Dufouil, C., Lehericy, S., Chupin, M., Mangin, J.F., Payoux, P., Adel, D., Legrand, P., Catheline, D., Kanony, C., Zaim, M., Molinier, L., Costa, N., Delrieu, J., Voisin, T., Faisant, C., Lala, F., Nourhashémi, F., Rolland, Y., Van Kan, G.A., Dupuy, C., Cantet, C., Cestac, P., Belleville, S., Willis, S., Cesari, M., Weiner, M.W., Soto, M.E., Ousset, P.J., Andrieu, S., 2014.

MAPT STUDY: A MULTIDOMAIN APPROACH FOR PREVENTING ALZHEIMER'S DISEASE: DESIGN AND BASELINE DATA. J. Prev. Alzheimer's Dis. 1, 13–22.

Villeneuve, S., Reed, B.R., Madison, C.M., Wirth, M., Marchant, N.L., Kriger, S., Mack, W.J., Sanossian, N., DeCarli, C., Chui, H.C., Weiner, M.W., Jagust, W.J., 2014. Vascular risk and Aβ interact to reduce cortical thickness in AD vulnerable brain regions. Neurology 83, 40–7. doi:10.1212/WNL.0000000000000550

Walther, K., Birdsill, A.C., Glisky, E.L., Ryan, L., 2010. Structural brain differences and cognitive functioning related to body mass index in older females. Hum. Brain Mapp. 31, 1052–64. doi:10.1002/hbm.20916

West, R.L., 1996. An application of prefrontal cortex function theory to cognitive aging. Psychol. Bull. 120, 272–292. doi:10.1037//0033-2909.120.2.272

Whitmer, R.A., Gunderson, E.P., Barrett-Connor, E., Quesenberry, C.P., Yaffe, K., 2005. Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. BMJ 330, 1360. doi:10.1136/bmj.38446.466238.E0

WHO, 2014. Global status report on noncommunicable diseases 2014. Geneva,Switzerland.

WHO, 2011. Global Health and Aging.

WHO, 2009. Interventions on diet and physical activity: what works/methodology.

Wyss-Coray, T., 2016. Ageing, neurodegeneration and brain rejuvenation. Nature 539, 180–186. doi:10.1038/nature20411

Zhou, J., Gennatas, E.D., Kramer, J.H., Miller, B.L., Seeley, W.W., 2012. Predicting regional neurodegeneration from the healthy brain functional connectome. Neuron 73, 1216–27. doi:10.1016/j.neuron.2012.03.004

A. Supplemental Materials

Publication2- Kharabian Masouleh et. al., 2016

Apolipoprotein E (APOE) genotyping

To assess individual APOE status, we used genomic DNA isolated from peripheral leukocytes using an automate protocol on the Qiagen Autopure LS (Qiagen, Hilden, Germany). DNA purity and yield was determined on a NanoDrop spectrophotometer. Genotyping of the APOE allele status (E2, E3, E4) was performed on a Roche Lightcylcer 480 according to the method of (Aslanidis and Schmitz, 1999).

Voxel Based Morphometry (VBM)

T1-weighted magnetic resonance images (MRIs) were processed using a VBM approach in SPM8 (www.fil.ion.ucl.ac.uk/spm). For VBM analysis of the cerebrum, individual whole brain images were segmented into gray matter maps and co-registered to a study-specific Dartel-template. In order to improve tissue segmentation and inter-individual alignment of the cerebellar lobules, individual cerebella were coregistered to a cerebellum-only atlas template (Diedrichsen, 2006; Diedrichsen et al., 2009). In detail, The following two approaches were performed:

For cerebral VBM, freely available VBM8 package (http://dbm.neuro.uni-jena.de/vbm/) integrated in the SPM8 software with default parameters was used. Whole brain Images were bias-corrected, tissue classified, and preregistered to standardized Montreal Neurological Institute (MNI) space using linear (12-parameter affine) transformations, within a unified model (Ashburner and Friston, 2005). A subgroup of 496 participants equally

matched for gender, age and body mass index (BMI) were used to create a group-specific Dartel template. The segmented images were then warped using high dimensional deformations to the created template. Gray matter segments were modulated by the Jacobian determinant of the deformations to account for local expansion and compression introduced by nonlinear transformation and then smoothed with an isotropic Gaussian kernel of 8mm full width half maximum (FWHM) (Ridgway et al., 2009).

Cerebellar VBM was carried out using Spatially Unbiased Infra-Tentorial (SUIT) toolbox (version 2.5, http://www.icn.ucl.ac.uk/motorcontrol/ imaging/suit.htm) (Diedrichsen, 2006). To ensure that the infratentorial cerebellum was isolated from the surrounding tissue, we used the isolate function within the SUIT toolbox, generating segmentation cerebellum maps. We additionally used freesurfer (version 5.0, https://surfer.nmr.mgh.harvard.edu) generated cerebellar masks to further improve participant-specific isolation function. This minimized the required manual corrections of the isolated maps. In the next step the cropped anatomical images were normalized to the SUIT template and the segmentation maps were modulated and resliced into the SUIT atlas space. Eventually, to preserve precision in the definition of cerebellar structures, a smaller smoothing was used (i.e. 4 mm FWHM isotropic Gaussian kernel (Reetz et al., 2012)). As modulation of the cerebellar gray matter maps included both linear and non-linear effects, a measure of total cerebellar volume (TCV) was estimated from the sum of gray and white matter segments, and then included as a nuisance regressor, in statistics concerning cerebellar gray matter volume (GMV).

Additional statistics

Interaction effects of BMI by sex and BMI by arterial hypertension were investigated in full-factorial models. To test if effects of BMI on GMV were dependent on obesity status, we additionally grouped participants using cut-offs by the World Health Organisation (WHO, 2006) in three groups, i.e. lean (BMI ≤ 25 kg/m², n = 175), overweight (25 kg/m² < BMI ≤ 30 kg/m², n = 277) and obese (BMI > 30 kg/m², n = 165) and tested for an interaction in a separate full factorial model. Note that only one participant of our cohort would fall in an underweight category (i.e. BMI < 18.5, WHO, 2006), this participant was grouped into the lean group. Lastly, we tested if restricting our analysis to participants without diabetes (n = 518) or to those without anti-hyperlipidemic medication (n=480) would result in similar effects of BMI on GMV. These additional analyses have been performed to further demonstrate that effects found with respect to obesity are not due to, or biased by, possible effects of co-morbidities and related medication intake.

Supporting References

Ashburner, J., Friston, K.J., 2005. Unified segmentation. Neuroimage 26, 839–51.

Aslanidis, C., Schmitz, G., 1999. High-Speed Apolipoprotein E Genotyping and Apoli- poprotein B3500 Mutation Detection Using Real-Time Fluorescence PCR and Melting Curves. Clin. Chem. 45, 1094–1097.

Diedrichsen, J., 2006. A spatially unbiased atlas template of the human cerebellum. Neuroimage 33, 127–138.

Diedrichsen, J., Balsters, J.H., Flavell, J., Cussans, E., Ramnani, N., 2009. A probabilistic MR atlas of the human cerebellum. Neuroimage 46, 39–46.

Reetz, K., Dogan, I., Rolfs, A., Binkofski, F., Schulz, J.B., Laird, A.R., Fox, P.T., Eickhoff, S.B., 2012. Investigating function and connectivity of morphometric findings--exemplified on cerebellar atrophy in spinocerebellar ataxia 17 (SCA17). Neuroimage 62, 1354–66.

Ridgway, G.R., Omar, R., Ourselin, S., Hill, D.L.G., Warren, J.D., Fox, N.C., 2009. Issues with threshold masking in voxel-based morphometry of atrophied brains. Neuroimage 44, 99–111.

WHO, 2006. Global Database on Body Mass Index - World Health Organization.

Supplementary Tables for Publication2

Supplementary Table 1. Characteristics of participants included in the main study compared to the subgroup with defined APOE-status, and compared to those participants that were excluded.

		Main sample n = 617	Subgroup with defined APOE genotype n = 485	p (main vs. APOE)	Participants excluded n = 368	p (main vs. excluded)
sex (female)		258 (41.8%)	208 (42.9%)	0.72[1]	181 (49.2%)	0.024[1]
Age (y)		68.7 ± 5 (60-79)	69.5 ± 4 (60-79)	0.0014[2]	69.5 ± 6 (60-79)	0.021[2]
BMI (kg/m²)		27.5 ± 4 (16.8-41)	27.46 ± 4 (16.8-41.3)	0.81[2]	28.4 ± 4.25 (19-46.5)	0.001[2]
Education (%)	no SS-LD	0.5	0.6	0.83[3]	1.1	< 0.001[3]
	SS-LD	10.4	10.5		17.9	
	advanced SS-LD	6.3	6.2		8.4	
	advanced technical SS-LD	42.6	41.4		46.7	
	technical college-ED	5.2	5.4		3.5	
	university-ED	35	35.9		22.3	

Data are mean (SD) (minimum-maximum) or median (IQR) (minimum-maximum). [1] chi-squared test, [2] independent t-test, [3] Mann-Whitney-U test. Abbreviations: APOE: Apolipoprotein E, SS-LD: secondary school-leaving degree, which represents the end of 9th grade. The university entrance degree equals to 12-13 years of education, in Germany. ED: entrance degree, BMI: body mass index, VBM: Voxel Based Morphometry.

Supplementary Table 2. Participants' characteristics compared between APOE4-allele carriers and non-carriers.

	APOE non-carriers n = 388 (164 women)	APOE e4-carriers n = 97 (44 women)	p
Age (y)	69.5 ± 4.2 (60-79)	69.7 ± 4.1 (60-79)	0.7*
BMI (kg/m^2)	27.5 ± 4.1 (16.8-40.0)	27.3 ± 4.1 (19.3-41.4)	0.7*
Women (%)	42.3	45.4	0.6**
Mean systolic BP (mmHg)	136.1 ± 16.9 (89-194)	138.6 ± 18.1 (100-197)	0.2*
Education (%) [without SS-LD / SS-LD / advanced SS-LD / advanced technical SS-LD / technical college-entrance degree / university-entrance degree]	0.3 / 11.6 / 6.4 / 41.0 / 5.9 / 34.8	0 / 8.2 / 5.2 / 43.3 / 3.1 / 40.2	0.3***
Depression scale (CES-D) [score]	9.2 ± 5.2 (0-34)	9.6 ± 5.3 (0-33)	0.5*
Smoking (%) [current / previous / never]	6.4 / 30.7 / 62.9	5.2 / 39.2 / 55.7	0.27**
Arterial hypertension (%) [yes]	56.4	60.8	0.4**
Cardiovascular diseases (%) [any]	18.3	23.7	0.2**
Diabetes status (%) [none / type 1-medicated / type 2-medicated / type 2-non-medicated]	84.3 / 1.0 / 11.6 / 3.1	81.5 / 1.0 / 16.5 / 1.0	0.5***
Anti-hyperlipidemic medication (%) [yes]	24.2	23.7	0.9**
Estrogen supplement (% females)[yes]	7.3	6.8	0.6**
White matter hyperintensities (%) [Fazekas score 0 / 1 / 2 / 3]	25.2 / 57.0 / 17.3 / 0.5	24.8 / 60.8 / 14.4 / 0.0	0.7***

* independent t-test, ** Chi-square test, *** Mann-Whitney-U test

Data are shown as mean ±SD (minimum-maximum) unless indicated otherwise.

Abbreviations: APOE: Apolipoprotein E, BMI: body mass index, BP: Blood pressure, CES-D: center for epidemiologic studies depression scale, SS-LD: secondary school-leaving degree, which represents the end of 9th grade. The university entrance degree equals to 12-13 years of education, in Germany.

Supplementary Table 3. Bivariate and partial correlations between cognitive performance and GMV in BMI-associated regions (defined by voxel-level FWE-corrected threshold of p<0.05 and a cluster extent of at least 50 voxels)

	r, p-value			
	bivariate n = 617	**model-1** n = 617	**model-2** n = 617	**model-3** n = 485
Executive functions:				
Paracingulate gyrus (L)	0.1, 0.013[a]	0.105, 0.009 [a]	0.07, 0.07	0.063, 0.17
Planum Temporale (R)	0.091, 0.024 [a]	0.096, 0.017 [a]	0.07, 0.07	0.087, 0.06
Verbal memory:				
Ventral-medial PFC	0.23, $<10^{-3}$ [a]	0.13, 0.001 [a]	0.109, 0.008 [a]	0.119, 0.01 [a]
Frontal Orbital cortex (R)	0.159, $<10^{-3}$ [a]	0.116, 0.004 [a]	0.09, 0.026 [a]	0.08, 0.086
Paracingulate gyrus (L)	0.204, $<10^{-3}$ [a]	0.118, 0.004 [a]	0.086, 0.034 [a]	0.107, 0.02 [a]
Precentral gyrus (L)	0.197, $<10^{-3}$ [a]	0.072, 0.075	0.041, 0.3	0.024, 0.6
Insular cortex (L)	0.192, $<10^{-3}$ [a]	0.103, 0.01 [a]	0.093, 0.02 [a]	0.086, 0.06
Superior Temporal gyrus (R)	0.176, $<10^{-3}$ [a]	0.077, 0.057	0.062, 0.13	0.072, 0.12
Planum Temporale (L)	0.114, 0.005[a]	0.086, 0.032 [a]	0.075, 0.07	0.062, 0.18
Planum Temporale (R)	0.174, $<10^{-3}$ [a]	0.085, 0.034 [a]	0.076, 0.06	0.084, 0.07
Parahippocampal gyrus (R)	0.135, 0.001[a]	0.028, 0.5	0.011, 0.78	0.01, 0.8
Parahippocampal gyrus (L)	0.148, $<10^{-3}$ [a]	0.057, 0.16	0.047, 0.25	0.043, 0.35
Occipital fusiform gyrus (L)	0.125, 0.002[a]	0.05, 0.2	0.05, 0.23	0.064, 0.17
Occipital fusiform gyrus (R)	0.087, 0.03 [a]	0.042, 0.3	0.015, 0.7	0.019, 0.7

Intracalcarine cortex (R)	0.146, <10^{-3a}	0.034, 0.4	0.034, 0.4	0.058, 0.21
Thalamus (R)	0.188, <10^{-3a}	0.112, 0.005 [a]	0.113, 0.006 [a]	0.103, 0.025 [a]
Processing speed:				
Ventral-medial PFC	0.152, <10^{-3a}	0.082, 0.042 [a]	0.067, 0.1	0.02, 0.66
Paracingulate gyrus (L)	0.124, 0.002^{a}	0.066, 0.1	0.053, 0.2	-0.012, 0.78
Precentral gyrus (L)	0.155, <10^{-3a}	0.072, 0.076	0.064, 0.12	0.066, 0.155
Insular cortex (R)	0.165, <10^{-3a}	0.095, 0.019 [a]	0.087, 0.033 [a]	0.067, 0.145
Insular cortex (L)	0.105, 0.009^{a}	0.045, 0.261	0.043, 0.3	0.02, 0.64
Planum Temporale (L)	0.097, 0.016^{a}	0.065, 0.106	0.066, 0.102	0.076, 0.099
Planum Temporale (R)	0.115, 0.004^{a}	0.051, 0.203	0.047, 0.24	0.05, 0.28
Parahippocampal gyrus (R)	0.096, 0.017^{a}	0.036, 0.37	0.024, 0.56	0.03, 0.51
Occipital fusiform gyrus (L)	0.141, <10^{-3a}	0.088, 0.028 [a]	0.087, 0.03 [a]	0.053, 0.25
Intracalcarine cortex (R)	0.149, <10^{-3a}	0.085, 0.035 [a]	0.078, 0.055	0.067, 0.147

a: Significant at $p < 0.05$.

Abbreviations: GMV: gray matter volume, BMI: body mass index, PFC: prefrontal cortex, L:left, R: right. r: Pearson's correlation coefficient.
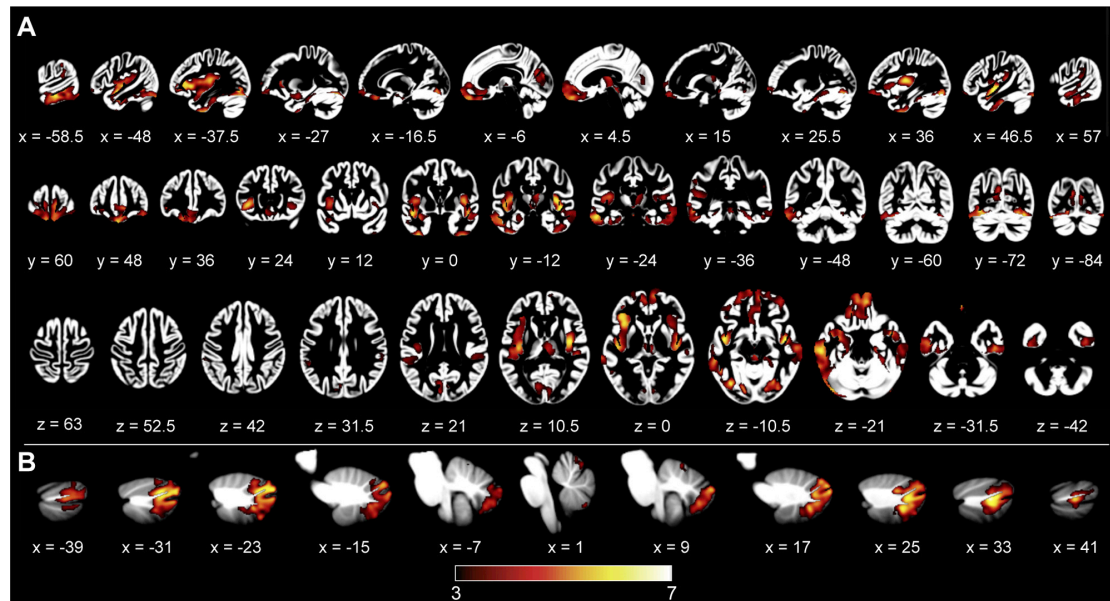
**Supplementary Figure 1. Body mass index (BMI)-associated gray matter alterations (model-2)**

Gray matter voxels negatively associated with BMI defined using multiple regression analysis with covariates in model 2 (n = 617). Significant clusters, surviving a voxel level threshold of p < 0.001 (uncorrected) and cluster level threshold of p < 0.05 (FWE-corrected) are displayed in the Cerebrum (B) as well as the Cerebellum (C), superimposed on a study-specific gray matter template. Color bar shows the t-value at significant voxels.
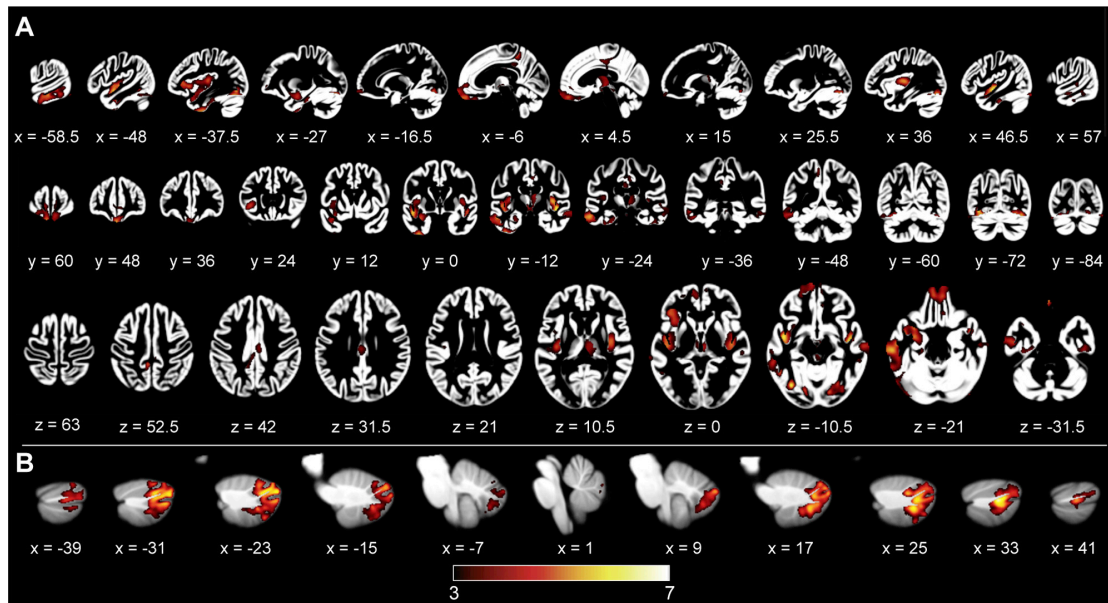
Supplementary Figure 2. Body mass index (BMI)-associated gray matter alterations (model-3)

Gray matter voxels negatively associated with BMI defined using multiple regression analysis with covariates in model 3 (n = 485). Significant clusters, surviving a voxel level threshold of P<0.001 (uncorrected) and cluster level threshold of p < 0.05 (FWE-corrected) are displayed in the cerebrum (B) as well as the cerebellum (C), superimposed on a study-specific gray matter template. Color bar shows the t-value at significant voxels.
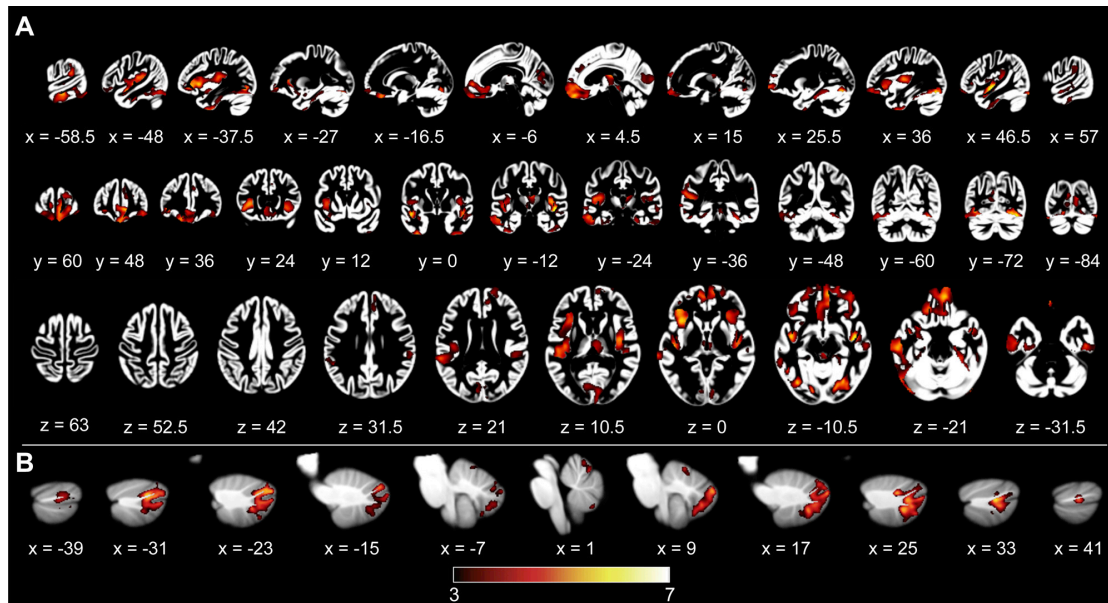
Supplementary Figure 3. Body mass index (BMI)-associated gray matter alterations in participants without diabetes

Gray matter voxels negatively associated with BMI defined using multiple regression analysis with covariates in model 1, in participants without diabetes (n = 518). Significant clusters, surviving a voxel level threshold of $p < 0.001$ (uncorrected) and cluster level threshold of $p < 0.05$ (FWE-corrected) are displayed in the cerebrum (B) as well as the cerebellum (C), superimposed on a study-specific gray matter template. Color bar shows the t-value at significant voxels.
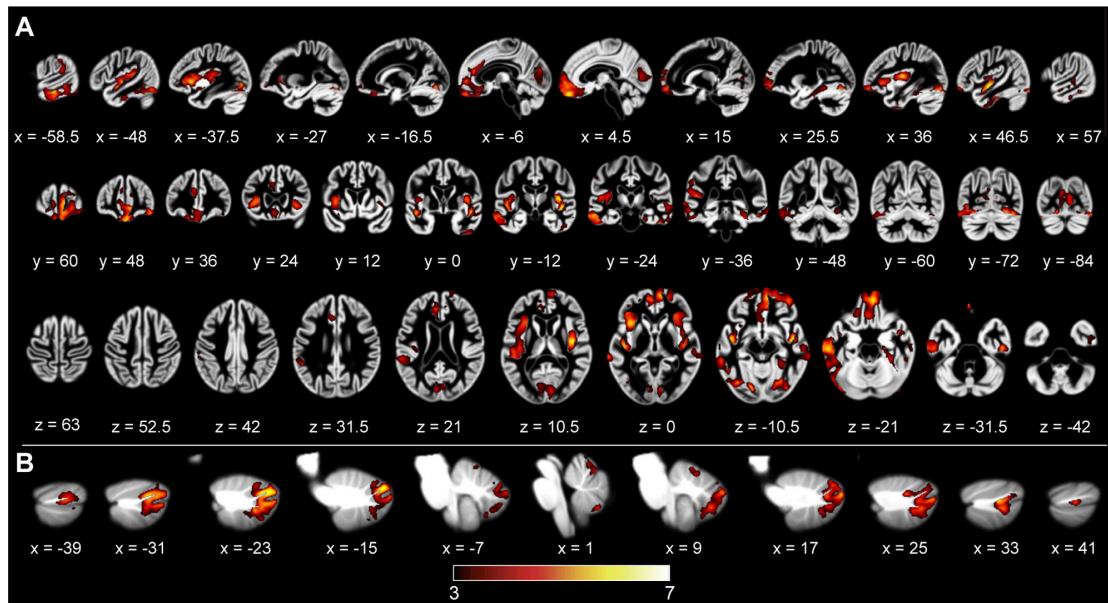
Supplementary Figure 4. Body mass index (BMI)-associated gray matter alterations in participants without intake of anti-hyperlipidemic medication

Gray matter voxels negatively associated with BMI using multiple regression analysis with covariates in model 1, in participants without intake of anti-hyperlipidemic medication (n = 480). Significant clusters, surviving a voxel level threshold of p < 0.001 (uncorrected) and a cluster level threshold of p < 0.05 (FWE-corrected), are displayed in the cerebrum (A) and cerebellum (B), superimposed on a study-specific gray matter template. Color bar shows the t-value at each significant voxel.
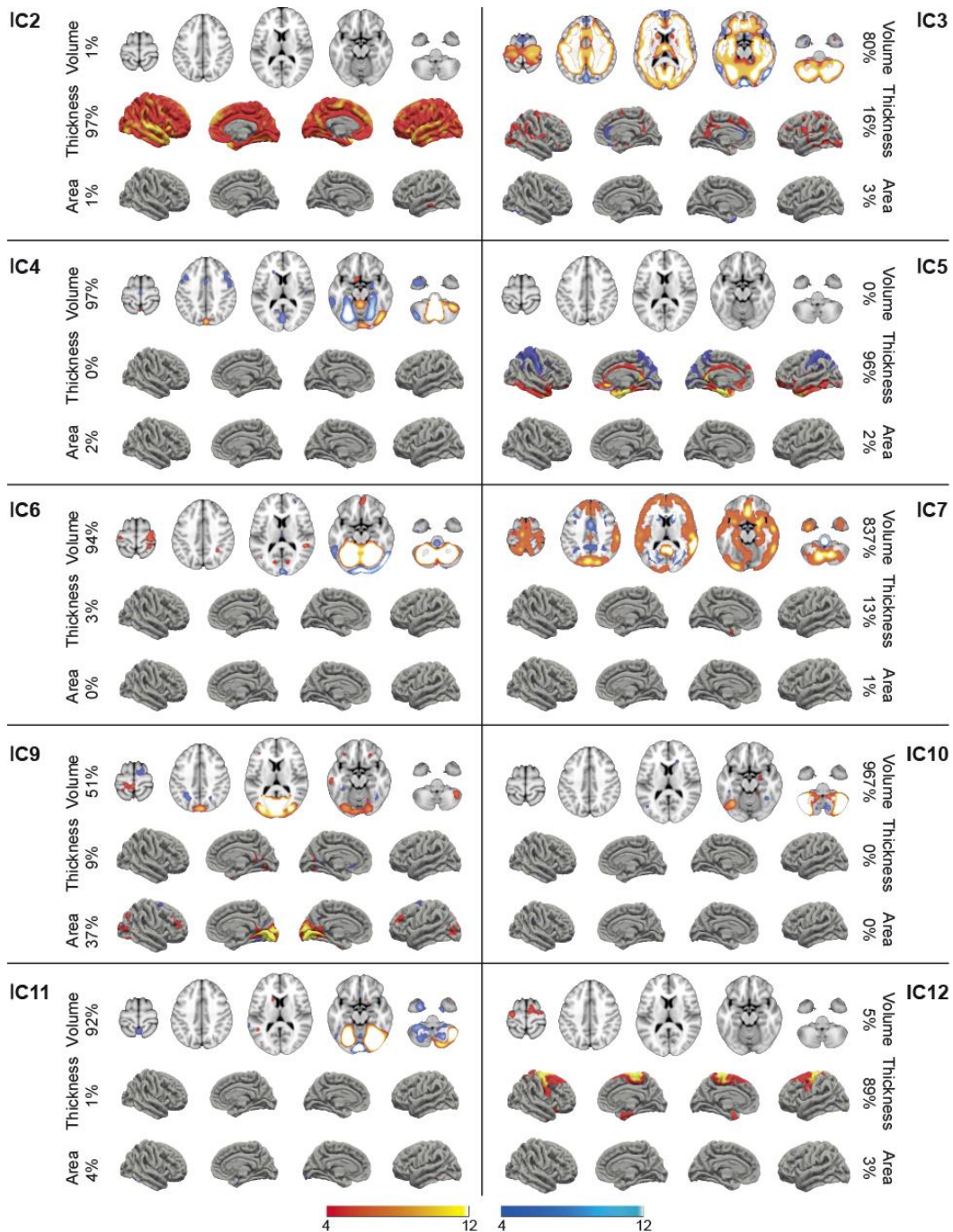
supplementary Figure 5. Spatial maps of independent components (ICs).
Spatial maps of the selected ICs give the regional extent and contribution of each gray matter modality, i.e., volume, thickness and area (z > 4), to the generation of the IC. Colors indicate positive (red/yellow) or negative (blue/light-blue) co-variations within the network, percentages give the amount of weighted variance that is explained by each modality. Maps are drawn on a standard brain.

B. Declaration of Authenticity

Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

....
Datum

....
Unterschrift
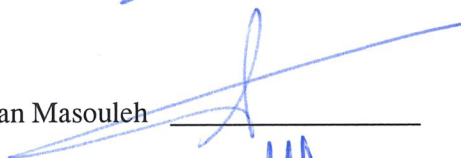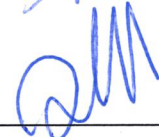
C. Author contributions to the publications

*"Components of a Mediterranean diet and their impact on cognitive functions in aging", by Sebastian Huhn (**SH**), Shahrzad Kharabian Masouleh (**SKM**), Michael Stumvoll (**MS**), Arno Villringer (**AV**), A. Veronica Witte (**AVW**)*, (Frontiers in Aging Neuroscience, 2015).*

*Corresponding author

Principal investigators: AV, MS, AVW

Conception and Design of the paper: SH, AVW

Literature Review: SH, SKM

Data Interpretation: SH, AVW

Figure creation: SH

Drafted manuscript: SH

Critical revision: SH, SKM, AV, AVW

Signatures

Leipzig, den _09.02.18_   Sebastian Huhn   _____

Leipzig, den _9.2.18_   Shahrzad Kharabian Masouleh   _____

Leipzig, den _9.2.18_   Arno Villringer   _____

Leipzig, den _9.2.18_   A. Veronica Witte   _____

"Higher body mass index in older adults is associated with lower gray matter volume: Implications for memory performance", by Shahrzad Kharabian Masouleh **(SKM)**, Katrin Arélin **(KA)**, Annette Horstmann **(AH)**, Leonie Lampe **(LL)**, Judy A. Kipping **(JAK)**, Tobias Luck **(TL)**, Steffi G. Riedel-Heller **(SGR-H)**, Matthias L. Schroeter **(MLS)**, Michael Stumvoll **(MS)**, Arno Villringer **(AV)**, A. Veronica Witte **(AVW)** *, (neurobiology of ageing, April 2016)
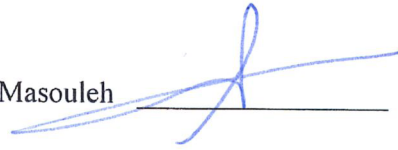
*Corresponding author

design of the study and data collection: LL, KA, AH, TL, MLS, SGR-H, MS, AV

Data analysis: SKM, JAK

Data interpretation: SKM, AV, AVW

Figure creation: SKM, AVW

Drafted manuscript: SKM, AVW

Critical revision: SKM, KA, LL, AH, JAK, TL, SGR-H, MLS, MS, AV, AVW

Signatures:

Leipzig, den ___9.2.18___ Shahrzad Kharabian Masouleh _____

Leipzig, den _____ Katrin Arélin _____

Leipzig, den ___9.2.2018___ Annette Horstmann _____

Leipzig, den ___9.2. 2018___ Leonie Lampe _____

Leipzig, den _____ Judy A. Kipping _____
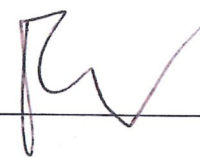
Leipzig, den ___6.2.19___ Tobias Luck _____
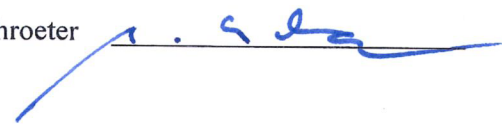
Leipzig, den ___0.2.18___ Steffi G. Riedel-Heller _____

Leipzig, den ___18.2. 18___ Matthias L. Schroeter _____

Leipzig, den _____ Michael Stumvoll _____

Leipzig, den ___9.2 2018___ Arno Villringer _____

Leipzig, den ___9 2 2018___ A. Veronica Witte _____

109

"Higher body mass index is associated with reduced default mode connectivity in older adults",

Frauke Beyer (**FB**), Shahrzad Kharabian Masouleh (**SKM**), Julia M. Huntenburg (**JMH**), Leonie Lampe (**LL**), Tobias Luck (**TL**), *Steffi G.* Riedel-Heller (**SGR-H**), Markus Loeffler (**ML**), *Matthias L. Schroeter (**MLS**), Michael Stumvoll (**MS**), Arno Villringer (**AV**), A. Veronica Witte (**AVW**)**, (*Human Brain Mapping, 2017)

*Corresponding author

Conception:

design of the study and data collection:	LL, TL, SGR-H, ML, MLS, AV
Data analysis:	FB, SKM, JMH, LL
Data interpretation:	FB, SKM, AVW
Figure creation:	FB
Drafted manuscript:	FB, SKM, AVW
Critical revision:	FB, SKM, JMH, LL, TL, SGR-H, ML, MLS, MS, AV, AVW

Signatures:

Leipzig, den _____9.2.18_____   Frauke Beyer   _____

Leipzig, den _____9.2.18_____   Shahrzad Kharabian Masouleh   _____
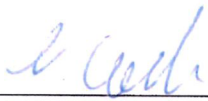
Leipzig, den _____   Julia M. Huntenburg   _____

Leipzig, den _____9.2.18_____   Leonie Lampe   _____

Leipzig, den _____6.2.18_____   Tobias Luck   _____
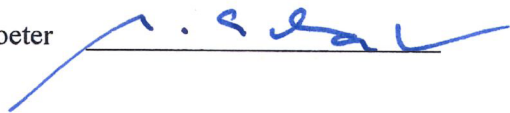
Leipzig, den _____6.2.15_____   Steffi G. Riedel-Heller   _____

Leipzig, den _____   Markus Loeffler   _____
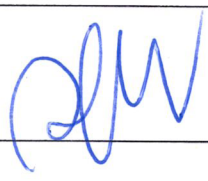
Leipzig, den _____12.2.18____   Matthias L. Schroeter   _____

Leipzig, den _____   Michael Stumvoll   _____

Leipzig, den _____9.7.2018____   Arno Villringer   _____

Leipzig, den _____9.7.2018____   A. Veronica Witte   _____

111

"Cardiovascular risk factors are associated with grey matter structural networks in ageing", by Shahrzad Kharabian Masouleh (SKM), Frauke Beyer (FB), Leonie Lampe (LL), Markus Loeffler (ML), Tobias Luck (TL), Steffi G. Riedel-Heller (SGR-H), Matthias L. Schroeter (MLS), Michael Stumvoll (MS), Arno Villringer (AV), A. Veronica Witte (AVW)*, (journal of cerebral blood flow and metabolism, 2017)

*Corresponding author

Conception:

design of the study and data collection:	LL, TL, SGR-H, ML, MLS, AV
Data analysis:	SKM, FB, LL
Data interpretation:	SKM, AVW
Figure creation:	SKM
Drafted manuscript:	SKM, AVW
Critical revision:	SKM, FB, LL, TL, SGR-H, ML, MLS, MS, AV, AVW
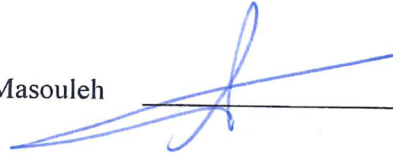
Signatures:

Leipzig, den ___9.2.18___   Shahrzad Kharabian Masouleh _____

Leipzig, den ___9.2.18___   Frauke Beyer _____

Leipzig, den ___9.2.18___   Leonie Lampe _____

Leipzig, den _____   Markus Loeffler _____
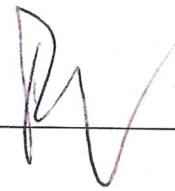
Leipzig, den ___6.2.18___   Tobias Luck _____
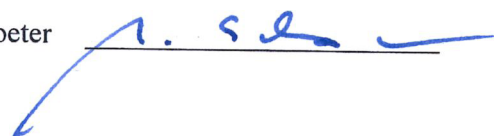
Leipzig, den ___8.2.18___   Steffi G. Riedel-Heller _____

Leipzig, den ___12.2.18___   Matthias L. Schroeter _____
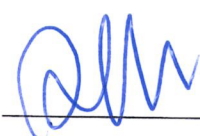
Leipzig, den _____   Michael Stumvoll _____

Leipzig, den ___9.2.2018___   Arno Villringer _____

Leipzig, den ___9.2.2018___   A. Veronica Witte _____

113

D. Curriculum Vitae

Shahrzad Kharabian Masouleh

Max Planck Institute for Human Cognitive and Brain Sciences

Stephanstraße 1A

04109 Leipzig

Germany

kharabian@cbs.mpg.de

Academic Education:

01.2017 – Present

Postdoctoral Researcher

Institute of Neuroscience and Medicine, Brain & Behaviour (INM-7), Research Centre Jülich, Jülich, Germany

10.2011 – 12.2016

Department of Neurology – Ageing and obesity Group

Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany, 2011-2016

> **Thesis:**
>
> Metabolic modifiers of gray matter structure and its cognitive outcome in older individuals.

10.2007 – 12.2009 M.Sc.

Biomedical Engineering (Bioelectric), Sharif University of Technology, Tehran, Iran, Tehran, IRAN.

> **Thesis:**
>
> Fetal R Detection from Mixed Maternal and Fetal MCG Signals.

10.2003 – 09.2007 B.Sc.

Biomedical Engineering (Bioelectric), Amirkabir University of Technology, Tehran, IRAN, 2003-2007.

Thesis:

Time Alignment in speech using genetic algorithm.

Research Experience

- Research assistant in the Hertie institute, Tuebingen, Germany (April 2011- September 2011).

- Research assistant in the Brain Imaging Lab (BIC), Frankfurt am Main, Germany (September 2010- May 2011).

- Research assistant in Biomedical Signal and Image processing Lab. Electrical Engineering School. Sharif University of Technology, Tehran, Iran (2007-2009).

Conference Papers

Kharabian S., Shamsollahi M.B., Sameni R., **"Fetal R detection from multichannel abdominal ECG recordings in low SNR"**, 31st Annual International Conference of the IEEE EMBS, Minneapolis, Minnesota, USA, September 2-6, 2009.

Ayat M., Shamsollahi M. B., Mozaffari B., **Kharabian S.**, "**ECG denoising using modulus maxima of wavelet transform",** 31st Annual International Conference of the IEEE EMBS, Minneapolis, Minnesota, USA, September 2-6, 2009.

Poster Presentations

Kharabian S., Horstmann A., Arélin K., Schäfer A., Neumann J., Mueller K., Riedel-Heller S., Schroeter M. L., Stumvoll M., Villringer A. (2013), **"Obesity and ageing related changes in brain structure and function."** Poster presented at 29th Annual Meeting of the German Society for Obesity, Hannover, Germany.

Kharabian S., Herzig S., Schroeter M., Gorgolewski K., Klose L., Tenckhoff H., Berg T., Wiese M., Thöne-Otto A., Margulies D., Villringer A. (2014), **"Functional connectivity changes in patients with chronic Hepatitis C",** 20th Annual Meeting of Human Brain mapping, Hamburg, Germany.

Kharabian Masouleh S., Lampe L, Beyer F, Schroeter M. L., Witte V. A., Gorgolewski K., Steele C.J., Villringer A., Bazin P.L. (2016), "**Fine-grained localization of relation between white matter hyperintensities and gray matter volume",** 22th Annual Meeting of Human Brain mapping, Geneva, Switzerland.

Kharabian Masouleh S., Beyer F., Lampe L., Loeffler M., Luck T., Riedel-Heller S. G., Schroeter M. L., Stumvoll M., Villringer A., Witte V. A., "**Cardiovascular risk factors are associated with grey matter structural networks in ageing",** 23th Annual Meeting of Human Brain mapping, Vancouver, Canada.

Computer Skills

Programming:

Python, MATLAB, bash, R, C++, Orcad, Micro Controler Assembly (8051, 8086), SPSS.

Neuroimaging Toolboxes:

FSL, SPM, Freesurfer, AFNI, LIPSIA, Fieldtrip (MEG).

E. List of Publications

Lampe L., **Kharabian Masouleh S.**, Kynast J., Steele C.J., Arélin K., Fritzsch D., Schroeter M. L., Villringer A., Witte V. A., Bazin P.L., "**Lesion location matters: The relationships between white matter hyperintensities on cognition in the healthy elderly**", journal of cerebral blood flow and metabolism, 2017.

Kharabian Masouleh S., Beyer F., Lampe L., Loeffler M., Luck T., Riedel-Heller S. G., Schroeter M. L., Stumvoll M., Villringer A., Witte V. A., "**Cardiovascular risk factors are associated with grey matter structural networks in ageing**", journal of cerebral blood flow and metabolism, 2017.

Beyer F., **Kharabian Masouleh S.**, Huntenburg M. J., Lampe L., Luck T., Riedel-Heller S. G., Schroeter M. L., Stumvoll M., Villringer A., Witte A. V., "**Higher body mass index is associated with reduced default mode connectivity in older adults**", Human Brain Mapping, 2017.

Liem F., Varoquaux G., Kynast J., Beyer F., **Kharabian Masouleh, S.**, Huntenburg J.M., Lampe L., Rahim M., Abraham A., Craddock R.C., Riedel-Heller S., Luck T., Loeffler M., Schroeter M.L., Witte A.V., Villringer A., Margulies D.S., "**Predicting brain-age from multimodal imaging data captures cognitive impairment**", Neuroimage 2017.

Kharabian Masouleh S.*, Herzig S.*, Schroeter M., Klose L., Tenckhoff H., Berg T., Wiese M., Thöne-Otto A., Margulies D., Villringer A. "**Functional connectivity changes in patients with chronic Hepatitis C**", Journal of Viral Hepatitis, Nov 2016.

Kharabian Masouleh S., Arélin K., Horstmann A., Lampe L., Kipping J.A., Luc T., Riedel-Heller S. G., Schroeter M. L., Stumvoll M., Villringer A., Witte A. V., "**Body mass index, brain structure and cognition in healthy older adults - a population based study**", neurobiology of ageing, April 2016.

Huhn S., **Kharabian Masouleh S.**, Stumvoll M., Villringer A., Witte V., "**Components of a Mediterranean diet and their impact on cognitive functions in aging**", Frontiers in Aging Neuroscience 2015.

F. Acknowledgements