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The impact of nutrition on hippocampal function

Results of a literature review and a randomized controlled trial

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Abbreviations

AD	Alzheimer's Disease
CVLT	California Verbal Learning Task
DWI	Diffusion-Weighted Imaging
BIA	Bioelectrical Impedance Analysis
HbA1c	Glycated Hemoglobin
MD	Mean Diffusivity
MMSE	Mini-Mental Status Examination
PREDIMED	Prevención con Dieta Mediterránea = Prevention with the Mediterranean Diet
RAGE	Receptor for Advanced Glycation End Products
RAVLT	Rey Auditory Verbal Learning Test

"Let food be thy medicine, and medicine be thy food"

Hippocrates

I Introduction

Nutrition as a modifiable lifestyle factor bears a great potential for the prevention of cognitive decline and related diseases, such as Alzheimer's disease (AD) (von Arnim et al., 2010;Carrera-Bastos et al., 2011;Hu et al., 2013;Barnard et al., 2014b;Alzheimer's Association, 2017;Aridi et al., 2017). Early affected in AD are brain structures such as the hippocampus (Wisse et al., 2015). The hippocampus shows high metabolic needs, life-long plasticity, and possibly the ability for adult neurogenesis, which might all be essential for successful learning and memory (Walhovd et al., 2015). These properties could render the hippocampus especially vulnerable to degeneration in aging, while at the same time they seem to make this brain structure especially susceptible to various (lifestyle-) interventions with the aim to prevent or slow cognitive decline (for review see Walhovd et al. (2015)). As of yet, no cure for AD is available, which makes it imperative to fully exploit these interventions as potential preventive measures (Ising et al., 2015). Amongst others, this includes nutrition.

The sum of ingested food can influence the homeostasis of bodily functions for the good or bad. On the one hand, poor- or malnutrition leads to nutrient deficiencies and negative biochemical changes on a neural level. This can be the root for diseases and impaired cognitive functions as explained in more detail over the course of this thesis. On the other hand, meeting required amounts of macro- and micronutrients could be a preventive factor. Therefore, it is essential to adhere to a balanced diet over the course of life in order to meet the individual nutritional requirements.

This, however, is increasingly difficult in Western societies, which are characterized by the predominant consumption of a high fat sugar diet – the so-called Western diet (Yeomans, 2017). This dietary pattern is accompanied by a sedentary lifestyle in a fast-living environment (Cordain et al., 2005). In combination, unfavorable lifestyle and diet might give rise to the increasing incidence of cognitive impairment and dementias, such as AD (Cooper et al., 2015;Xu et al., 2015;Cao et al., 2016;Falck et al., 2017).

In contrast, the traditional Mediterranean diet could serve as a good example for a nutrient-rich diet with properties beneficial for overall and cognitive health (for review see e.g. (Lourida et al., 2013;Gotsis et al., 2015;Aridi et al., 2017)). In an attempt to understand the underlying mechanisms of these positive features, researchers

focused on single dietary components. In a literature review (Publication 1 Huhn et al., (2015)), we identified the polyphenol resveratrol as one of these promising components of the Mediterranean diet. It bears biochemical traits that might affect cognitive health and was therefore used to target memory functions in a randomized controlled trial in elderly humans (Publication 2 Huhn et al., (2018)).

In this thesis, I will first discuss distinct features of the hippocampus, which render it susceptible to (lifestyle-) interventions and introduce cognitive tests to assess hippocampus functionality. Furthermore, I will describe state-of-the-art neuroimaging measures to gain *in-vivo* evidence for possible intervention effects on the brain level. Afterwards, I will employ the Western and Mediterranean diet as examples of common dietary patterns. In this context, certain biochemical changes evoked by these diets will be elaborated, as possible underlying mechanisms of dietary effects on cognitive health. To go even more into details of underlying mechanisms, I will focus on a single component of the Mediterranean diet – resveratrol. Therefore, I will summarize specific biochemical properties of resveratrol, as well as data from human studies with regard to resveratrol's effects on cognitive health. The section about resveratrol is completed with the evaluation of the results of Publication 2 Huhn et al., (2018), which are an important contribution to this field of research. Finally, in a critical discussion I will deploy the presented data and findings of Publication 2 to optimize the design of future studies. All this will hopefully advance the future of research about the impact of nutrition on memory functions in order to maintain cognitive health over the course of life.

1. Distinct features and functions of the human hippocampus

The hippocampus is characterized by several properties that could help to explain its vulnerability to degeneration in aging and the susceptibility to intervention effects (for review see Walhovd et al. (2015)). These features include the potential ability for adult neurogenesis, high metabolic demands, and life-long plasticity.

The hippocampus stands out as one of two sites in the brain with the potential ability for adult neurogenesis (for detailed discussion see (Deng et al., 2010;Walhovd et al., 2015)). Adult neurogenesis describes the life-long generation of neurons and might contribute to structural plasticity and network maintenance within the brain (Mu and Gage, 2011;Sorrells et al., 2018). Enhanced neurogenesis might be an early response mechanism of the brain in the development of AD, and dysfunctions of neurogenesis could contribute to the development of memory impairments (Mu and

Gage, 2011;Lazarov and Marr, 2013). Nevertheless, the knowledge is mainly based on animal studies and the transfer to humans is complicated. Significant differences in the manifestation of the neurogenesis become evident and the quantification of neurogenesis in humans is only established post-mortem (Lazarov and Marr, 2013;Bergmann et al., 2015). Importantly, recent publications emphasized that adult neurogenesis might only exist to a far lesser extent than previously assumed and conclusions need to be drawn with caution (Snyder, 2018;Sorrells et al., 2018).

Furthermore, the hippocampus is characterized by high metabolic demands. These could be related to the neurogenesis, but might also be caused by general maintenance functions (McEwen and Reagan, 2004;Walhovd et al., 2015). Both, high metabolic demands and neurogenesis, seem to make the hippocampus especially susceptible to change (for review see (Walhovd et al., 2015). This change involves molecular and structural changes of the hippocampus and could be in positive or negative terms (Mufson et al., 2015). Also referred to as synaptic plasticity, these changes are supposed to be a crucial mechanism for successful learning and memory, but the exact processes determining synaptic plasticity remain to be established (for review see (Roelfsema and Holtmaat, 2018)).

On a functional level, the hippocampus is, amongst others, involved in processes, such as learning, memory, and pattern separation, which can be assessed with specific neuropsychological tests (Brickman et al., 2014;Eichenbaum and Cohen, 2014). The role of the hippocampus for learning and memory was well established since the milestone publication by Scoville (1957) (Squire and Zola-Morgan, 1991;Gabrieli, 1998;Eichenbaum, 2000; 2004;Squire, 2004). The hippocampal memory system is composed of the hippocampus itself, the adjacent parahippocampal region and cerebral cortical areas (Eichenbaum, 2000). It is one of several memory systems that operate in parallel (Squire, 2004). The integrity of the memory system can be assessed experimentally, e.g. with tests that include free recall, cued recall or recognition (Gabrieli, 1998). An example of such a test is the California Verbal Learning Task (CVLT), which is also used in Publication 2 Huhn et al., (2018). The CVLT is a multiple-trial list-learning task, which assesses recall and recognition of two 16-item word lists over immediate and delayed memory trials (Niemann et al., 2008). It is widely used to draw conclusions about verbal learning and memory performance (Elwood, 1995). A detailed description of the test administration can be found in Publication 2.

To assess pattern separation, we employed the ModBent task according to Brickman et al. (2014) in Publication 2. Pattern separation can be described as the ability to discriminate among similar experiences, including visually similar objects (Yassa and Stark, 2011). The Dentate Gyrus – a region within the hippocampus – seems to be responsible for this feature and adult neurogenesis could mechanistically be involved (Yassa and Stark, 2011;Brickman et al., 2014). For more details on test administration see Appendix A1. Besides the neuropsychological tests, which assess the functionality of the hippocampus, it is also possible to acquire *in vivo* information about its anatomy and microstructure with neuroimaging.

2. *In vivo* analysis of the Human Hippocampus

Regarding its anatomy, the hippocampus is a bilateral brain structure located in the medial temporal lobe of the mammalian brain (Mu and Gage, 2011). It is a heterogeneous structure that comprises interconnected subfields with distinctive histological and functional features (Maruszak and Thuret, 2014). The subfields are the Cornu Ammonis areas 1-4, Dentate Gyrus and the subiculum (see Figure 1 and 2). Although overall hippocampus volume declines with age, different subfields appear to be more susceptible to underlying vascular changes or neuropathologies (Shing et al., 2011;Maruszak and Thuret, 2014). Thus, analyzing the hippocampus not exclusively as a whole entity, but also on subfield level might yield more specific insights. This can be achieved with state-of-the-art neuroimaging.

Over the last decades, magnetic resonance imaging (MRI) developed to be an established and reliable method for, non-invasive, *in-vivo* examination of the brain. MRI uses the inherent magnetic properties of hydrogen protons as part of the water molecules abundantly present in the human body. These magnetic properties can be manipulated by adding external magnetic fields and radio-frequency pulses. During the further procedures, various signals are recorded and ultimately translated into images. There are several imaging contrasts, which highlight different tissue properties such as relaxation rates and water content (Marques et al., 2010;de Carvalho Rangel et al., 2011). Among the most common contrasts is T1-weighting, which allows an anatomical characterization of brain structures. Furthermore prevalent is diffusion-weighting that allows mapping of white matter tracts based on directed diffusion of water molecules along these tracts. White matter structures consist mainly of myelinated axons that connect different areas of the brain. It is opposed to grey matter, which contains mainly neurons and other brain cells.

2.1. T1-weighted anatomical images of the hippocampus

Anatomical images are oftentimes based on the T1 relaxation time. This parameter reflects the longitudinal relaxation of protons after a radiofrequency pulse emitted from the scanner excited them. Hydrogen protons are abundant in the brain as part of water and other biological structures. From these T1-weighted images, information such as the volume of brain structures can be derived.

Standard magnetic resonance imaging at 1.5 or 3 Tesla field strength reaches spatial resolutions of around 1 mm isotropic (equal in all directions), given whole brain coverage and acquisition times of around 10 minutes. The resolution usually refers to the size of a voxel, which represents a unit of a three-dimensional space. Hence, for research with a special interest in small brain structures (e.g. the hippocampus), higher resolutions add an extraordinary benefit. With the 7 Tesla field strength used in Publication 2, a resolution of 0.7 mm isotropic was achieved, which allows detailed segmentations of the brain (see Figure 1). Thereby, even a segmentation of the hippocampus on subfield level is meaningful as hallmark anatomical features can be distinguished. For methodological details see Publication 2 Huhn et al., (2018).

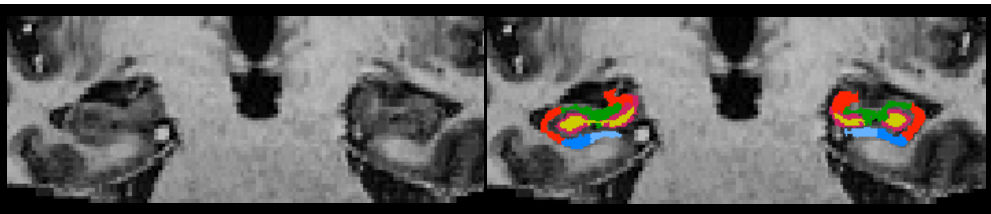

Figure 1: T1-weighted images of the hippocampus acquired at 7 Tesla.
This is an example of a 7 Tesla T1-weighted image without (left) and with (right) hippocampal subfield delineation. Figure is from Publication 2 Huhn et al., 2018. Color-coding: Cornu Ammonis (CA) 1 = red; CA 2/3 = green; CA 4 = yellow; Dentate Gyrus = purple; presubiculum = light blue; subiculum = dark blue.

2.2. Diffusion-weighted imaging of hippocampus microstructure

Diffusion-weighted imaging (DWI) is used to visualize water diffusion within the brain. In general, water molecules are constantly in random, so-called Brownian motion due to the thermal energy (carried by these molecules). Without boundaries, the molecular motion is the same in all directions. Within the brain, however, boundaries, such as the lipophilic cell membranes, or myelin sheaths around fiber tracts limit the range of motion of water molecules and diffusion becomes more pronounced in one direction. This anisotropic motion represents the respective fiber tracts and can be used to visualize them. In clinical practice, this is for example used to detect damaged brain tissue, e.g. ischemia after stroke. Here, in the acute phase diffusion

within the damaged tissue is more restricted, while in the long term the before limited range of movement becomes free and un-directional (Horsfield and Jones, 2002). Similarly, in cognitive decline, and ultimately dementia, the integrity of axonal tracts declines and results in more isotropic diffusion. Such changes are detectable early in the course of the disease and could be further developed to valuable biomarkers (Amlien and Fjell, 2014).

Diffusion-weighted images can also be used to evaluate microstructure of brain regions that are considered as grey matter (see Figure 2). Here, lower mean diffusivity (MD) is discussed to display less "integrity", e.g. fewer or less-intact cell boundaries due to disruption or break-down of cytoarchitecture demyelination processes, compared to higher MD (den Heijer et al., 2012;Van Camp et al., 2012;Weston et al., 2015). This evaluation of the "integrity" of grey matter structures is important for our research with regard to the hippocampus as it could be affected by different diets (e.g. the Western or Mediterranean diet) but also by interventions, such as Publication 2.

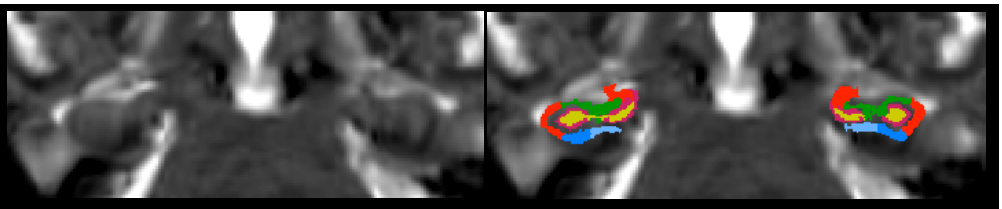

Figure 2: Diffusion-weighted image of the hippocampus acquired at 7 Tesla.

This is an example of a 7 Tesla diffusion-weighted image without (left) and with (right) hippocampal subfield delineation. Figure is from Publication 2 Huhn et al., 2018. Color-coding: Cornu Ammonis (CA) 1 = red; CA 2/3 = green; CA 4 = yellow; Dentate Gyrus = purple; presubiculum = light blue; subiculum = dark blue.

3. The Western diet and its unfavorable impact on the brain

Nowadays, a so-called “Western diet” predominates in countries such as the USA, Australia, and wide parts of Europe (Cordain et al., 2005). A certain set of problems is related to this eating pattern and comprises both, an unfavorable behavior and food composition. Characteristic behavior includes a sedentary lifestyle in a fast-living environment (Carrera-Bastos et al., 2011). In this context, it is oftentimes undervalued to spent time to prepare a wholesome meal. This is combined with a rapidly advancing food industry, which provides omnipresent, fast and conveniently available meals (Carrera-Bastos et al., 2011). However, the nutritious value of the industrialized and highly palatable food is increasingly marginal (Cordain et al.,

2005;Carrera-Bastos et al., 2011). Furthermore, the purchasable plethora of dietary supplements can lead to temptation to simply compensate unbalanced diets with the "correct" combination of pills (Dickinson et al., 2014).

The composition of the Western diet is characterized by a high proportion of (saturated and trans) fatty acids and refined sugars (Yeomans, 2017) (also see Figure 3). Therefore, it is also known as a high fat sugar diet (Yeomans, 2017). This composition has negative biochemical consequences on a systemic level, causing oxidative stress, and bearing an inflammatory potential (Yeomans, 2017). Several mechanisms underlying these consequences have been proposed and a few examples will be elaborated on and afterwards related to their impact on the brain.

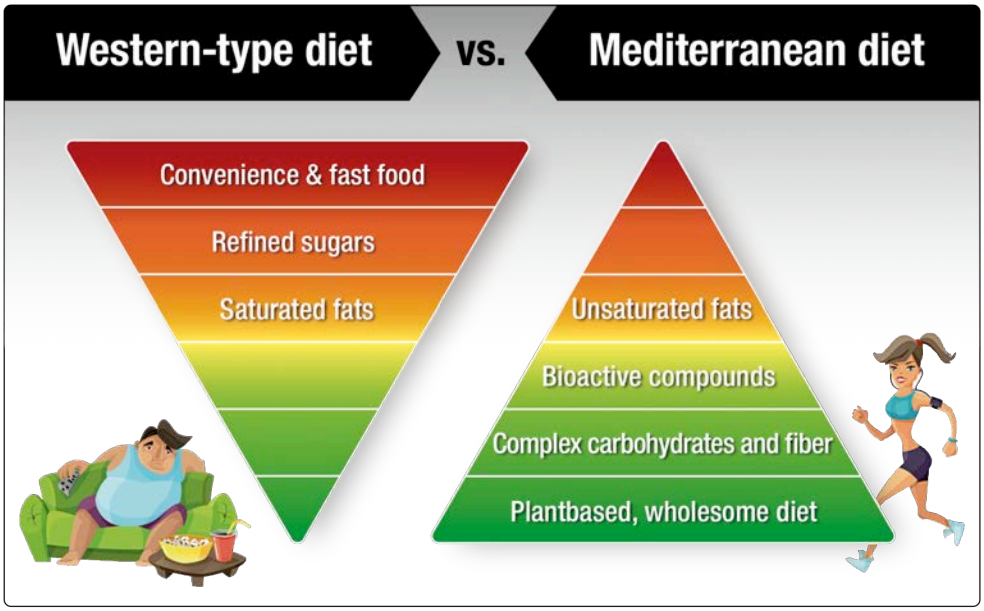

Figure 3: Comparison of key components of the Mediterranean and Western diet.

The width of the pyramids relates to the consumed amount in each diet (e.g. a lot of complex carbohydrates and fibers are consumed in the Mediterranean diet (light green), but only little convenience and fast food (dark red). Colorscale represents transition from beneficial to unfavorable food items: green = beneficial effects, high consumption recommended; red = unfavorable effects assumed, consumption should be limited. Furthermore the Western-type diet is characterized by a sedentary lifestyle, while the Mediterranean diet is usually related to a physically active lifestyle.

Chronically high glucose levels can be responsible for the dysfunction of the mitochondrial electron transport chain and thus cause higher levels of reactive oxygen species (Francis and Stevenson, 2013;Freeman et al., 2014). These in turn potentially damage important cellular bio-macromolecules, such as lipids, sugars, proteins, and polynucleotides (Sayre et al., 2008;Liochev, 2013). Emerging damages

can result in increased mutation rates, growth inhibition, and changes in the insulin-signaling pathway (Droge and Schipper, 2007;Liochev, 2013).

In addition, a high amount of dietary lipids contributes to the genesis of oxidative stress, and inflammation. Lipids are metabolized into free radicals through the lipid peroxidation pathway (Sies et al., 2005;Freeman et al., 2014). The inflammatory potential might be caused by hypercholesterolemia, which is known as a possible inducer of neuro-inflammation, the activation of microglia and the immune system (Freeman et al., 2014;Guillemot-Legris and Muccioli, 2017).

Both, oxidative stress and (neuro-) inflammation appear to be linked to cell death (Andersen, 2004;Sayre et al., 2008;Heneka et al., 2015;Ray et al., 2016). The brain is especially susceptible to these damages compared to other organs, as neurons as post-mitotic cells are more prone to accumulate DNA damage (Andersen, 2004). This is additionally combined with a high metabolic rate (i.e. high levels of reactive oxygen species) and relatively reduced capacity of the brain for cellular regeneration (Andersen, 2004).

In the case of oxidative stress, a higher amount of reactive oxygen species leads to increased oxidative alterations of lipids and proteins (Pedersen et al., 1998;Andersen, 2004;Sayre et al., 2008). Lipid oxidation, in turn, results in highly-reactive byproducts (Pedersen et al., 1998). These can furthermore modify amino acids within proteins and thus result in protein conjugates (Andersen, 2004). These protein conjugates and proteins damaged directly by reactive oxygen species are likely to become misfolded and show an impaired degradation (Andersen, 2004). The subsequent accumulation of soluble (e.g. amyloid-β) and insoluble aggregates (e.g. amyloid plaques and tau-filaments), as for example observed in AD, can be toxic and finally result in neuronal cell death and neurodegeneration (Bloom, 2014).

Furthermore, cell-surface molecules, such as the receptor for advanced glycation end products (RAGE) can recognize amyloid-β (Sayre et al., 2008;Ray et al., 2016). Subsequent activation of RAGE and also oxidative stress per se leads to the activation of microglia (Ray et al., 2016). Consequently, both activated RAGE and microglia release an increased amount of pro-inflammatory molecules and thus reinforce the development of neuroinflammation (Ray et al., 2016). As a consequence, the neurochemical foundations of learning and memory could be affected, such as the long-term potentiation of synapses (Cooke and Bliss, 2006;Wang et al., 2015). The damage to neurons and synapses, which is occurring

due to oxidative stress and neuroinflammation, ultimately changes neuronal plasticity and thus has the potential to change memory and cognitive processes (Kanoski and Davidson, 2011;Francis and Stevenson, 2013;Barnard et al., 2014a;Bloom, 2014;Freeman et al., 2014).

The hippocampus is strongly affected by the damages described above due to its vulnerability as described in section 1 (Morrison et al., 2010;Tucsek et al., 2014;Biessels and Reagan, 2015). Resulting alterations of this structure affect learning, (working) memory, and pattern separation (Francis and Stevenson, 2013;Brickman et al., 2014). A first longitudinal, observational human study showed an association between Western diet and hippocampal volume (Jacka et al., 2015). The authors report independent associations of a high intake of unhealthy food (i.e. Western diet) and a low intake of nutrient-dense food (i.e. fruits, vegetables) with lower volumes of the left hippocampus after 4 years in a sample with 255 elderly humans (>60 years) (Jacka et al., 2015). Underlying mechanisms triggered by a Western diet with an unfavorable dietary composition could comprise changes in gluco-regulation and levels of brain-derived neurotrophic factors (Kanoski and Davidson, 2011;Francis and Stevenson, 2013).

Summarizing, the Western diet leads to an increased intake of detrimental components (e.g. refined sugars and saturated fats), which in turn results in elevated oxidative stress and a high inflammatory potential. With subsequent changes in neuronal plasticity and the potential to induce neuronal cell death, this diet can have a negative biochemical impact on the brain and cognition.

4. A promising preventive strategy – the Mediterranean diet

4.1. Health-beneficial characteristics of the Mediterranean diet

One dietary pattern described to be of high value for cognitive health in multiple studies is the traditional Mediterranean diet as consumed in Greece, Crete, and Southern Italy during the 1960s (Davis et al., 2015). Its composition and related lifestyle-factors are almost the exact opposite of a Western diet (see Figure 3).

Even though differences exist regarding the exact quantities of food consumed as part of the Mediterranean diet, there is a consensus about its key components (Davis et al., 2015). These include a high intake of vegetables, fruits, cereals, legumes, nuts and olive oil (as principal source of fat), and moderate intake of fish, other meat, dairy products and red wine (normally consumed with meals) (Davis et al.,

2015;Yannakoulia et al., 2015). At the same time, sweets and refined sugar are of minor importance, and fast or convenient food is even completely omitted. This results in a beneficial profile on the macro- and micronutrient level. Being plant-based, the Mediterranean diet provides a lot of complex carbohydrates, fiber and bioactive compounds including polyphenols, phytosterols, B vitamins and folic acid (Davis et al., 2015). Furthermore, the diet includes sources of beneficial unsaturated fatty acids with fish and olive oil.

The Mediterranean diet influences the same biochemical pathways as described above for the Western diet. However, the components of the Mediterranean diet act beneficially, being anti-oxidative and anti-inflammatory (see Figure 4) (Psaltopoulou et al., 2013;Aridi et al., 2017). Many fruits and vegetables are rich in antioxidative components such as vitamins and carotenes (Crichton et al., 2013;Gotsis et al., 2015). These substances can function as a scavenger for free radicals and reactive oxygen species and thus attenuate oxidative stress (Gotsis et al., 2015). This limits the oxidation of lipids and proteins and avoids negative consequences such as the activation of microglia and the resulting impact on neuronal integrity as described in section 3. Furthermore, the high content of unsaturated fats and fiber elicits an anti-inflammatory response (Mori and Beilin, 2004;Aridi et al., 2017). This is mediated by lowering the levels of the nuclear transcription factor kappa B and the tumor necrosis factor alpha, both of which play a crucial role in systemic inflammation (Gotsis et al., 2015). Anti-inflammatory substances furthermore stimulate phagocytosis and thereby help to remove misfolded and potentially toxic substances (e.g. amyloid-β) (Heneka et al., 2015). This helps to maintain neuronal plasticity and can thus be considered neuroprotective (Heneka et al., 2015).

Within the framework of food synergy, it was suggested that the single constituents multiply their positive consequences by additive or synergistic effects on health (Jacobs and Tapsell, 2013). This is based on the assumption that within the matrix of a whole food/diet the composites are coordinated in a meaningful way (Jacobs and Tapsell, 2013). Nuts, for example, are high in unsaturated fatty acids. These are prevented from oxidation by a high amount of antioxidative compounds, which are present in nuts as well (Jacobs et al., 2009).

Besides its favorable composition, the Mediterranean diet is accompanied by a distinct lifestyle that might furthermore have a beneficial impact on overall and brain health. This includes engaging in physical activity and social support, sharing food as

part of lengthy meals and post-lunch siestas (Yannakoulia et al., 2015). These lifestyle-behaviors are evidence-based linked with cognitive health and could multiply their impact in synergistic-associations. For more details see the review by Yannakoulia et al. (2015).

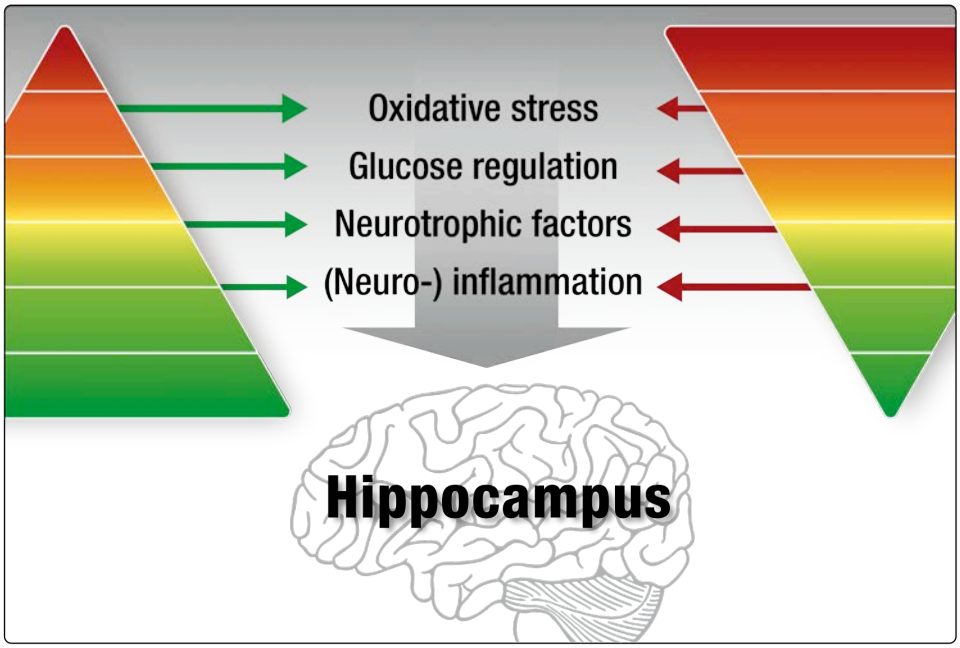

Figure 4: The impact of Mediterranean and Western diet on brain health.

Shown are the biological consequences of the Mediterranean and Western diet according to proposed underlying biochemical pathways. Green arrows indicate beneficial influence of the Mediterranean diet; red arrows indicate negative influence of a Western-type diet. The hippocampus is one of the most likely affected areas within the brain.

4.2. Effectiveness of the Mediterranean diet

The effectiveness of the Mediterranean diet was investigated in several observational, cross-sectional, and longitudinal studies. A recent systematic review with 31 studies concluded that especially cohort studies conducted in the Mediterranean area or randomized controlled trials showed promising results for the beneficial effect of the Mediterranean diet on cognition (Aridi et al., 2017). Here, the available randomized controlled trials should be further evaluated. As part of the parallel-group, multicenter, randomized trial Prevención con Dieta Mediterránea (PREDIMED)-study Martinez-Lapiscina et al. (2013) compared two types of the Mediterranean diet with either added extra-virgin olive oil or mixed nuts against a low-fat diet control group in 522 elderly participants at high vascular risk. After 6.5 years

of intervention, both Mediterranean diet groups showed higher performance in the mini-mental status examination (MMSE) as well as in visuo-spatial and executive skills compared to control (Martinez-Lapiscina et al., 2013). The MMSE is widely used in clinical practice to screen for mental impairment, but its sensitivity and the value for longitudinal studies seems to be limited (Strauss, 2006). It is fast, and easily administered and uncomplicated to score, and assesses amongst others hippocampus-related features, such as immediate and delayed recall (Strauss, 2006). In the analyses, the authors took into account multiple potential confounders including age, family history of cognitive impairment or dementia, apolipoprotein E genotype, education, physical activity and energy intake (Martinez-Lapiscina et al., 2013). Importantly, the neglected to look into blood pressure, smoking, (over-) weight, or diabetes and cognitive performance was assessed only at follow up. Thus, the analyses were not adjusted for important confounders and baseline differences cannot be ruled out.

Another randomized controlled trial in the PREDIMED-study was conducted with 447 cognitively healthy volunteers in Barcelona and used the same intervention as in Martinez-Lapiscina et al. (2013) (Mediterranean diet with either extra-virgin olive oil or nuts and low-fat control diet). A cognitive test-battery was assessed both, at baseline and follow up and included among others the MMSE and Rey auditory verbal learning test (RAVLT). The RAVLT is similar to the CVLT and can be used to evaluate verbal learning and memory (Schmidt, 1996). After 4.1 years of intervention, data was available for 334 participants. Performance improvements within both Mediterranean diet groups were only observed in the RAVLT and the color trait test (assessing attention, mental flexibility, and motor function), while the control group declined in cognitive performance (Valls-Pedret et al., 2015). However, the study comprises a subsample of a larger clinical trial and analyses were only specified *post-hoc* (Valls-Pedret et al., 2015). In both PREDIMED trials described here, it is difficult to attribute possible beneficial effects to single components of the Mediterranean diet (e.g. nuts or olive oil) or even single substances within these components. To disentangle the effects of dietary patterns, single component or nutrient studies can be employed.

Summarizing, cognitive improvements after Mediterranean diet were especially observed in randomized controlled trials in at-risk populations. Nevertheless, these trials also showed serious limitations and should be confirmed in larger samples with

improved design and *a-priori* hypotheses. While, results were limited to a crude overall cognitive score (MMSE) and auditory memory, other cognitive domains (e.g. working memory and semantic fluency) were not significantly improved in the randomized controlled trials. Also, evidence derived from cohort studies in non-Mediterranean areas and cross-sectional studies were inconclusive. For a detailed review see Aridi et al. (2017). Furthermore, Aridi and colleagues emphasize the importance of a consensus on the definition of the Mediterranean diet, applied scoring systems and the variety of cognitive test batteries to develop comprehensive and meaningful studies (Aridi et al., 2017).

Nonetheless, the Mediterranean diet is a cost-effective and low-risk intervention that can be recommended for future trials investigating the prevention of cognitive decline with modifiable lifestyle factors and could have implications for cognitive health (Psaltopoulou et al., 2013;Tuso et al., 2013).

5. Exploring the mechanisms with single food and nutrient studies

Besides analyzing whole dietary patterns, studies attempted to disentangle the framework and focus on single foods or even isolated nutrients (see e.g. Krikorian et al., (2010), Brickman et al., (2014), Wightman et al., (2015), which are further discussed in section 6.2.). This is a way forward to unveil underlying mechanisms of beneficial effects of dietary patterns and could in turn advance the knowledge to develop efficient strategies to prevent, slow or even reverse cognitive decline.

Both approaches, however, whole dietary pattern and single food or nutrient studies, have their advantages and disadvantages (see Figure 5). In single nutrient studies the dosage of a single nutrient can be manipulated, while the rest of the diet remains the same. This allows the identification of possible dose-dependent effects and eliminates effects of other nutrients, which could possibly mask or counterbalance certain effects. On the other hand, food synergy effects as described in section 4.1 get attenuated and a generalization of findings to the daily diet is more difficult. Another difference between the study approaches is the duration. With single food and nutrient studies, high dosages can easily be administered and therefore effects might be expected in a shorter time frame. However, one needs to keep in mind that the decline of cognition is a gradual process and related diseases such as AD have long latency periods and might develop over years (Alzheimer's Association, 2017). Therefore, long-term observational studies evaluated with high-quality food frequency questionnaires might be more accomplishable.

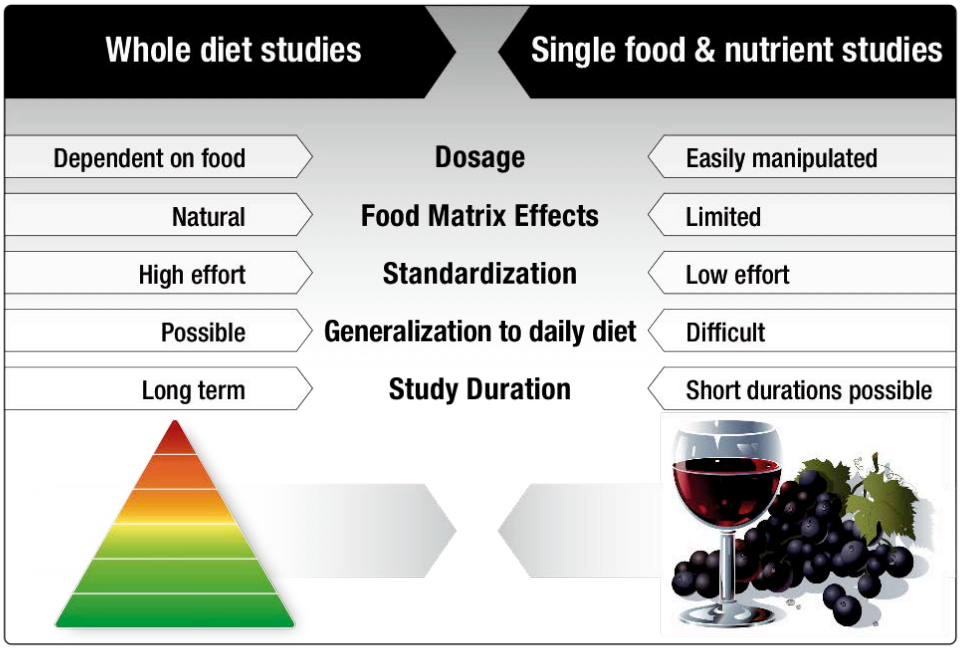

Figure 5: Comparison of whole diet and single food or nutrient studies.

The approaches, whole diet studies and single food or nutrient studies, can be compared according to the administered dosage, food matrix effects, the difficulty to standardize the approach, the possibilities for generalizations to the daily diet and the study duration. The Mediterranean and Western diets are examples for whole dietary approaches. Red wine and resveratrol are examples for a single foods and a nutrient.

In Publication 1 Huhn et al. (2015) we evaluated the literature regarding the effects of single components of the Mediterranean diet on cognition. To get a better understanding of this complex topic, we summarized current evidence for two components that distinguish the Mediterranean diet from other dietary patterns – polyunsaturated omega-3 fatty acids and polyphenols. We concluded that the evidence from animal studies and first interventional human trials for effects of polyphenols on cognitive functions is promising. Yet, only few controlled studies with considerable heterogeneity in design and quality were available (Crichton et al., 2013;Huhn et al., 2015). Therefore, we decided to conduct a randomized controlled trial with the most promising polyphenol resveratrol. With the “Resveratrol Study” we aimed to contribute to the body of evidence about the impact of polyphenols on cognitive functions (see Publication 2 Huhn et al., (2018)).

6. Resveratrol as promising nutrient for cognitive health

6.1. Possible mechanisms underlying the effects of resveratrol on cognition

Resveratrol can be found in natural dietary sources. The highest doses occur in red grapes, lingonberries, and cranberries, but can also be found in lower concentrations in peanuts and cocoa (Rothwell et al., 2013). The concentration of resveratrol varies with each plant and furthermore depends on external factors, such as the weather and exposure of the plant to stressors (Gambini et al., 2015). Therefore, within a scientific study, it is appropriate to use resveratrol supplements to ensure equal and standardized intake for all participants.

After ingestion resveratrol shows an oral absorption of around 75%, which is high compared to other polyphenols (Walle, 2011). After reaching the bloodstream via the gut, resveratrol is quickly metabolized in phase II in the liver into glucuronides and sulfites (Davinelli et al., 2012). Thus, the levels of free resveratrol in the blood are low. In contrast, accumulation seems to be higher within tissues and effects might therefore be evoked directly at the sites of action (Walle, 2011). Furthermore, resveratrol metabolites can function as a pool in the blood, from which active resveratrol could be released (Walle, 2011).

As a basis for research with humans, it is imperative that resveratrol is safe and well tolerated. This was investigated and confirmed for daily doses of resveratrol up to 5 g (for review see Cottart et al. (2014)). Only minor adverse events (e.g. flatulence, diarrhea) were described for chronic dosages higher than 0.5 g per day, and they always remained reversible (Cottart et al., 2014). However, in clinical populations, the administration of resveratrol should be considered carefully. A study with multiple myeloma patients had to be discontinued due to an unacceptable overall safety profile including severe adverse events and a possibly treatment-related death because of renal toxicity (Popat, 2013). Subjects in this trial received 5 g of SRT501, which is a micronized oral formulation with improved bioavailability (Popat, 2013). Furthermore, it needs to be considered that resveratrol might interact with estrogen-receptors due to the molecular similarity with estrogen (Bowers et al., 2000). But data is yet limited (Brown et al., 2010).

Regarding its biochemical properties, resveratrol was called a “promiscuous molecule” due to the number of binding partners, including enzymes and other proteins involved in inflammation, oxidation, regulation of metabolism, cell signaling and cell cycle regulation (Britton et al., 2015). Further discussed are resveratrol’s

antioxidative and anti-inflammatory actions, its implications as a calorie restriction mimetic, and neuroprotective agent.

The antioxidative properties of resveratrol are both, direct (based on inherent chemical properties) and indirect (based on activated pathways) (Manach et al., 2004;Iacopini et al., 2008;Spanier et al., 2009;McAnulty et al., 2013). Even though this was mainly established *in vitro*, human studies are in line with these findings (Pignatelli et al., 2006;Crichton et al., 2013). The anti-inflammatory potential of resveratrol was also initially shown *in vitro* (Jimenez-Gomez et al., 2013;Poulsen et al., 2015) and underlying pathways have been confirmed in animals subsequently (Wang et al., 2013). However, the effect remains to be fully established in humans (Poulsen et al., 2015). Given these properties, the intake of resveratrol might avoid the negative consequences of oxidation and inflammation on a biochemical level and maintain the neuronal integrity and synaptic plasticity. See section 3 and Figure 4 for further discussion of neuroprotective effects.

Resveratrol is also known to mimic the effects of calorie restriction. Calorie restriction per se is the deliberate reduction of calorie intake compared to baseline consumption, or ad libitum feeding in animals, without inducing malnutrition (Baur, 2010). Increased interest in calorie restriction was raised by the findings that it delays or prevents age-related diseases and even extends lifespan. This holds true for a variety of animal models ranging from yeast, worms and flies up to mammals and nonhuman primates (Wood et al., 2004;Baur, 2010;Russo et al., 2014;Ingram and Roth, 2015). The calorie restriction-mimicking effects of resveratrol seem to mainly arise from its potential to activate sirtuins (Kulkarni and Canto, 2015). Sirtuins are nicotinamide adenine dinucleotide (NAD^+) dependent deacetylases and adenosine diphosphate (ADP) ribosyltransferases and constitute the key mediators of calorie restriction (Hubbard and Sinclair, 2014). In humans, activation of the *silent mating type information regulation 2 homolog 1* (sirtuin1) results in beneficial alterations of DNA-repair, apoptosis, adult neurogenesis, and glucose-insulin homeostasis (Hubbard and Sinclair, 2014). Especially adult neurogenesis is a crucial, but controversially discussed process (Snyder, 2018). Adult-born neurons, seem to be more excitable and build more synaptic connections compared to mature neurons (Ho et al., 2013). In rats, resveratrol was found to increase hippocampal neurogenesis and prevent age-related memory decline (Kodali et al., 2015). This, in turn, could also influence functions of the hippocampus, such as early memory

formation, and pattern separation, but whether neurogenesis exists in humans is a highly debated topic (Ho et al., 2013;Snyder, 2018). Furthermore, insulin signaling within the hippocampus is involved in synaptic integrity, plasticity and function and impairments can lead to deficits in hippocampus-dependent learning (Biessels and Reagan, 2015). Thus, hippocampal insulin-signaling and neurogenesis have implications for cognitive processes, such as the formation of memories (Couillard-Despres et al., 2011;Cholerton et al., 2013;Ho et al., 2013;Lazarov and Marr, 2013;Biessels and Reagan, 2015). However, there remains controversy about whether adult hippocampal neurogenesis really extends to humans or can only be observed in animals (Snyder, 2018;Sorrells et al., 2018). Methodological challenges make human studies difficult to interpret and further investigations will be needed to determine if we need some conceptual recalibration with regard to adult hippocampal neurogenesis (Snyder, 2018).

Furthermore related to the activation of sirtuin1 by resveratrol, a beneficial effect on blood glucose levels and insulin sensitivity has been described (Liu et al., 2014). The glucose uptake itself is increased by resveratrol via an increased expression of the glucose transporter GLUT4 and the activation of glucose uptake in the absence of insulin as shown in animal models (Su et al., 2006;Chi et al., 2007;Jiang, 2008;Penumathsa et al., 2008). According to the meta-analysis by Liu et al. (2014) resveratrol ameliorates glucose control and insulin sensitivity in diabetic, but not in non-diabetic humans. However, even within a normal range of glucose levels, slight improvements in glucose control might already benefit memory performance (Kerti et al., 2013). In a cross-sectional study with 141 healthy, older individuals lower levels of glucose and glycated hemoglobin (HbA1c) – which is a long term marker of blood glucose concentration – were associated with improved performance in delayed word recall, word learning ability, and memory consolidation (Kerti et al., 2013). Both, hippocampus volume and microstructure were inversely correlated with levels of glucose and HbA1c (Kerti et al., 2013). Furthermore, pointing to underlying mechanisms, mediation analyses indicated that volume and microstructure of the hippocampus at least partly mediated the beneficial effects of lower HbA1c on memory (Kerti et al., 2013).

Nevertheless, whether all the calorie restriction-mimicking effects of resveratrol produce the same results as the actual reduction of caloric intake, remains to be fully established in human studies. In a trial with 50 healthy elderly subjects calorie

restriction was found to improve memory performance after 3 months of intervention (Witte et al., 2009). The normal- to overweight older participants were stratified into groups with either 30% of calorie restriction, 20% relative increased intake of unsaturated fatty acids or control (Witte et al., 2009). The reduced calorie intake was calculated relative to previous habits assessed with self-reported dietary records and necessary instructions were provided by clinical dietitians (Witte et al., 2009). After the intervention, verbal memory scores of the calorie restriction group showed a mean increase of 20% as compared to baseline (Witte et al., 2009). Regarding underlying mechanisms, a decrease in levels of fasting insulin and the inflammation marker high-sensitive c-reactive protein are pointed out (Witte et al., 2009).

Also, in another study, Prehn et al. (2016) described improved recognition memory in 19 postmenopausal obese women after 12 weeks of caloric restriction intervention. As possible underlying mechanisms, the findings were paralleled with changes of the hippocampus including increased gray matter volume and augmented resting-state functional connectivity to precuneus and angular gyrus, which are regions essential for memory formation (Prehn et al., 2016). Generalization of these results, however, is strongly limited due to the very specific study population and small sample size (Prehn et al., 2016).

In conclusion, the intake of resveratrol-containing food or supplements, could lead to beneficial changes on a biochemical level and ultimately maintain synaptic plasticity and neuronal integrity via various underlying mechanisms (for an overview see below Figure 6). This, in turn, could aid in the prevention of cognitive decline; yet, the evidence from human trials is still scarce.

6.2. Resveratrol intervention studies in humans

Most of the available studies on resveratrol that include cognitive measures used a food-based approach. They assessed, for example, the effects of chocolate, cocoa-flavonols, red wine, berry-juice or a pill-based nutraceutical (Nurk et al., 2009;Krikorian et al., 2010;Brickman et al., 2014;Small et al., 2014). These foods have a high concentration of resveratrol, but also contain other polyphenols and micronutrients (Bensalem et al., 2016). This renders it difficult to attribute the findings to resveratrol alone. Nevertheless, the studies concordantly reported beneficial effects of resveratrol-containing food. The results range over various cognitive domains (Nurk et al., 2009) including learning and memory performance (Krikorian et al., 2010), a pattern recognition task (Brickman et al., 2014) and processing speed

(Small et al., 2014). Taken together, the data suggest that dietary polyphenols, including resveratrol, can benefit brain health and cognitive functions (for reviews see (Bensalem et al., 2016;Figueira et al., 2017)).

The amount of studies investigating the impact of isolated resveratrol on cognition and underlying mechanisms is limited. Kennedy and colleagues described the effects of resveratrol from a more mechanistic point of view. Several of their studies show that acute resveratrol supplementation increases cerebral blood flow in a dose-dependent manner as measured with near-infrared spectroscopy (Kennedy et al., 2010;Wightman et al., 2014;Wightman et al., 2015). However, after chronic supplementation of 500 mg resveratrol for 28 days in 60 young (18-29 years) healthy participants Wightman et al. (2015) did not observe persistent changes of cerebral blood flow or clear changes in cognitive functions, such as learning and memory. Remarkably though, they observed increases in acute resveratrol metabolites in a subsample of seven participants, in contrast to previous acute dose research suggesting that plasma metabolites should not be present beyond 24 hours (Walle et al., 2004;Boocock et al., 2007;Wightman et al., 2015).

The group of Wong and Howe realized another approach linked to mechanistic insights. In several studies, they described the benefits of resveratrol for circulatory and cerebrovascular functions (Wong et al., 2011;Wong et al., 2013a;Wong et al., 2016;Evans et al., 2017). This is probably related to an improvement in endothelial vasodilator functions, which are crucial to enable the exchange of nutrients and waste products between blood and tissue (Wong et al., 2013b). However, these effects were found in at-risk populations, such as overweight, obese, diabetic individuals and post-menopausal women. In the latter population, additional improvements were described after resveratrol in overall cognition and verbal memory (Evans et al., 2017).

In another study 26 weeks of daily 200 mg resveratrol resulted in improved memory performance compared to placebo in 46 healthy, but overweight older individuals (Witte et al., 2014). Furthermore, they reported a reduced percentage of HbA1c, which again was correlated with a higher functional connectivity of the hippocampus as measured by resting-state functional magnetic resonance imaging (Witte et al., 2014). Notably, changes in functional connectivity seemed to mediate the increased memory performance. This furthermore implies that an ameliorated glucose metabolism could be one underlying mechanism of resveratrol's effects on cognitive

performance and that these changes might especially affect the hippocampus (Witte et al., 2014).

A recent meta-analysis concluded that resveratrol has no significant impact on memory and cognitive performance (Farzaei et al., 2017). However, doubts about the methodology and selection of studies for the meta-analysis have been raised by (Wong and Howe, 2018). They emphasize that their own studies have been omitted from the meta-analyses and the included studies have only been partly analyzed (Wong and Howe, 2018). Therefore, the results of the meta-analysis have to be interpreted with caution.

Summarizing, to achieve more conclusive results, future clinical trials are encouraged to include specific tests for cognitive functions, such as memory (Farzaei et al., 2017). This can, for instance, be achieved with a focus on the hippocampus as in the experimental work described in this thesis (see Figure 6 and Publication 2 Huhn et al. (2018)).

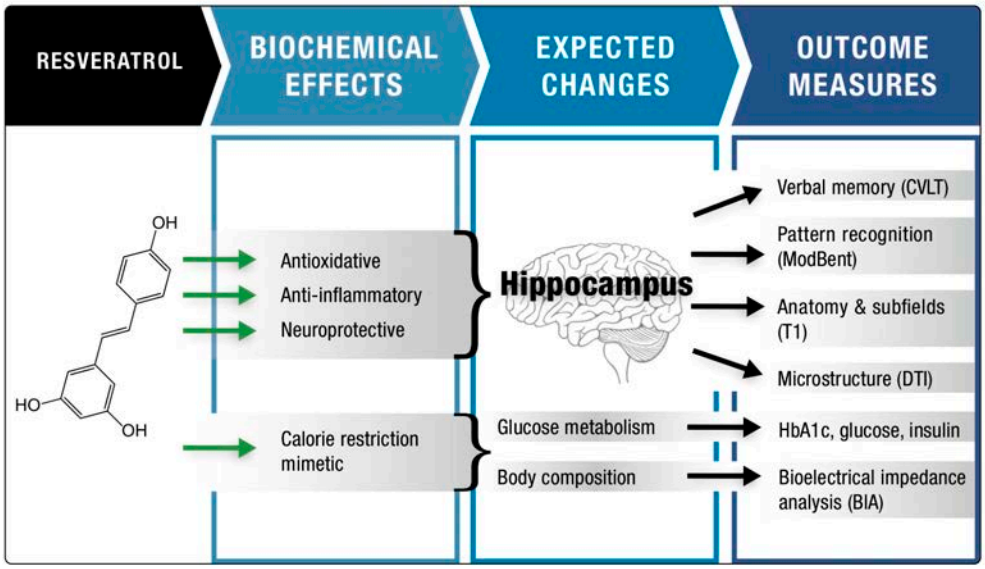

Figure 6: Measuring the effects of resveratrol in humans.

This flowchart shows the biochemical properties of resveratrol and their expected effects on the hippocampus, glucose metabolism, and body composition. Furthermore, it is exemplified which outcomes were assessed within the "Resveratrol Study" to measure the effects of resveratrol on cognition, neuroimaging modalities, blood parameters and anthropometric measures. CVLT = California Verbal Learning Task, DWI = Diffusion-weighted imaging, HbA1c = glycated hemoglobin, T1 = T1-weighted images.

7. Rationale for the experimental work – the "Resveratrol Study"

As argued before, nutrition can have a meaningful impact on cognition. However, underlying mechanisms are still elusive and remain to be established. One approach to advance in this endeavor is the analysis of single nutrients. In this regard, resveratrol has been identified as a promising candidate to prevent cognitive decline (for review see Publication 1). With its properties and underlying mechanisms, it might benefit brain health and cognitive functions.

Previous investigations on safety and tolerability of resveratrol form the basis for human research. First consequent studies with isolated resveratrol report enhanced memory performance, improved markers of glucose metabolism, and changes in cerebral blood flow or functional connectivity of the hippocampus (Kennedy et al., 2010;Witte et al., 2014). Nevertheless, findings are still conflicting with other studies reporting rather acute than chronic effects (Wightman et al., 2015). So far, however, studies are characterized by moderate sample sizes and inhomogeneous study designs rendering conclusions difficult (Farzaei et al., 2017). Difficulties to compare previous studies arise also from the heterogeneity in study design. Resveratrol might be differently effective in old versus young and normal-weight versus obese or diabetic individuals. In addition, the specificity of selected cognitive tests might vary in order to observe cognitive changes. Furthermore, the anatomical and functional complexity of the hippocampus and its substructures requires state-of-the-art, high-resolution magnetic resonance imaging to be appropriately addressed.

The "Resveratrol Study" aimed to advance the knowledge about effects of resveratrol on brain health with a special focus on hippocampal function. Therefore, we combined measures that were already sensitive to the effects of resveratrol in several previous publications. These included glucose, glycated hemoglobin, anthropometry, learning, memory, pattern separation, and neuroimaging at a higher field strength than in other studies. This was complemented with the assessment of various confounders ranging from apolipoprotein E genotype and further blood measures to physical activity, chronic stress and sleeping quality. With a thus optimized and improved overall design, the study contributes to the knowledge about effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults.

In more detail, we first of all aimed to test the assumption that a supplementation with 200 mg of the polyphenol resveratrol for 6 months in healthy, elderly individuals

would enhance memory performance measured with the CVLT in comparison to placebo. We furthermore aimed to more specifically assess the function of the hippocampus. Therefore, we included the ModBent task, which is a hippocampus-dependent memory task and assesses pattern separation skills (Brickman et al., 2014). To further acquire knowledge on the underlying mechanisms of resveratrol we hypothesized a beneficial impact of resveratrol on blood glucose metabolism as measured with the HbA1c, as well as changes in anatomical and functional connectivity measures of the hippocampus. In this regard, we used high-resolution magnetic resonance imaging at 7 Tesla, which allows subfield specific analyses at higher field strength than in any other previous study.

Summarizing, with this randomized controlled trial in healthy, elderly individuals we aimed to determine i) the effects of resveratrol supplementation on cognitive performance, ii) the related role of the hippocampus and iii) further underlying mechanisms of resveratrol's potential impact. For an overview see Figure 6. We thus hope to advance on the way to characterize the impact of nutrition on hippocampal function.

II Published Articles

Publication 1 – 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.

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

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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

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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).

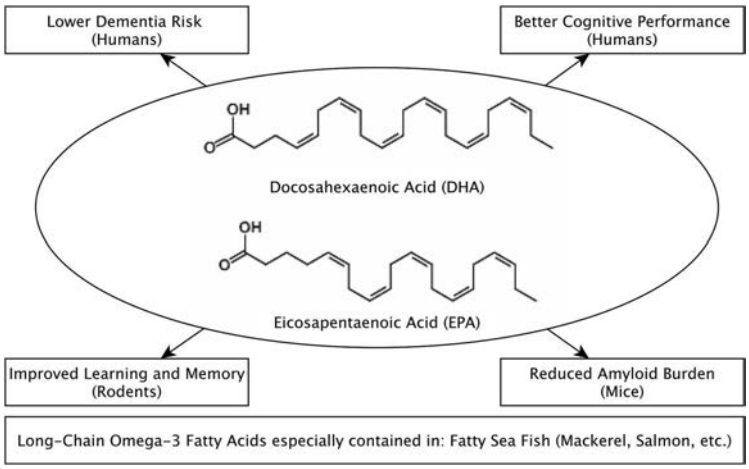

FIGURE 1 | Postulated effects of long-chain Omega-3 Fatty Acids (Eicosapentaenoic Acid, EPA and Docosahexaenoic Acid, DHA) with regard to brain health and their main dietary sources.

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

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

Author (year)	Participants sample size/age (years)		Duration	Intervention		Measured outcome		Results	
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

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).

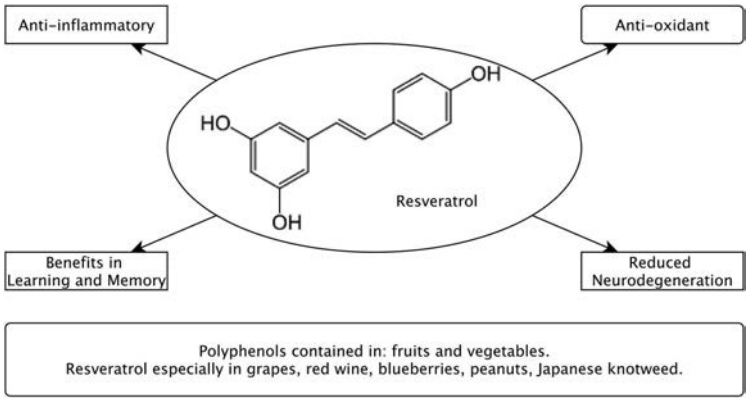

FIGURE 2 | Postulated effects of Polyphenols including Resveratrol with regard to brain health and their main dietary sources.

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.

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.

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Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults – A randomized controlled trial

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Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults – A randomized controlled trial☆

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ABSTRACT

Introduction: The polyphenol resveratrol has been suggested to exert beneficial effects on memory and the aging hippocampus due to calorie-restriction mimicking effects. However, the evidence based on human interventional studies is scarce. We therefore aimed to determine the effects of resveratrol on memory performance, and to identify potential underlying mechanisms using a broad array of blood-based biomarkers as well as hippocampus connectivity and microstructure assessed with ultra-high field magnetic resonance imaging (UHF-MRI).

Methods: In this double-blind, randomized controlled trial, 60 elderly participants (60–79 years) with a wide body-mass index (BMI) range of 21–37 kg/m^2 were randomized to receive either resveratrol (200 mg/day) or placebo for 26 weeks (registered at ClinicalTrials.gov: NCT02621554). Baseline and follow-up assessments included the California Verbal Learning Task (CVLT, main outcome), the ModBent task, anthropometry, markers of glucose and lipid metabolism, inflammation and neurotrophins derived from fasting blood, multimodal neuroimaging at 3 and 7 T, and questionnaires to assess confounding factors.

Results: Multivariate repeated-measures ANOVA did not detect significant time by group effects for CVLT performance. There was a trend for preserved pattern recognition memory after resveratrol, while performance decreased in the placebo group (n.s., $p = 0.07$). Further exploratory analyses showed increases in both groups over time in body fat, cholesterol, fasting glucose, interleukin 6, high sensitive C-reactive protein, tumor necrosis factor alpha and in mean diffusivity of the subiculum and presubiculum, as well as decreases in physical activity, brain-derived neurotrophic factor and insulin-like growth factor 1 at follow-up, which were partly more pronounced after resveratrol.

Discussion: This interventional study failed to show significant improvements in verbal memory after 6 months of resveratrol in healthy elderly with a wide BMI range. A non-significant trend emerged for positive effects on pattern recognition memory, while possible confounding effects of unfavorable changes in lifestyle behavior, neurotrophins and inflammatory markers occurred. Our findings also indicate the feasibility to detect (un)healthy aging-related changes in measures of hippocampus microstructure after 6 months using 7T diffusion MRI. More studies incorporating a longer duration and larger sample size are needed to determine if resveratrol enhances memory performance in healthy older adults.

☆ General Theme: Cognition and Aging.

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Abbreviations:

ASAT/ALAT	Alanine/Aspartate Aminotransferase
ApoE	Apolipoprotein E
BDI	Beck's Depression Inventory
BMI	Body Mass Index
CV	Coefficient of Variation
CVLT	California Verbal Learning Task
FC	Functional Connectivity
FWE	Family-Wise Error
HbA1c	Glycated hemoglobin
HDL/LDL	High/Low Density Lipoprotein
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MD	Mean Diffusivity
MMST	Mini Mental Status Test
RCT	Randomized Controlled Trial
SCD	Subjective Cognitive Decline
TFCE	Threshold-free Cluster Enhanced
TMT	Trail Making Task
UHF	Ultra-High Field
WHR	Waist Hip Ratio

Introduction

Food-derived polyphenols, common in the Mediterranean diet, have been suggested to exert beneficial effects on brain health (reviewed e.g. in Davinelli et al., 2012; Bastianetto et al., 2015; Huhn et al., 2015). One of the most extensively studied polyphenols is resveratrol, which occurs in various natural sources such as blueberries, peanuts, red grapes and red wine (Baur et al., 2006; Baur, 2010). *In vitro* as well as *in vivo* rodent and primate studies provided evidence for antioxidative, anti-inflammatory and calorie-restriction mimicking characteristics of resveratrol (Baur, 2010; Bastianetto et al., 2015; Kulkarni and Canto, 2015). These effects have been discussed to contribute to improvements in glucose-metabolism and cardiovascular factors (reviewed in Liu et al., 2014; Kakoti et al., 2015; Huang et al., 2016), and eventually to preserved brain structure and neuronal function (discussed in Davinelli et al., 2012; Huhn et al., 2015; Tellone et al., 2015; Wong and Howe, 2018).

While preclinical studies yielded exciting results, data from interventional human studies on the effect of polyphenols on brain structure and cognition is scarce. Using small- to moderate sample sizes, few randomized controlled trials (RCTs) in older adults reported improved memory performance after supplementary intake of berry juice or formulas with cocoa-flavonol or other polyphenol-containing ingredients (Krikorian et al., 2010; Brickman et al., 2014; Small et al., 2014). A memory-enhancing effect in older adults has also been reported in two RCTs for the intake of isolated resveratrol (150–200 mg/day for 3 or 6 months) (Witte et al., 2014; Evans et al., 2017). In contrast, studies in younger age or patient groups did not detect significant effects of resveratrol on cognitive functions (Turner et al., 2015; Wightman et al., 2015; Zortea et al., 2016; Kobe et al., 2017). Also, a recent meta-analysis (Farzaei et al., 2017) including 255 participants of four studies (Witte et al., 2014; Wightman et al., 2015; Evans et al., 2017; Kobe et al., 2017) concluded that resveratrol has no significant impact on cognitive performance.

Only few human studies so far included measures that could yield underlying mechanistic insights. Two studies led by Kennedy and colleagues suggested that acute doses of 250 mg or 500 mg resveratrol enhance cerebral blood flow (Kennedy et al., 2010; Wightman et al., 2015). Studies with a longer duration of resveratrol supplementation (3–6 months) reported improvements in cerebrovascular responsiveness to hypercapnia (Evans et al., 2017) and changes in magnetic resonance imaging (MRI)-based measures of functional connectivity (FC) of the hippocampus, a key region involved in memory processes (Witte et al., 2014; Kobe et al., 2017). Witte et al. (2014) could also observe decreased levels of glycated hemoglobin (HbA1c), a long-term marker of glucose control, after resveratrol supplementation, which in turn correlated with resveratrol-induced improvements in functional connectivity and verbal memory. However, the hypothesis that resveratrol enhances human memory performance via improvements or maintenance of hippocampus functioning in aging remains to be established, and potentially related mechanistic pathways are still debated (Huhn et al., 2015; Dias et al., 2016; Figueira et al., 2017). Notably, so far only few longitudinal studies in healthy elderly were able to show plastic changes in either regional hippocampus blood volume (Pereira et al., 2007; Brickman et al., 2014) or functional connectivity and volume (Erickson et al., 2011; Witte et al., 2014) that followed a dose-response relationship with memory improvements after plasticity-enhancing interventions such as physical exercise or polyphenol diets. Similarly, in longitudinal studies, systemic changes such as improvements in physical activity or glucose metabolism have only occasionally been linked to selective changes in the hippocampus (Erickson et al., 2011; Cherbuin et al., 2012; Maass et al., 2015; Prehn et al., 2016).

This might be explained in part by the regional complexity of the hippocampus and its sub-structures that have distinct morphological and functional properties, as indicated by preclinical and post-mortem studies (Mueller et al., 2011; Robinson et al., 2016). Using ultra-high field (UHF) MRI, though, it is now possible to delineate hippocampus subfields *in vivo* with higher signal-to-noise ratio and higher spatial resolution. This also enables to identify plastic changes at the subfield level more reliably (Iglesias et al., 2016; Giuliano et al., 2017). Implementing 7 Tesla (7T) UHF MRI in interventional studies would thus help to better understand if and how the hippocampus translates potential plasticity-enhancing effects of a systemic factor, such as diet, into specific improvements in cognition (in this case memory). Therefore, we aimed to examine the effects of resveratrol on memory performance in an independent sample of older adults by employing sensitive memory tests, state-of-the-art 7T UHF MRI, and a broad array of blood-based biomarkers. We hypothesized that 6 months of resveratrol supplementation leads to improvements in memory performance, assessed with the California Verbal Learning Task (CVLT; Niemann et al., 2008). Secondary hypotheses included an improvement in glucose metabolism, reflected in lower HbA1c levels after resveratrol supplementation, and improvements in pattern recognition memory (Brickman et al., 2014). In addition, we hypothesized resveratrol-induced improvements in functional connectivity of the hippocampus within the default-mode network, and in measures of regional hippocampus volume and microstructure, assessed using 7T UHF MRI.

Material and methods

Participants and study design

Sixty healthy elderly participants (60–78 years) were recruited via the Max Planck Institute's database and local advertisements in Leipzig, Germany. The research protocol was approved by the Ethics Committee of the University of Leipzig and was conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent and received reimbursement for participation. The trial was registered and a study protocol was uploaded at ClinicalTrials.gov with the identifier NCT02621554. Baseline assessments were acquired from April to July 2016 and follow-up from October 2016 to January 2017 at the Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany.

Potential participants were first interviewed via telephone to screen for eligibility criteria, i.e. over 60 years of age, body-mass index (BMI) between 22 kg/m^2 and 40 kg/m^2. Exclusion criteria were MRI

contraindications (e.g. metal implants, pacemaker, tattoos), history of stroke, current psychiatric disease, pregnancy, diabetes mellitus type 2 or other severe internal diseases (e.g. affecting the gastro-intestinal tract, lungs, heart, vascular system, liver or kidney), intake of antidepressants or antioxidative supplements, daily consumption of more than 50 g alcohol, 6 cups of coffee or 10 cigarettes (see Figure 2, all based on self-reported information).

At baseline visits, participants completed a medical interview including disease and medication anamnesis. To exclude subjects with objective cognitive impairments, a cut-off level for performance in the Mini Mental State Examination (MMSE; Folstein et al., 1975) of < 26 out of 30 possible points was employed.

Participants were instructed to keep their diet and amount of physical activity throughout the study duration unchanged.

Sample size determination and attrition

The number of subjects needed for this trial was determined with a power calculation (Faul et al., 2007; Faul et al., 2009). The primary outcome measure was a change in four subscales of the California Verbal Learning Task after 6 months resveratrol supplementation. Based on previous studies (Witte et al., 2009; Witte et al., 2013; Witte et al., 2014) an effect-size (d) of 0.4 was assumed. With a significance level $\alpha = 0.05/4 = 0.0125$ (Bonferroni-corrected for multiple comparisons) and a power of 95%, power analysis revealed a required sample size of 44 subjects. Assuming a drop out rate of 30% we aimed to recruit 60 participants.

During recruitment 193 participants were contacted. Of those, 66 did not meet inclusion criteria (29 with diabetes, other diseases or centrally active medication, 28 not MRI-suitable, 9 out of BMI range), 61 were not interested in participating in the study and another six were not included without further specified reasons (Figure 1). Thus, 60 subjects were included into the study and randomly assigned to either intervention group ($n = 30$) or placebo ($n = 30$) by a researcher who was not involved in data acquisition. The intervention/placebo period was designed as a parallel-group and double blind trial with 60 subjects with balanced (1:1) randomization to either placebo or resveratrol group, stratified for age (60–70 or >70 years) and sex with a block size of four. A web-based randomization system (www.randomization.com) was used. The last participant was assigned in a way to get balanced groups with 30 subjects irrespective of the randomization. After baseline-assessments and randomization two participants of the placebo group were excluded due to medication matching exclusion criteria. Another five participants dropped out during the intervention period and were unavailable for follow-up measurements. The dropout reasons in the resveratrol group were a sudden decrease in eyesight ($n = 1$; after 18 weeks of pill intake), a skin rash ($n = 1$; after 5 weeks of pill intake) and without further specification ($n = 1$). The two dropouts in the placebo group were due to personal reasons ($n = 1$) and lost contact ($n = 1$). Other reported adverse events that did not lead to exclusion or dropouts included stomach aches ($n = 1/1$; resveratrol/placebo), diarrhea ($n = 3/1$), dizziness ($n = 1/3$), vomiting ($n = 0/1$), skin changes ($n = 3/3$), mood changes (improvements $n = 1/1$; decline $n = 0/3$), hot flashes ($n = 0/1$), loss of hair ($n = 1/0$), reflux ($n = 0/1$), rotating vertigo ($n = 0/1$), tight feeling in the chest ($n = 0/2$), blood pressure fluctuations ($n = 0/1$).

Intervention and compliance

Subjects were instructed to take two pills per day (one in the morning and one in the afternoon) over 26 weeks. Resveratrol pills per day contained 2×100 mg $= 200$ mg resveratrol (3,5,4′-trihydroxy-trans-stilbene) and 2×160 mg $= 320$ mg quercetin to increase bioavailability (Smoliga and Blanchard, 2014) in line with (Witte et al., 2014). Placebo pills were identical in color and shape, but contained exclusively the filling material (microcrystalline cellulose). Resveratrol was produced using a yeast fermentation process and all pills were manufactured by Evolva SA (Basel, Switzerland) and provided at no costs. Sponsoring occurred without any terms or research assignments. At baseline participants received a pill supply for 18 weeks and another pill supply at an interim visit. At interim and follow-up visits, pill count and anamnis of adverse events took place. Subjects and investigators were blinded for the duration of the study to the treatment group. To estimate compliance, remaining capsules at interim and follow-up visits were counted and participants were asked to keep a pill diary. After follow-up, diaries and pill counts of 41 participants were available. Additional 12 diaries/pill counts were available for interim visits.

Outcome measures

For all participants at baseline and follow-up visits, we assessed neuropsychological tests, blood parameters, anthropometric measures, and neuroimaging (see Figure 2). All measures were executed according to pre-specified protocols by trained staff. Cognitive testing was performed on assessment day 2 before the 7T MRI scanning procedure. Due to limited scanning slots assessment time was at baseline between 8 a.m. and 4 p.m. and at follow up between 9 a.m. and 4 p.m.

Neuropsychological testing

We assessed verbal memory performance, i.e. learning ability, delayed recall, retention of words/forgetting rate and recognition according to previous studies (Witte et al., 2014, Kobe et al., 2017), with the German version of the California Verbal Learning Task (CVLT) (Niemann et al., 2008). The investigator read out loud a wordlist, consisting of 16 words and participants had to remember as many words as possible. Words belonged to one of four categories (e.g. spices, drinks, toys). The same list was repeated over 5 consecutive immediate recall trials (trials 1–5). Then, a second distractor list was presented in the same way for one trial. Afterwards, an immediate free recall trial occurred and was followed by a cued recall trial, where participants were asked to recall words according to the four categories. After a delay of 15–20 min in which participants underwent anthropometric measurements (see below), they were asked to recall the words of the first list in a long delayed free recall trial. Subsequently, the investigator read out loud a list of 48 words in a recognition trial and participants had to decide whether the word belonged to list A (yes/no-answer). The outcome "*Learning ability*" was defined as sum of correctly given words after trials 1–5. "*Delayed recall*" was the number of correct words in the long delayed recall trial. "*Forgetting rate*" was calculated by subtracting the number of correct words of trial 5 from the delayed free recall. "*Recognition*" was calculated by the number of correctly identified words of the recognition trial minus false-positives. Two parallel versions at random order were used at baseline and follow up. Assessment time of day for cognitive testing did not correlate with baseline task performance in the CVLT (all $p > 0.05$) and assessment time of day did not change between baseline and follow up dependent on group ($p = 0.77$).

In addition, attention and mental flexibility were assessed using the Trail Making Task (TMT) (Reitan and Wolfson, 1985). Briefly, participants had to connect numbers or letters that were randomly distributed over a sheet of paper with a pencil in ascending order as fast as possible. Part A comprised numbers (1–25), in part B participants had to alternate between numbers (1–13) and letters (A–L). Outcome measures were reaction time (in seconds) to complete part A and part B, and their ratio (time part B / time part A).

ModBent task

Pattern recognition performance was assessed with the ModBent Task (Brickman et al., 2014). The first part of this computer-based test consisted of 41 trials. During each trial the participants were asked to memorize a so-called Lissajous-figure that was presented on screen for 10 s. A Lissajous-figure is a sinusoidal curve derived in a mathematically

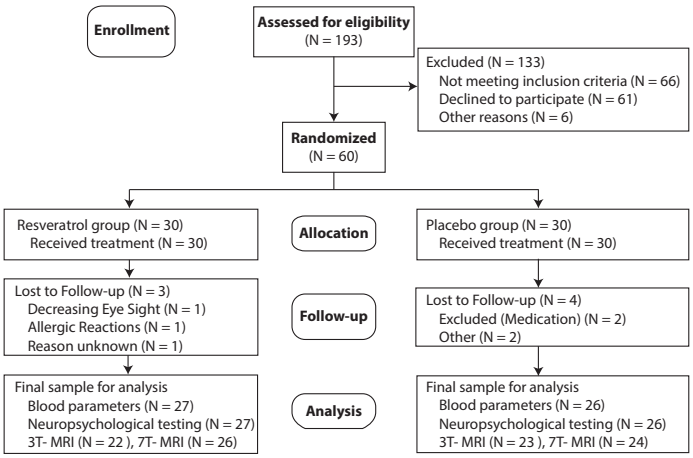

Figure 1. Flowchart Participants. 193 participants were assessed for eligibility. 133 had to be excluded because they did not meet inclusion criteria (n = 66), declined to participate (n = 61) or due to other reasons (n = 6). 60 participants could then be randomized to placebo (n = 30) and resveratrol group (n = 30). Three participants receiving resveratrol were lost to follow-up because of a decrease in eyesight (n = 1), an allergic reaction (n = 1) and without further specifications (n = 1). Two participants belonging to the placebo group had to be excluded after baseline assessment, because of intake of medication that did not meet inclusion criteria of the study protocol. Finally, analysis for blood parameters was available for n = 26/24 (resveratrol/placebo), neuropsychological tests n = 27/26 and magnetic resonance imaging (MRI) with good image quality at 3 Tesla (3T) n = 22/23 and at 7 T n = 26/24.

controlled manner and figures differ in order, e.g. number of horizontal and vertical nodes (see Figure 2). After a delay of 1 s participants had to choose the previously presented figure out of two figures from the same Lissajous-order. The task included 41 different figures and different parallel versions were used at baseline and follow-up. The second part of the ModBent Task contained 82 recognition trials. Participants were presented one figure at a time and had to decide whether the object was identical to any previous target stimulus. Outcome measures included the sensitivity index (d'), calculated as d' = z (hits) - z (false alarms), according to signal detection theory (Hochhaus, 1972) and mean reaction time in correct rejection trials (Brickman et al., 2014). Further details are provided in the supplementary material. Five participants had to be excluded from the analysis due to systematic response bias (always pressing "yes" or "no") in either baseline or follow-up measure, one had to be excluded due to a program error at follow-up, leaving n = 44 for analysis.

Blood parameters and anthropometric measurements

Overnight fasted blood samples were collected and immediately submitted to the laboratory. To assess glucose-metabolism, glycated hemoglobin (HbA1c, Institute for Medical Diagnostics (IMD) Berlin-Potsdam, Germany), glucose (Cobas 8000, Roche diagnostics, Mannheim, Germany) and insulin (Liaison, DiaSorin, Dietzenbach, Germany) were measured. In addition, liver parameters, lipid-metabolism (total cholesterol, high- and low-density lipoproteins (HDL, LDL), triacylglycerides), inflammatory markers (interleukin 6 (IL-6) and high-sensitive C-reactive protein (hsCRP)) were determined by standard clinical chemistry procedures (Cobas 8000, Roche Diagnostics, Mannheim; Germany).Main target parameters as tumor necrosis factor (TNF α, interassay coefficient of variation (CV) 2.10–9.01%), brain derived neurotrophic factor (BDNF, CV 5.90–15.97%) (both from R&D Systems, Wiesbaden, Germany), leptin (CV 6.06–10.58%; Mediagnost, Reutlingen, Germany) and insulin-like growth factor 1 (IGF-1, CV 3.5–7.2%; iSYS, IDS, Frankfurt/Main, Germany) were measured by immunoassays. Blood count measurements were performed by the SYSMEX system (Norderstedt, Germany). IL-6 measures below detection limit were set to 1.50 pg/ml (lowest value). Due to technical reasons, HbA1c measures were missing in 3 subjects (n (resveratrol) = 2; n (placebo) = 1) and insulin in one resveratrol subject.

Unconjugated resveratrol and its main metabolites (sulfated, glucuronated, sulfo-glucuronated) were determined in serum samples by 3S-Pharma, Bucharest, Romania using high performance liquid chromatography. Details can be found elsewhere (Liu et al., 2010; Sergides et al., 2016). All values below detection threshold were set to 0. Furthermore, no baseline serum samples were available for two participants of the resveratrol group. Therefore, values were substituted by group medians (i.e. 0).

Anthropometric measures included weight (kg), height (m), waist- and hip-circumference (cm) to calculate body-mass index (BMI, kg/m^2) and waist-hip-ratio (WHR). Furthermore, a bioelectrical impedance analysis (BIA) was performed to assess percentage of body-fat (Biacorpus RX4004M with phasertab-electrodes, MediCal Healthcare GmbH, Karlsruhe, Germany). The measurement was bilateral with eight electrodes (two attached to each hand and foot of the participant). Systolic and diastolic blood pressure was measured according to guidelines of the European Society of Hypertension (O'Brien et al., 2005).

Confounder assessment

Apolipoprotein E (ApoE) genotyping was performed with genomic DNA extracted from peripheral blood samples at The Medical Research Centre of the University of Leipzig. The rs7412 and rs429358 polymorphisms were genotyped using the KASPar SNP Genotyping assay (KBioscience Ltd, Hoddesdon, UK) according to the manufacturer's instructions on an ABI Prism 7500 Sequence Detecting System (Life technologies, Foster City, CA, USA). Genotype frequencies were in Hardy-Weinberg-equilibrium. To assess genotyping reproducibility, a random 10% selection of the sample was re-genotyped in both SNPs; all genotypes matched initial designated genotypes. To assess subjective cognitive decline (SCD), participants were asked about a recent memory decline and if they worried about those changes (Jessen et al., 2014). Participants who answered both questions in the affirmative were classified as having SCD. In addition, subjects filled in computer-aided questionnaires about education (6 levels: no degree, 9, 10, 12, 13 years of school, university degree), depressive symptoms (German version of Beck's Depression Inventory (BDI; Hautzinger et al., 1994), a multiple-choice vocabulary intelligence test (MWT-B; Lehrl, 1999), the International Physical Activity Questionnaire (IPAQ; IPAQ Group, 2002), the Trier Inventory for chronic stress (TICS; Schulz et al., 2004), Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989), and a validated Food Frequency Questionnaire (FFQ) used within the German Health Examination Survey for Adults (DEGS1) of the Robert-Koch Institute (Robert-Koch-Institute, 2009; Haftenberger et al., 2010; Gosswald et al., 2012).

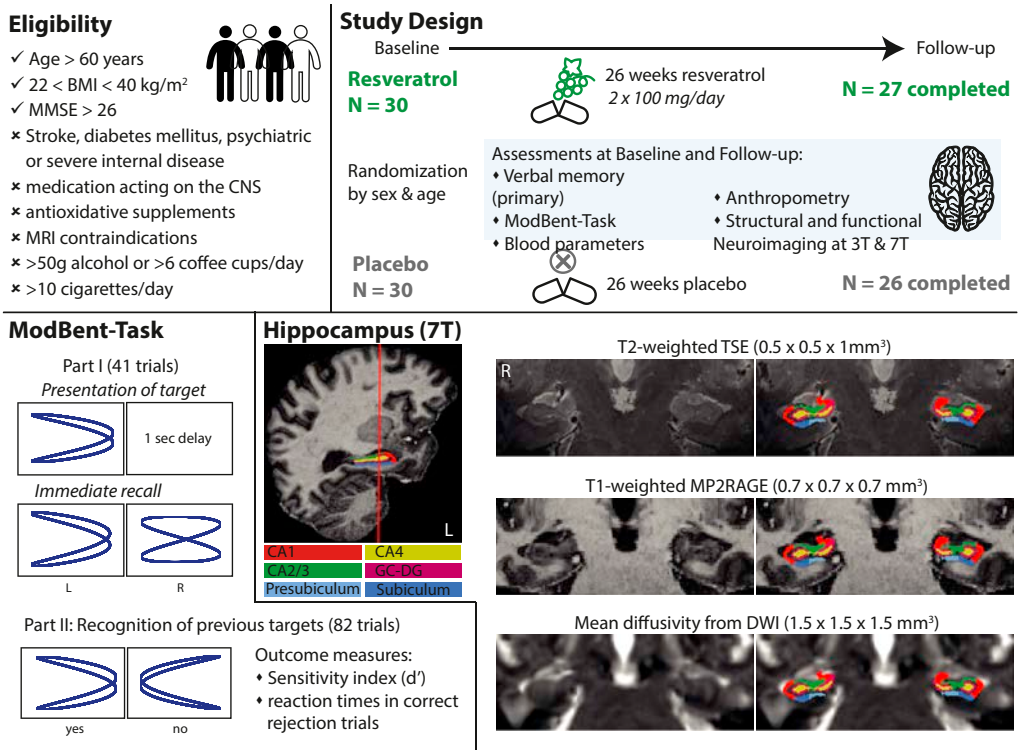

Figure 2. Study design and outcome measures. Healthy elderly subjects were screened for eligibility based on inclusion and exclusion criteria, and 60 participants were randomly assigned to either resveratrol or placebo group. Baseline and follow-up measurements included neuropsychological testing, fasting blood draw (glucose- and lipid-metabolism, inflammatory markers, neurotrophic factors), anthropometric measures (weight, height, waist- and hip-circumference, body fat), as well as multimodal neuroimaging. 27 participants receiving resveratrol and 26 participants receiving placebo completed 26 weeks of pill intake and were included in primary analyses of verbal memory performance. In addition, pattern recognition performance was tested using the ModBent task. Volume and mean diffusivity of hippocampus subfield was assessed using high-resolution MRI at 7 T. Abbreviations: BMI: body mass index, CA: cornu ammonis, CNS: central nervous system, DG: dentate gyrus, DWI: diffusion weighted imaging, MMSE: Mini Mental Status Examination, MP2RAGE: Magnetization-Prepared 2 RApid Gradient Echoes MRI: magnetic resonance imaging, T: Tesla, TSE: turbo spin echo.

Magnetic resonance imaging (MRI) acquisition and analysis

Anatomical imaging

Anatomical MRI for hippocampal volumetry was acquired at a Siemens Magnetom 7 T system (Siemens Healthineers, Erlangen, Germany) using a 32-channel head array coil (NOVA Medical Inc., Wilmington MA, USA). High-resolution T1-weighted images were acquired using a MP2RAGE (Marques et al., 2010) protocol (repetition time (TR) = 5000 ms; inversion time (TI) 1/2 = 900/2750 ms; echo time (TE) = 2.45 ms; image matrix: 320 × 320 × 240; voxel size 0.7 mm × 0.7 mm × 0.7 mm; flip angle 1/2 = 5°/3°; parallel imaging using GRAPPA (Griswold et al., 2002) with acceleration factor = 2). T2-weighted imaging slabs perpendicular to the anterior-posterior axis of the hippocampus were acquired using a Turbo-Spin Echo Sequence (TR = 13000 ms; TE = 14 ms; image matrix: 384 × 384; 50 slices; voxel size: 0.5 mm × 0.5 mm × 1 mm; refocusing flip angle = 120°; turbo factor = 8; parallel imaging using GRAPPA with acceleration factor = 2). Briefly, the bias-field corrected T1-weighted images provided by the MP2RAGE sequence were skull-stripped using CBS Tools (Bazin et al., 2014) and processed with the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/) in a longitudinal stream (Reuter et al., 2012). Hippocampal subfield segmentation was performed using the MP2RAGE and TSE images in a multimodal approach, which was initialized by the output of the longitudinal stream (Iglesias et al., 2015). For details, see **Supplementary information**. Out of 13 subfields and structures segmented by the algorithm, we considered six main subfields (Cornu Ammonis 1, 2/3, 4, dentate gyrus, presubiculum and subiculum) for further analysis (Erickson et al., 2011; Brickman et al., 2014). Four participants had to be excluded from 7T data analysis (n = 1 not MRI suitable at follow-up, n = 2 withdrew 7T MRI consent at follow-up, n = 1 poor data quality), leaving 49 for analysis (n = 25 resveratrol, n = 24 placebo).

Diffusion-weighted imaging analysis

Diffusion weighted images (DWI) were collected at 7T with a single shot echo planar imaging (EPI) sequence (TR = 6000 ms; TE = 62.8 ms; image matrix = 128 × 128, 60 slices; voxel size = 1.2 mm × 1.2 mm × 1.2 mm, 67 diffusion directions, b = 1000 s/mm^2, parallel imaging using GRAPPA with acceleration factor = 2). The imaging slab was chosen to cover the bilateral hippocampus in all participants. In order to correct for image distortions, an additional volume with no diffusion weighting (b = 0) but with opposite

phase-encoding direction was acquired. Briefly, diffusion weighted images were preprocessed using FSL (Smith et al., 2004) and mean diffusivity (MD)-slabs were registered to the T1-weighted MP2RAGE image using CBS Tools (Bazin et al., 2014) and ANTS (Avants et al., 2011). For details, see **Supplementary information**. Then, median hemisphere-averaged values of MD values > 0 and $< 0.002\,mm^2/s$ of the six hippocampus subfields were extracted to increase signal-to-noise ratio. One additional participant had to be excluded based on poor data quality, leaving 48 for analysis (n = 25 resveratrol, n = 23 placebo).

Resting state functional connectivity

To achieve whole brain coverage resting state fMRI was performed on a 3T Siemens Verio Scanner with a 32 channel head coil. T1-weighted images were acquired using an MP-RAGE sequence and the Alzheimer's Disease Neuroimaging Initiative standard protocol (TR = 2300 ms; TI = 900 ms; TE = 2.98 ms; image matrix = 256 × 240 × 176; voxel size = 1.0 mm × 1.0 mm × 1.0 mm; flip angle = 9°. T2*-weighted functional images were acquired using a multi-band echo-planar-imaging sequence with the following parameters: TR = 1400 ms; TE = 30 ms; image matrix = 88 × 88; 64 slices; voxel size = 2.3 mm × 2.3 mm × 2.3 mm; flip angle = 69°; multiband factor = 4; 550 volumes).

Briefly, preprocessing of the functional images was performed using FSL and included correction for motion, distortion and further nuisance variables and a bandpass-filtering between 0.01 and 0.1 Hz. FreeSurfer-derived masks of the left and right hippocampus at 3T images, divided into anterior and posterior division (Lerma-Usabiaga et al., 2016), were registered to the functional images (2 mm isotropic) and connectivity was estimated by correlating the mean time series in left and right posterior and anterior hippocampus with all other voxels in the brain. Connectivity maps were standardized, transformed to MNI space using ANTS (Avants et al., 2011) and smoothed with a Gaussian kernel of 6 mm full-width-at-half-maximum. A reproducible pipeline is available at https://github.com/fBeyer89/RSV_rsanalysis, for further details see **Supplementary Information**. In addition, as motion is an important confounder in rs-fMRI we performed a sensitivity analysis following the scrubbing approach described in (Power et al., 2014). To verify the selection of anterior and posterior hippocampus regions of interest, we calculated the within-subject differences of anterior and posterior hippocampus connectivity for the right and left hippocampus separately using available baseline MRI data (n = 51, 2 excluded due to strong head motion defined as exceeding a maximum framewise displacement of 3 mm). For longitudinal analysis, another 6 participants were excluded from analysis due to motion, leaving 45 for analysis (n = 22 resveratrol, n = 23 placebo).

Statistical analysis

Main analysis

To test the effects of resveratrol on memory performance, a multivariate repeated-measures analysis of variance ($MANOVA_{RM}$) was conducted with time (baseline, follow-up) as within-subject factor, group (resveratrol, placebo) as between-subject factor and the four primary outcome measures of the CVLT test (learning ability, forgetting rate, delayed recall and recognition) as dependent variables. The multivariate design allowed to assess potential intervention effects on the four outcomes with the same model. We additionally performed paired sample t-tests/Wilcoxon signed rank tests for within-group pre-post comparison and corrected for age, sex and education in a second analysis, as these variables are known to influence cognitive performance.

Exploratory analyses

In addition, performance of TMT and ModBent task, as well as changes in anthropometrics and blood parameters were compared using $ANOVA_{RM}$ to check for time and group effects, except for inflammatory markers (hsCRP, IL-6, TNF-a) which were tested using non-parametric tests on the differences between baseline and follow-up due to their skewed distribution. Volumes and MD of hippocampal subfields were compared using $MANOVA_{RM}$. Paired samples *t*-test and Wilcoxon signed rank test were used for within group comparisons as appropriate. Additionally, independent sample t-tests, Mann Whitney U-tests or χ^2-tests were performed to check for baseline differences between groups. All variables were checked for assumptions of normal or near-normal distribution (unimodal, |skewness| and |kurtosis| < 1). Significance level was set to $\alpha < 0.05$ unless indicated otherwise (two-sided). Statistical analysis was performed using SPSS (IBM, version 24).

Whole-brain connectivity analyses

First, anterior-posterior connectivity difference maps were tested using a one-sample-t-test in RANDOMISE with positive and negative contrast. Then, to test time by intervention-group interaction we calculated post – pre-difference maps of left, right, anterior and posterior hippocampus connectivity and compared them between groups using a two-sample independent *t*-test with FSL's RANDOMISE (5000 permutations) (Winkler et al., 2014). Significant results were based on threshold-free cluster enhanced (TFCE), family wise error (FWE) corrected p-values of $p < 0.05$ and differences were visualized based on thresholded t-maps with $|t| > 3$.

Results

Baseline characteristics

At baseline, the intervention and placebo group did not differ significantly in sex, age, years of education, MMSE, ApoE-status, SCD, depressive symptoms, verbal intelligence (measured with a vocabulary test), perceived stress, sleep quality, and BMI (see Table 1 for details). All participants met the MMSE inclusion criterion.

Compliance and change in mood and lifestyle factors

According to self-reported diaries and capsule counts, adherence to the capsule intake instructions was overall high in both groups (mean pill intake of > 94% ± 0.06; range: 74–100%). In serum, resveratrol and its metabolites could not be detected at baseline except in one participant of the resveratrol group showing a very low value of unconjugated resveratrol. At follow-up, there was a highly significant increase in serum measures of resveratrol and its metabolites in the intervention group, whereas values were again low in the placebo group ($ANOVA_{RM}$, all $F_{(1, 51)} > 21$, all $p < 0.001$, Table 2). While the biological activity of resveratrol and its metabolites is still incompletely understood, serum measures might be regarded as rather short-term markers of resveratrol intake (see Walle 2011 for further discussions). Evaluation of self-reported FFQ data did not indicate that participants in either group got high amounts of dietary resveratrol across the intervention/placebo period (see Supplementary Information).

Depressive symptoms, perceived stress, sleep quality and diet did not change during the intervention period according to self-reported information (all $p > 0.49$). However, there was a significant time effect on physical activity showing less physical activity at follow-up in both groups ($F_{(1, 51)} = 13.068$, $p < 0.001$, n = 53). This effect remained after exclusion of eight participants, whose data was rated as implausible due to extreme over-reporting ($F_{(1, 51)} = 12.449$ $p < 0.001$, n = 45, Supplementary S1). Please note that after baseline assessments the IPAQ data entry format was changed with the intention to avoid over-reporting: After baseline evaluation a tendency towards over-reporting was noticed that was related to the data entry format as hours and minutes. The correct way to enter the data for a 90 min workout had been: hours = 1, minutes = 30. However, during data curation we noticed that participants, who engaged in an activity for 3 h entered in the first field indicating the hours "3" and in the second field indicating minutes "180", obviously transforming hours to minutes. To avoid such an over-reporting the data entry format for follow-up was changed to minutes

Table 1
Baseline characteristics dependent on group. Data is given as mean ± standard deviation (SD) and range (minimum – maximum). a) Two missing values (1 resveratrol, 1 placebo) due to missing blood samples b) Verbal intelligence was measured using a vocabulary test c) presented values refer to the screening scale for chronic stress with low values referring to low stress d) presented values refer to the PSQI standard outcome; low values represent good quality of sleep e) Chi Square Test f) Independent Sample *t*-test, g) Mann-Whitney test.

Parameter	Resveratrol	Placebo	p-value
n (female/male)	27 (14/13)	26 (14/12)	0.88 [e]
Age (years)	68.60 ± 4.92 (61–78)	67.54 ± 5.07 (60–77)	0.46 [g]
Education (years)	15.20 ± 3.8 (10–18)	15.46 ± 3.89 (9–18)	0.89 [g]
Mini Mental Status Examination (score)	28.70 ± 1.2 (26–30)	28.88 ± 1.03 (26–30)	0.67 [g]
Apolipoprotein E Status (ApoE ε 4-Carrier n, %) [a]	9, 34.6%	8, 32%	0.84 [e]
Subjective Cognitive Decline (n, %)	Yes = 10 (37%) No = 17 (63%)	Yes = 10 (38.5%) No = 16 (61.5%)	0.91 [e]
Beck's Depression Index (score)	4.7 ± 3.2 (0–13)	5.46 ± 4.84 (0–16)	0.48 [f]
Verbal Intelligence [b]	119.8 ± 12.0 (97–143)	117.2 ± 13.5 (94–143)	0.46 [f]
Stress (values 0–48) [c]	8.93 ± 5.31 (0–21)	11.42 ± 6.2 (0–26)	0.178 [g]
Sleep (values 0–21) [d]	5.19 ± 3.08 (2–13)	5.42 ± 2.89 (1–12)	0.622 [g]
Body-Mass Index (BMI, kg/m^2)	26.5 ± 3.8 (22.2–36.2)	26.9 ± 4.6 (21.1–37.6)	0.86 [g]
Primary outcome measures of the California Verbal Learning Task			
Learning sum	44.52 ± 9.1 (18–62)	45.96 ± 8.9 (22–65)	0.69 [g]
Delayed recall	9.22 ± 2.9 (3–16)	9.08 ± 2.7 (4–15)	0.98 [g]
Forgetting rate	1.81 ± 3.3 (-5–7)	2.58 ± 2.5 (-3–7)	0.47 [g]
Recognition	12.56 ± 3.1 (4–16)	12.54 ± 2.6 (7–16)	0.67 [g]
Secondary outcome measures			
ModBent (d')	0.95 ± 0.66 (0–2.95)	1.34 ± 0.85 (-0.42–3.17)	0.052 [g]
Trail Making Task A (in seconds)	43 ± 13 (28–84)	41 ± 14 (16–79)	0.66 [g]
Trail Making Task B (in seconds)	89 ± 29 (45–161)	80 ± 25 (40–127)	0.33 [g]

only. Unfortunately, this might have introduced a systematic bias, as the subjective evaluation of an activity given in minutes only might be different from a time given in hours and minutes.

Memory performance

There was no significant time by intervention group interaction in the main analysis of performance of the CVLT after the intervention/placebo period according to $MANOVA_{RM}$ ($F_{(4,\ 48)} = 1.29$, $p = 0.29$; Figure 3). Correcting for age, sex and education did not change this result ($F_{(4,\ 45)} = 1.40$, $p = 0.25$). We observed a significant overall effect of time ($F_{(4,\ 48)} = 2.94$, $p = 0.03$) showing higher learning ($F_{(1,\ 51)} = 4.40$, $p = 0.041$) and delayed recall ($F_{(1,\ 51)} = 4.06$, $p = 0.049$) and a trend for lower forgetting rate ($F_{(1,\ 51)} = 3.39$, $p = 0.072$) in the whole group at follow-up. In addition, we detected an effect of the order of the parallel test versions that were used in the CVLT (time by order, $F_{(4,\ 46)} = 3.2$, $p = 0.021$). This might have contributed to our results, as random assignment to the parallel versions at baseline yielded in a different distribution of version order between groups (resveratrol/placebo, order 1: n = 18/9, order 2: n = 9/17). In exploratory analyses separately for the two version order subgroups, we did not observe significant time by group interactions (all $p > 0.6$).

We found no effects of APOE-genotype.

With regard to pattern recognition memory performance measured using the ModBent task, we observed a trend for a time by intervention group interaction for d' ($ANOVA_{RM}$ $F_{(1,\ 42)} = 3.46$, $p = 0.07$; Figure 4). Within-group analyses revealed decreases in d' in the placebo group ($t_{(21)} = 2.24$, $p = 0.036$), whereas the resveratrol group did not change ($p = 0.8$). Reaction times did not change significantly ($ANOVA_{RM}$ $p > 0.8$).

For the Trail making test, there was a significant effect of time for part A ($ANOVA_{RM}$, $F_{(1,\ 51)} = 5.1$, $p = 0.028$), indicating decreases in reaction times at follow up in the whole group (Table 2). No further time effects for part B and for ratio B/A nor group by time interactions for the three subscores emerged ($ANOVA_{RM}$, time: all $p > 0.12$, time by intervention group interactions: all $p > 0.4$). Correcting for age and sex did not change these results.

Fasting blood levels and anthropometric measures

For details on blood measures, see Table 2. There was no significant time by intervention group interaction for HbA1c levels ($ANOVA_{RM}$ $F_{(1,\ 48)} = 1.83$, $p = 0.18$; Figure 5, Table 2). Also within groups no significant changes of HbA1c were observed (resveratrol and placebo $p > 0.2$). In an exploratory analysis, we considered 'change in physical activity', 'change in BMI' as well as 'change in caloric intake' (as a proxy of dietary intake) as covariates in the model. When adding these covariates, there was a trend for a time by intervention group interaction effect on HbA1c values, indicating reductions in the resveratrol group at follow up ($F_{(1,\ 45)} = 3.9$, $p = 0.054$). When additionally adjusting for age and sex, the interaction term reached significance ($F_{(1,\ 43)} = 4.67$, $p = 0.036$).

In addition, there was a significant time effect for fasting glucose ($ANOVA_{RM}$ $F_{(1,\ 51)} = 13.97$, $p < 10\text{-}3$), showing increases in glucose levels (whole group, mean increase: 0.24 mmol/L ± 0.46 SD), while insulin did not change significantly (all $p > 0.27$). Further comparisons revealed a significant time by intervention group interaction for cholesterol ($ANOVA_{RM}$ $F_{(1,\ 51)} = 9.47$, $p = 0.003$), showing increases in the resveratrol group ($p = 0.006$, $t_{(26)} = 3.0$), while values of the placebo group slightly decreased (trend, $p = 0.087$). A significant decrease over time was observed for IGF-1 and BDNF levels in the whole group (all $ANOVA_{RM}$, IGF-1: $F_{(1,\ 51)} = 11.442$, $p = 0.001$, BDNF: $F_{(1,\ 51)} = 16.463$, $p < 0.001$). The decline in IGF-1 was more pronounced again in the resveratrol group ($t_{(26)} = 3.68$, $p < 10^{-3}$).

Considering inflammatory markers, we noticed overall increases in hsCRP, IL-6 and TNF-α in both groups (Wilcoxon-signed rank test, hsCRP, $Z = 2.26$, $p = 0.024$; IL-6, $Z = 3.88$, $p < 10^{-3}$; TNF-a, $Z = 2.73$, $p = 0.006$; Table 2). In addition, IL-6 levels showed larger increases over time in the resveratrol group compared to placebo (Mann-Whitney *U* test, $Z = 2.20$, $p = 0.028$).

At follow-up, participants also showed a significant increase in body weight and body fat in both groups. They gained on average 0.55 kg weight and 0.54% fat (weight: $ANOVA_{RM}$ $F_{(1,\ 51)} = 4.06$, $p = 0.049$; body fat: $ANOVA_{RM}$ $F_{(1,\ 51)} = 5.84$, $p = 0.019$), which was more pronounced in the resveratrol group (Table 2). An overall effect of time on diastolic blood pressure was not significant ($p = 0.11$), yet within-group comparisons indicated a decrease in diastolic blood pressure in the placebo group ($Z = 2.06$, $p = 0.039$). No further changes were observed.

Hippocampus subfield measures

$MANOVA_{RM}$ revealed no significant time ($F_{(6,\ 42)} = 0.96$, $p = 0.46$) or time by intervention group effect ($F_{(6,\ 42)} = 0.46$, $p = 0.84$) on hippocampus subfield volumes (Supplementary Table S1). Similar results were observed for MD ($MANOVA_{RM}$, time: $F_{(6,\ 41)} = 1.20$, $p = 0.33$, time by intervention group: $F_{(6,\ 41)} = 0.32$, $p = 0.93$, Supplementary Table S2), however, MD values seemed to decrease with time in the subiculum and presubiculum in both groups (Figure 6, univariate $ANOVA_{RM}$ time effect: subiculum $p = 0.049$, presubiculum $p = 0.012$). See Supplementary Figures S1-S2 for details on subject's variability.

Table 2
Changes in fasting serum levels and anthropometric measures according to groups. Bold numbers indicate significant differences. Data is given as mean ± standard deviation and range (minimum – maximum). a) Three participants were excluded due to missing values b) One participant excluded due to technical problems c) Dependent Samples T-Test, d) Wilcoxon Sign-Rank Test, e) all values below lower level of detection; Abbreviations: BDNF = brain-derived neurotrophic factor, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein, hsCRP = high-sensitivity C-reactive protein, IGF = insulin-like growth factor, LDL = low-density lipoprotein, MET = Metabolic Equivalent, TNF = tumor-necrosis factor

Parameter	Resveratrol (n = 27)			Placebo (n = 26)		
	Pre	*Post*	*p, T(df) or p, Z*	*Pre*	*Post*	*p, T(df) or p, Z*
HbA1c (%) [a]	5.63 ± 0.25 (5.23–6.27)	5.61 ± 0.30 (5.04–6.22)	0.202 [c]	5.55 ± 0.23 (5.14–6.21)	5.56 ± 0.2 (5.19–5.97)	0.564, c
Glucose (mmol/L)	5.17 ± 0.45 (4.38–6.05)	5.45 ± 0.66 (4.37–7.36)	**0.008**, −2.67 [d]	5.36 ± 0.57 (4.42–6.91)	5.55 ± 0.57 (4.35–6.81)	**0.029**, −2.19, d
Insulin (pmol/L) [b]	61.5 ± 39.41 (12.10–157.30)	72.6 ± 50.11 (9.10–231.40)	0.3[d]	65.36 ± 56.67 (13.60–286.80)	59.68 ± 39.41 (14.30–193.70)	0.53, d
Total Cholesterol (mmol/L)	5.74 ± 0.92 (4.15–7.66)	6.02 ± 1.07 (4.10–8.23)	**0.006**, −3.0 (26) [c]	5.99 ± 0.93 (4.74–8.32)	5.70 ± 0.83 (3.92–7.38)	0.087, c
LDL/HDL-ratio	2.5 ± 1.2 (0.82–4.86)	2.6 ± 1.2 (0.86–5.11)	0.7 [c]	2.3 ± 0.79 (0.69–4.04)	2.1 ± 0.7 (0.70–3.39)	0.1, c
Triacylglycerides (mmol/L)	1.22 ± 0.71 (0.57–3.60)	1.37 ± 0.87 (0.62–4.19)	0.77 [d]	1.16 ± 0.61 (0.53–2.89)	1.15 ± 0.45 (0.59–2.23)	0.35, d
Interleukin-6 (pg/ml)	2.25 ± 1.35 (1.50–6.76)	3.5 ± 2.16 (1.50–9.88)	**0.002**, −3.10 [c]	2.53 ± 3.25 (1.50–18.21)	3.12 ± 4.2 (1.50–23.17)	**0.032**, −2.143, d
HsCRP (mg/L)	2.33 ± 2.22 (0.28–8.90)	4.9 ± 8.02 (0.47–42.46)	**0.022**, −2.28 [c]	1.93 ± 2.39 (0.16–11.27)	3.82 ± 11.28 (0.24–58.58)	0.39, d
TNF-α (pg/ml)	0.89 ± 0.29 (0.57–1.77)	0.99 ± 0.43 (0.51–2.29)	0.099 [d]	0.76 ± 0.21 (0.48–1.20)	0.81 ± 0.2 (0.38–1.08)	**0.020**, −2.329 [d]
Leptin (μg/L)	13.46 ± 13.15 (0.57–50.73)	13.37 ± 12.82 (0.2–48.31)	0.719 [d]	11.43 ± 8.22 (1.01–24.25)	11.92 ± 9.11 (1.47–31.90)	0.675, d
IGF-1 (μg/L)	106.5 ± 24.5 (67.10–155.90)	96.2 ± 24.9 (57.80–138.90)	**0.001**, 3.7 (26) [c]	107.9 ± 34.5 (50.30–189.80)	103.3 ± 32.5 (38.20–172.70)	0.19 [c]
BDNF (ng/ml)	22.21 ± 6.12 (11.1–41.5)	25.48 ± 7.7 (14.3–46.4)	**0.015**, −2.43 [d]	26.3 ± 6.4 (10.9–39.8)	29.6 ± 6.1 (17.0–41.5)	**0.014**, −2.451, d
Unconjugated Resveratrol (ng/mL)	0.05 ± 0.18 (0.0–0.95)	4.90 ± 5.57 (0.0–19.52)	**0.001, -3.92** [d]	0.16 ± 0.81 (0.0–4.13)	0.0 ± 0.0 [e]	0.32, −1.00 [d]
Glucuronated Resveratrol (ng/mL)	0.26 ± 0.50 (0.0–1.94)	894.19 ± 593.07 (0.89–2308.54)	**0.001, -4.52** [d]	0.21 ± 0.50 (0.0–2.13)	0.73 ± 0.65 (0.0–2.88)	**0.007, -2.69** [d]
Sulfated Resveratrol (ng/mL)	0.39 ± 0.79 (0.0–2.42)	336.16 ± 217.91 (0.0–757.46)	**0.001, -4.52** [d]	0.12 ± 0.36 (0.0–1.39)	0.08 ± 0.29 (0.0–1.34)	0.50, −0.67 [d]
Sulfo-Glucuronated Resveratrol (ng/mL)	0.59 ± 0.99 (0.0–4.24)	155.85 ± 168.25 (1.26–748.73)	**0.001, -4.35** [d]	0.32 ± 0.49 (0.0–1.60)	0.21 ± 0.33 (0.0–1.37)	0.49, −0.686 [d]
Weight (kg)	75.54 ± 14.29 (57.0–112.0)	76.35 ± 14.61 (57.4–118.0)	0.062, −1.95 [c]	76.02 ± 17.32 (52.0–137.0)	76.29 ± 17.36 (52.6–138.0)	0.279, d
Body-Mass Index (kg/m^2)	26.76 ± 3.91 (22.2–36.2)	26.95 ± 4.06 (21.5–38.1)	0.113 [d]	26.94 ± 4.46 (21.1–37.6)	27.01 ± 4.28 (22.0–37.7)	0.424, d
Body fat (%)	29.07 ± 8.07 (15.7–42.8)	29.71 ± 7.97 (11.9–43.1)	**0.028**, −2.20 [d]	30.43 ± 8.47 (14.8–47.8)	30.87 ± 8.31 (16.3–44.9)	0.073, −1.791 d
Systolic Blood Pressure (mm Hg)	139.10 ± 16.17 (109.0–173.3)	139.95 ± 17.49 (116.7–185.3)	0.748 [c]	141.35 ± 17.63 (115.3–191.3)	138.08 ± 15.76 (112.7–183.7)	0.174, d
Diastolic Blood Pressure (mm Hg)	86.30 ± 8.86 (68.0–102.0)	85.89 ± 7.65 (74.0–101.0)	0.773, c	89.92 ± 11.40 (66.0–113.0)	87.38 ± 10.86 (70.0–118.0)	**0.039**, −2.061 [d]
Trail Making Task A (sec)	42.65 ± 12.83 (27.8–83.6)	38.96 ± 17.83 (18.68–112.62)	0.15, d	40.66 ± 13.72 (16.0–79.0)	35.90 ± 19.18 (16.56–54.72)	0.16, d
Trail Making Task B (sec)	88.56 ± 28.88 (45.4–160.9)	86.08 ± 28.06 (42.0–156.03)	0.52, d	80.32 ± 24.91 (40.0–127.4)	75.47 ± 25.38 (28.97–135.90)	0.27, d
Trail Making Task B/A	2.16 ± 0.73 (1.23–4.32)	2.43 ± 0.93 (1.25–4.25)	0.12, d	2.06 ± 0.53 (1.32–3.24)	2.14 ± 0.58 (1.40–3.73)	0.79, d

Resting state functional connectivity of the hippocampus

At baseline, the anterior hippocampus was significantly stronger connected to the parahippocampal gyrus and the temporal lobe than the posterior hippocampus (TFCE, FWE-corrected, $p < 0.05$; Figure 7). The posterior hippocampus was more connected to precuneus and angular gyrus but this effect did not survive correction for multiple comparisons (TFCE, uncorrected $p < 0.05$). The results were similar for both hemispheres.

In the longitudinal analysis, we found no significant difference in hippocampus connectivity change between the intervention and control group at a FWE-corrected level of $p < 0.05$ using TFCE. These results remained unchanged after removing volumes affected by motion in 28 subjects.

Discussion

In this randomized controlled interventional study we did not detect significant effects of 26 weeks resveratrol intake compared to placebo on verbal memory performance measured using the CVLT in healthy elderly. In exploratory analyses, we observed a non-significant trend for stable performance in a pattern recognition task in the resveratrol group, while performance decreased in the placebo group. HbA1c levels as well as hippocampus volume, microstructure and functional connectivity did not change significantly compared to placebo. In contrast, we noticed increases in serum cholesterol only in the resveratrol group and increases in weight, body fat, fasting glucose and inflammatory markers, as well as decreases in physical activity and neurotrophic factors in both groups.

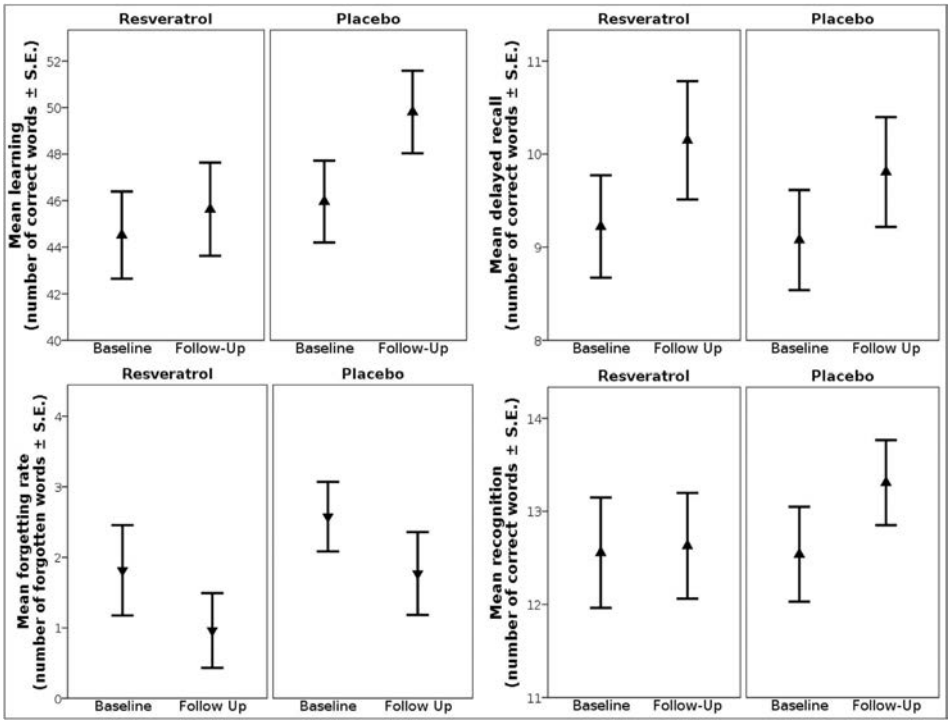

Figure 3. Memory performance was measured with the California Verbal Learning Task, at baseline and after 6 months of either resveratrol (n = 27) or placebo intake (n = 26). Multivariate analyses detected an overall positive effect of time in both groups (p = 0.03), but no time by intervention group interaction. Triangles represent mean, error bars represent standard error (S.E.).

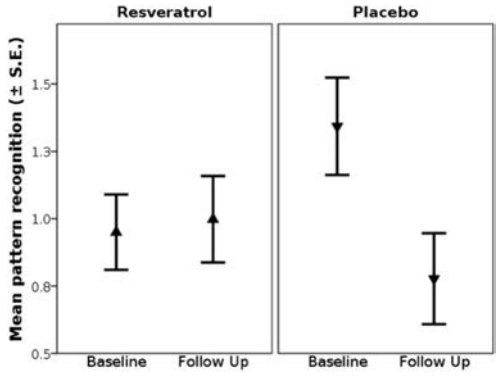

Figure 4. Pattern recognition memory performance, measured using the Mod-Bent task, at baseline and after 6 months of either resveratrol (n = 22) or placebo intake (n = 22), showing a trend for a time by intervention group interaction for sensitivity index d' ($ANOVA_{RM}$ $F_{(1, 42)} = 3.46$, p = 0.07). Triangles represent mean, error bars represent standard error (S.E.).

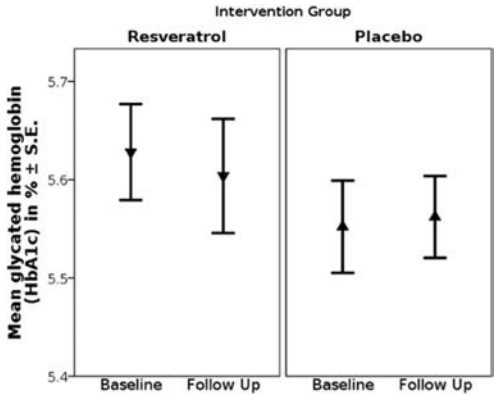

Figure 5. Glycated Hemoglobin (HbA1c) levels. There was no significant effect of time on HbA1c levels between or within groups ($ANOVA_{RM}$ p = 0.18, F = 1.83, Paired samples t-tests p > 0.2). Triangles represent mean, error bars represent standard error (S.E.).

Resveratrol and memory performance

Considering verbal memory performance, we could not replicate previous results of a significant improvement after resveratrol compared to placebo (Witte et al., 2014; Evans et al., 2017). An increase in memory after resveratrol has also been reported in a variety of experimental animal studies (Ingram et al., 2007; Dal-Pan et al., 2011; Kodali et al., 2015). The significant overall effect of time on learning, delayed recall and forgetting rate can be a simple learning sequence effect and should be interpreted with caution. However, in the current study we noticed that the order of parallel test versions significantly influenced test-retest performance over time, and that version order was unevenly distributed between groups at baseline. Though subgroup analyses stratified by version order did not show significant effects of the intervention, test-retest effects and order effects might have introduced additional confounding. In addition, some previous RCTs in healthy young adults, in mild cognitive impairment patients or in patients with schizophrenia were not able to show significant effects of resveratrol on verbal memory either (Turner et al., 2015; Wightman et al., 2015; Zortea et al., 2016; Kobe et al., 2017; however note that sample sizes were not always powered for cognitive effects).

It has also been discussed that polyphenols exert region-specific effects on the hippocampus, which would not necessarily translate into improvements in simple word list learning, but rather in pattern recognition memory (Brickman et al., 2014). Brickman et al. (2014) observed that a flavanol-containing diet improved reaction times in pattern recognition memory assessed using the ModBent task, but not in verbal memory performance of the modified Rey auditory learning task. Using the same ModBent task, we observed a trend for resveratrol-induced benefits on pattern recognition measured with d prime (d') (Hochhaus, 1972), but not in reaction times. Due to the small sample size available for that analysis in our study and the exploratory nature, interpretation of these results remain difficult. In sum, future studies need to further examine possible effects of resveratrol on memory before definite conclusions can be drawn.

Changes in biomarkers and lifestyle behavior

Considering possible mechanistic pathways, we could not confirm that resveratrol supplementation led to an improved long-term glucose metabolism measured using HbA1c. Preclinical studies have reported extensively that resveratrol improved glucose tolerance and insulin sensitivity (for review, see Liu et al., 2014). However, this effect has not been fully established in humans. More specifically, "at risk" populations with overweight, obesity or type II diabetes did benefit from resveratrol intake (Timmers et al., 2011; Bhatt et al., 2012; Witte et al., 2014), while those with normal weight did not (Yoshino et al., 2012; Poulsen et al., 2013; Liu et al., 2014). Thus, resveratrol might have failed to induce net improvements in glucose metabolism in our group comprising of healthy participants with a wide BMI range (i.e., 21.1–37.6 kg/m^2, none diabetic), which then would not have induced systematic structural or cognitive changes in the whole group.

We furthermore observed unfavorable changes in a series of biomarkers. Participants in both groups showed increases in weight and body fat at follow-up, as well as lower physical activity. This might hint at external factors, such as seasonal changes in physical exercise and diet that occurred during intervention time. Considering that baseline assessments were in spring and early summer, whereas follow-up assessments took place in autumn and winter, a change to an unhealthier and more sedentary lifestyle to the end of the year seems likely. Moreover, participants might have drawn their own conclusions regarding study participation: they might have thought of taking a "magic pill" and therefore might have reduced their regular engagement in physical exercise and healthy dietary habits. Eventually, this behavioral change might have provoked increases in low-grade inflammation as seen in higher levels for hsCRP, IL-6 and TNF-a, and in glucose, but also decreases in neurotrophic factors IGF-1 and BDNF. In sum, all of these factors are known to affect brain structure and function (Mattson et al., 2004; Cherbuin et al., 2012; Wyss-Coray and Rogers, 2012), and could therefore represent additional confounding factors. Note that HbA1c levels did not increase in the resveratrol group despite increases in body fat and BMI, and that the interaction term of time by group reached significance for changes in HbA1c in a model that accounted for changes in physical activity, BMI and dietary caloric intake as well as age and sex.

The observed increase in cholesterol in the resveratrol group might also be explained by higher dietary fat intake, as suggested by correlations with increases in self-reported caloric intake ($r = 0.41$, $p = 0.035$) and body fat mass ($r = 0.5$, $p = 0.008$) in that group. Yet, an increase in cholesterol has also been observed previously in the resveratrol-group only (Witte et al., 2014), and measures of resveratrol metabolites in the current study at follow-up correlated with increases in cholesterol (sulfated resveratrol, $r = 0.43$, $p = 0.024$; glucorunated resveratrol, $r = 0.39$, $p = 0.046$). However, to our knowledge no cholesterol-increasing effect of resveratrol has been described. Rather the opposite has been reported e.g. in animals (Cho et al., 2008; Do et al., 2008) and in human cell cultures (Voloshyna et al., 2013). We also evaluated changes in HDL, LDL and LDL/HDL-ratio (which is an indicator for the risk of coronary-heart diseases) according to current clinical praxis and findings of the Framingham study (e.g. Nam et al., 2006). The respective results did not raise clinical concerns. However, future studies should take potential effects of resveratrol on cholesterol metabolism into account.

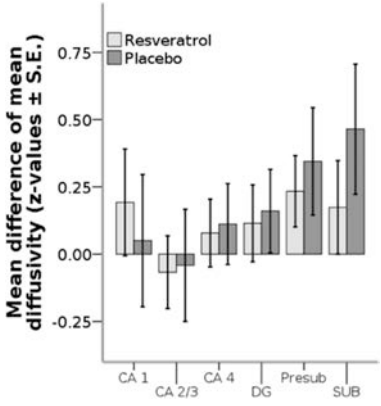

Figure 6. Differences in mean diffusivity between baseline and 6 months follow-up measures of hippocampus subfields. Values are z-transformed to reach comparability between subfields. Bars give means, error bars represent standard error (S.E.). CA 1 = Cornu Ammonis 1, CA 2/3 = Cornu Ammonis 2/3, CA 4 = Cornu Ammonis 4, DG = Dentate Gyrus, Presub = Presubiculum, SUB = Subiculum.

Hippocampus microstructure and connectivity

We could not detect significant effects of resveratrol compared to placebo on volume and microstructure of the hippocampus. This is in line with two previous trials using MRI at lower field strength (Witte et al., 2014; Kobe et al., 2017). Yet, this might contradict the hypothesis of increased resveratrol-induced plasticity in hippocampus microstructure, as shown in higher neurogenesis, microvasculature, and reduced glial activation measured *post mortem* in older rats after resveratrol injections (Kodali et al., 2015; Dias et al., 2016). In particular, MRI-derived hippocampal MD (Weston et al., 2015) has been discussed as an inverse measure of intact cellular barriers stemming from neurons, vasculature or

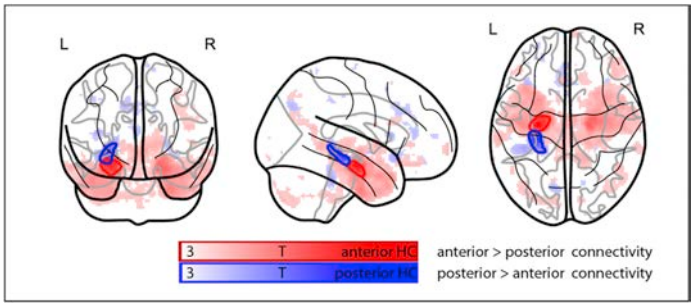

Figure 7. Functional connectivity differences of the anterior and posterior left hippocampus: while the anterior hippocampus (red) was more strongly connected to the temporal lobe, the posterior hippocampus (blue) showed stronger connectivity to the precuneus and angular gyrus. Hippocampus region of interests (delineated in bold) derived from the mean of all individual hippocampus masks transformed to MNI space and thresholded at 0.2.

astrocytes (den Heijer et al., 2012; Van Camp et al., 2012) and might therefore be an even more sensitive plasticity measure compared to volumetric measures. This has been supported by several studies in beginning Alzheimer's pathology and memory decline (Fellgiebel et al., 2004; Kantarci et al., 2005; Muller et al., 2005; Muller et al., 2007). However, in our study, there were no resveratrol-induced changes on hippocampus MD, despite implementing recent advances in UHF MRI and sensitive state-of-the-art preprocessing which integrated information from two modalities and made use of the longitudinal design (Iglesias et al., 2016). Yet univariate analyses suggested an increase in MD in the presubiculum and subiculum subfields in the current healthy sample after only 6 months of time, which is possibly due to (un)healthy aging effects. Therefore, our findings support the notion that MD measures outperform volumetry in detecting subtle changes in hippocampus microstructure. Again, the above-discussed lifestyle-changes might have prevented us from observing significant effects of resveratrol on MD.

With regard to resting-state fMRI, we could not replicate previous findings of resveratrol-induced improvements in functional connectivity of the hippocampus (Witte et al., 2014, Kobe et al., 2017). Disadvantageous connectivity changes had previously been discussed as early signs of functional reorganization that well precede structural and cognitive changes (Sheline et al., 2010; Pievani et al., 2011; Prvulovic et al., 2011; Kobe et al., 2017; Prehn et al., 2017). However, controlled studies on the effects of plasticity-enhancing interventions on functional connectivity measures are still scarce to date, and methodological limitations in functional MRI might have reduced the potential to detect significant effects. For example, we noticed high head motion in >15% of our participants, and the 2 mm × 2 mm × 2 mm resolution of connectivity maps did not allow analysis at the subfield-level. This might have limited statistical power to detect potential effects in our sample, even more so when considering previous results of increased blood volume after polyphenol supplementation that was restricted to the dentate gyrus region of the hippocampus (Brickman et al., 2014). Upcoming improvements in UHF whole-brain fMRI (Robinson et al., 2015) might help to further establish if resveratrol exerts beneficial effects on hippocampus connectivity.

Potential adverse events

Some minor adverse events were reported during our trial in both groups, such as stomachaches, skin changes and mood changes. However, diarrhea was reported more often and with higher severity in the resveratrol group and might be linked to the intervention. Especially as adverse events caused by resveratrol have been described to affect the abdomen (e.g. flatulence, mild diarrhea), but at the same time to remain moderate and reversible (Cottart et al., 2014). This is in line with several studies including larger sample sizes that reported resveratrol to be safe and well tolerated until a dose of 5 g per day (Almeida et al., 2009; Anton et al., 2014; Cottart et al., 2014; Turner et al., 2015). Even though minor adverse events were observed with doses higher than 0.5 g resveratrol per day after consumption for several weeks and up to one year (Cottart et al., 2014), this is still more than twice the amount of resveratrol in our study. Yet, two participants belonging to the resveratrol group dropped out, one due to a decrease in eyesight after 18 weeks of resveratrol intake and the other one because of a skin rash that is unlikely to be caused by resveratrol as it occurred five weeks after supplementation start. The eyesight improved again after ending the treatment, whereas we do not have further information regarding the skin rash. To our knowledge no negative impact of resveratrol on eyesight has been reported yet. In contrast, it has been reported to reduce oxidative damage in human retinal pigment epithelial cells and therefore to inhibit cataract formation (King et al., 2005; Zheng et al., 2010). Negative effects regarding the skin have been reported previously, but do not have to be caused by resveratrol (Brown et al., 2010; Howells et al., 2011). In sum, our findings implicate no major unintended effects of daily 200 mg resveratrol intake over the course of 6 months in healthy elderly. However, we cannot rule out that resveratrol led to a sudden, transient decrease in eyesight in one of our participants, a notion that future studies should take into account.

Limitations

Some limitations have been identified that might help to interpret our results and to improve future studies. First, our study comprised of healthy older adults with a wide BMI range. However, resveratrol seemed to be effective especially in overweight and obese people. Additionally, the study sample consisted mainly of highly educated participants with good task-performance already at baseline. Therefore, there was little scope to improve in cognitive and neuropsychological tasks. Furthermore, a different duration of the intervention or a change in dosage of resveratrol could lead to other results. In future studies, participants could be followed-up even longer after study cessation as post-intervention effects might occur. This was however not feasible in our study. In addition, the sample size might have been too small to detect significant effects in exploratory analyses including those of the ModBent task, HbA1c, MRI and subgroups. Second, resveratrol pills also contained quercetin to increase the bioavailability of resveratrol (De Santi et al., 2000; Skroza et al., 2015). As quercetin itself is a bioactive compound, it is not impossible that it affects cognition (e.g. memory recall) or other outcomes itself (Nakagawa et al., 2016). Nevertheless, quercetin did not have a significant effect on neurocognitive functioning in a large (n = 941) placebo-controlled, double-blind study with 12 weeks of quercetin-supplementation (500 mg or 1 g per day) (Broman-Fulks et al., 2012), rendering a confounding effect of quercetin in the current study unlikely. Third, the evaluation of compliance was largely based on self-reported pill-diaries at interim and follow-up visit, and measures of lifestyle behavior (physical activity, dietary intake) were based on self-reported questionnaires. Self-reported data is always prone to reporting errors according to social desirability (Herbert et al., 1995;

Adams et al., 2005). Therefore, we cannot exclude that a low unintentional compliance to the study instructions might have introduced additional confounding. Data from resveratrol metabolites are yet difficult to interpret as they might rather reflect acute changes and might not be suitable as long-term marker (Walle et al., 2011). Also, the change of seasons from spring/summer at baseline to autumn/winter at follow-up might have strongly influenced diet and physical activity of our subjects. Also, holidays such as Thanksgiving, Christmas or Easter can strongly influence eating behavior, underlining the importance to incorporate information on seasons and holidays into the study design of dietary interventions. This could mean to conduct the study within one season (without holidays) or to design the studies in waves (e.g. wave 1 starts in summer, wave 2 in winter) and to control for those effects.

Strengths of our study include the interventional design, a well-characterized sample and in-depths memory, blood samples and hippocampus phenotyping with cutting-edge UHF MRI.

Conclusions

This randomized interventional trial in healthy elderly with a wide BMI range failed to show significant effects of 6 months resveratrol supplementation on verbal memory performance, while pattern recognition performance tended to remain stable in the resveratrol group compared to decreases after placebo (non-significant trend). Additional confounding factors might be study duration or administered dosage of resveratrol, or possible ceiling effects in cognitive tasks, but also unfavorable seasonal changes in lifestyle behavior in both groups, as indicated by higher weight, body fat and sedentary behavior at follow-up assessments. These changes were paralleled by increases in cholesterol in the resveratrol group and increases in fasting glucose, inflammatory markers and lower neurotrophins in both groups, factors known to be detrimental for neuronal tissue and brain functions. Those negative effects might be due to resveratrol intake or lifestyle changes. Moreover, though we could not detect resveratrol-induced plasticity in hippocampus microstructure or functional connectivity, our findings further underscore the feasibility to assess hippocampus microstructure at the subfield level to identify subtle changes in hippocampus microstructure related to aging and/or lifestyle factors by implementing longitudinal UHF MRI. Future studies incorporating additional memory tasks of distinct sub-domains, a longer intervention period and larger sample sizes, and a rigorous control of adjacent lifestyle changes might help to determine whether resveratrol exerts beneficial effects on memory and the hippocampus in normal aging.

Declarations and conflict of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2018.03.023.

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III Discussion and Outlook

In Publication 1 Huhn et al., (2015), we summarized current evidence for effects of polyunsaturated omega-3 fatty acids and polyphenols on cognition. Afterwards, we decided to analyze the effects of resveratrol on memory performance, hippocampus connectivity, and microstructure in older adults in a randomized controlled trial. The resulting “Resveratrol Study” is presented in Publication 2 Huhn et al., (2018). Its results do not support the assumption that resveratrol improves memory performance in healthy, elderly individuals. Neither cognitive performance nor blood glucose metabolism was improved by resveratrol supplementation and also no changes in microstructure or volume of the hippocampus were observed. These findings are extensively discussed in the publication given above. The following chapter focuses on implications from our experimental work to inform and optimize future studies.

1. Questions and implications from the experimental work

Several questions arise from the “Resveratrol Study”. In the following I address i) the implications of the study population for the experimental findings, ii) the comparability of resveratrol supplementation to whole food effects, and iii) meaningful selection of outcome measures.

Implications of the study population for experimental findings

The characteristics of our study population have implications for the interpretation of the results. First of all, our participants were highly educated and already performed above average in the cognitive tests. Considering this, it might be difficult to observe improvements at all, as ceiling effects might occur. Secondly, our participants were healthy and did not belong to an at-risk population. Resveratrol was found to be effective especially in at-risk populations, such as overweight and diabetic subjects or individuals with mild cognitive impairment. Even if underlying mechanisms are not fully understood, it is possible that these populations share some commonalities, which make them more susceptible to beneficial effects of resveratrol. For instance, one could assume impaired glucose metabolism as common denominator. Glucose metabolism is impaired in diabetic patients and also in overweight and obese (Alberti et al., 2005;Geijselaers et al., 2015). Furthermore, an impaired glucose metabolism seems to be related to the progression from mild cognitive impairment to AD (Morris et al., 2014). Consequently, cognitive benefits could arise, if resveratrol beneficially

affected glucose metabolism in these individuals and patients. In contrast, no changes would be expected in overall healthy participants without glucose impairment as in the “Resveratrol Study”. Even though a few overweight subjects were included in our study, they were too few for meaningful subgroup analyses. However, given the highly conflicting and inconsistent data, it is also possible that resveratrol has only a very subtle effect on the assessed outcomes or no effect at all.

Comparability of resveratrol supplementation to whole food effects

Dietary interventions oftentimes try to evoke the effects of a life-long diet in a comparably short period (6 months in the “Resveratrol Study”). For this reason, many supplementation studies use dosages that are much higher than the intake via natural foods, based on the motto “the more, the better”. For example, the daily dosage of 200 mg resveratrol in our study is comparable to multiple bottles of red wine, tens of kilograms of blueberries or even hundreds of kilograms of strawberries, pistachios or chocolate (Rothwell et al., 2013). However, it is possible that there is a “too much” of resveratrol and very high dosages will not yield the same effects as the amount via the reasonable consumption of natural foods. Furthermore, it might not only be the dosage per se that triggers effects, but also the overall consumption period. Thus, a life-long diet with small amounts of resveratrol might be more effective than a relatively short period with high dosages. So far only acute effects of different resveratrol dosages have been well described (Kennedy et al., 2010;Wong et al., 2013b). However, this might be different for chronic dosages and also u-shaped dose-response curves need to be considered as assumed for other substances, e.g. alcohol (Kurth et al., 2006). It might, therefore, be informative to investigate especially low dosages of resveratrol, which are closer to the intake via natural sources, such as in Wong et al. (2013b). Admittedly, the lowest dosage used in this study is 30 mg, which is still equal to several bottles of red wine or kilograms of berries and nuts (Wong et al., 2013b). To our knowledge, the dose-response relation of resveratrol has not yet been addressed comprehensibly and should be subject to future studies.

Another caveat of single-nutrient studies is the (partial) loss of food synergy effects. As discussed in Introduction section 4.1., additive and synergistic effects of single nutrients can arise, when they are consumed within the food matrix (Jacobs et al., 2009). This matrix comprises the composite of all naturally occurring biological food

constituents, which are coordinated in a meaningful way (Jacobs and Tapsell, 2013). Losing these significant interrelations might also change the effectiveness of resveratrol. This renders it more difficult to uncover possible underlying mechanisms of dietary patterns like the Mediterranean diet.

On the way to a more meaningful selection of outcome measures

The choice of outcome measures for intervention studies is of great importance. Therefore, I would first emphasize to focus on the further investigation of mechanisms underlying the influence of resveratrol. Knowledge gained from that could prove the meaningfulness of already assessed cognitive performance outcomes or reveal further relevant measures. In previous studies on resveratrol and related resveratrol-containing foods, a wide and unspecific array of neuropsychological tests was employed. In contrast, in our study, we incorporated tasks that especially focus on hippocampus functions. This includes the CVLT and the ModBent task. The latter task was designed to assess the functions of the hippocampus and especially the dentate gyrus subfield. Thus, our outcome measures were chosen in line with previous findings and our hypotheses that resveratrol would affect the microstructure or volume of the hippocampus (also see Figure 6). However, our study is also limited with regard to a few aspects. The CVLT was probably limited due to the parallel test versions, which were significantly different regarding their difficulty. The version order was unevenly distributed between the intervention groups at baseline and thus influenced test-retest performance in our study. For future studies, it might therefore be important to pay closer attention to the order of parallel versions of cognitive tasks and to balance them between the intervention groups. Furthermore, the ModBent Task could be improved with more detailed instructions or neuroimaging during task performance, as done by Brickman et al., (2014). Also, the imaging modalities showed missing values due to missing follow-up scans, strong head motion or poor data quality. Taken together, CVLT and ModBent task seem to be appropriate tasks to assess hippocampus function, such as memory and pattern separation. However, close attention should be paid to the implementation of these tests in a study setting.

2. Optimizing the design of future studies

Several implications for the optimization of future trials arise from our study, but also from subsequent thoughts. Based on the previous paragraph, it would be insightful to

investigate, whether resveratrol affects the glucose metabolism in healthy participants without pre-existing impairments. The subsequent choice of cognitive tasks should also be well considered and matched to the hypothesized underlying mechanisms. For instance, related to hippocampal changes, future studies could assess verbal memory performance or pattern separation (e.g. ModBent task) and ensure complete data sets. Furthermore, the assessments should be difficult enough to avoid ceiling effects and allow changes also in well-educated samples.

However, there are more challenges that researchers will have to address in future trials. Already in the experimental work, we extensively discussed the importance of the time of year for longitudinal studies (including holidays entailing special food choices, e.g. Christmas), the following-up on participants after study cessation, determination of reliable compliance markers and extensive assessment of confounding factors (also see Figure 7). In line with that, more studies should combine the assessment of resveratrol metabolites and cognitive assessments (as e.g. done in Publication 2) to fully understand the complex pharmacokinetics and bioavailability. It is furthermore important to go beyond the effects of isolated resveratrol and keep the broader picture of a wholesome diet in mind. Here, I want to restate the importance of food matrix effects. They could be assessed in contrast to the effects of isolated resveratrol in a parallel group design. Thus, participants could be assigned to groups with different isolated resveratrol-dosages, but also paralleled with resveratrol-containing foods in a dose-dependent manner. Therefore, the resveratrol content of berries, grapes, and nuts could be manipulated with different growing conditions (Carrizzo et al., 2013;Gambini et al., 2015). Using natural foods with known resveratrol content will keep the food matrix intact, but would still be controlled enough to draw meaningful conclusions. Opening up the scope even more it might be insightful to compare the effects of resveratrol within different dietary patterns, such as the Western or Mediterranean diet. A further general approach could include overall dietary changes to prove that changes in glucose metabolism are already effective in improving cognitive functions. This could include the limitation of sugar within the diet or sugar substitution with e.g. stevia.

Another important addition to our work, would be the investigation of other brain areas that are either related to memory functions (e.g. prefrontal cortex) or medial temporal lobe areas, which could be especially plastic and susceptible to

interventions (Walhovd et al., 2015). For detailed discussion on this topic see review by (Walhovd et al., 2015).

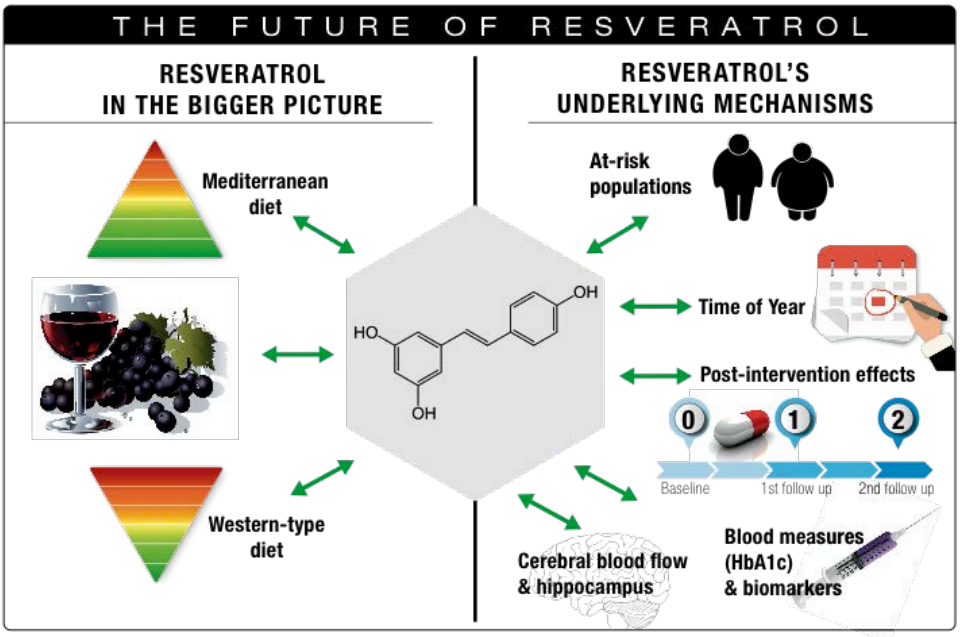

Figure 7: The future of resveratrol research.

Future studies are encouraged to investigate resveratrol in the bigger picture of single foods (e.g. wine, berries, chocolate) and whole dietary patterns (e.g. Western and Mediterranean diet). Furthermore, the elucidation of underlying mechanisms of resveratrol is crucial for future trials. It is important to determine the effects of resveratrol in at-risk populations as compared to healthy populations, the influence of the time of year on longitudinal studies, and effects of resveratrol after study cessation with more than one follow-up. Based on these results appropriate outcome measures can be chosen. Suggestions for targets are the hippocampus, blood-based measures and the establishment of reliable biomarkers for compliance. HbA1c = glycated hemoglobin.

In summary, we did not find evidence for a beneficial effect of resveratrol on blood parameters (e.g. glucose), anthropometric measures, cognitive performance and magnetic resonance imaging measures in our experimental work. Nevertheless, important implications arise from our work to shape the future of resveratrol research in a more goal-directed manner. I would propose to investigate at-risk populations and compare the effects of resveratrol with healthy populations. Upcoming trials should furthermore be encouraged to disentangle the complexities of underlying mechanistic pathways. In this regard, it would be interesting to see whether especially the anti-inflammatory and anti-oxidative effects of resveratrol are important or other mechanisms, such as the activation of sirtuins. All this might lead to significant insights and advances on the effects of resveratrol.

In view of the strength and details of our study, resveratrol might in the end not have considerable effects on the assessed functions of the hippocampus, such as learning, memory and pattern separation. Well-designed future trials will hopefully elucidate the effects of whole dietary patterns and single components of the Mediterranean diet on cognition. This could help to substantiate further evidence for the impact of nutrition on hippocampal function.

IV Summary

Zusammenfassung der Arbeit
Dissertation zur Erlangung des akademischen Grades Dr. rer. med.

"The impact of nutrition on hippocampal function -
Results of a literature review and a randomized controlled trial"

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Einreichung:	Mai 2018

1. English summary

Nutrition as a modifiable lifestyle factor bears a great potential for the prevention of cognitive decline and related diseases, such as Alzheimer's disease (AD; e.g. Aridi et al., 2017;Barnard et al., 2014b). An early affected brain structure in AD is the hippocampus, which seems to be vulnerable to degeneration in aging, but at the same time especially susceptible to various (lifestyle-) interventions (Wisse et al., 2015;Walhovd et al., 2015). Distinct features of the hippocampus could be responsible for this susceptibility and comprise its potential ability for adult neurogenesis (the continued production of neurons throughout life), high energy demands, and life-long plasticity (Walhovd et al., 2015). On a functional level, the hippocampus is involved in learning and memory, but also in pattern separation (Brickman et al., 2014;Eichenbaum and Cohen, 2014). These functions can be measured with specific neuropsychological tests, while magnetic resonance imaging allows assessing anatomy and microstructure of the hippocampus *in vivo*.

As of yet, no cure for AD is available and potential preventive measures are highly needed (Ising et al., 2015). Therefore, modifiable lifestyle factors, such as nutrition should fully be exploited. Nowadays, however, a so-called "Western diet" predominates in countries such as the USA, Australia and wide parts of Europe (Cordain et al., 2005). Related problems arise including a sedentary lifestyle and an unfavorable dietary composition that could ultimately promote the development of AD (Cordain et al., 2005;Carrera-Bastos et al., 2011). Due to the high consumption of refined sugar and (saturated and trans) fatty acids, the Western diet is also known as a high fat sugar diet (Yeomans, 2017). Consequently, following this dietary pattern results in negative biochemical effects on a systemic level (Yeomans, 2017).

Metabolizing the high amount of fat and sugar leads, for example, to increased levels of free radicals and reactive oxygen species (Francis and Stevenson, 2013;Freeman et al., 2014). These, in turn, damage important cellular bio-macromolecules, such as lipids, sugars, and polynucleotides and can lead to conjugation and loss of function of proteins (Andersen, 2004;Sayre et al., 2008). Further, it can result in impaired degradation of proteins and the accumulation of potentially toxic products (e.g. tau and amyloid-β in AD). These products and otherwise occurring damages can ultimately lead to cell death (Sayre et al., 2008;Bloom, 2014;Heneka et al., 2015;Ray et al., 2016). The brain is especially susceptible to these harms, as neurons are post-mitotic, hence more prone to accumulate DNA damage, and show an overall high metabolic rate compared to other organs (Andersen, 2004). Furthermore, oxidative stress can activate microglia, which together with the metabolism of lipids results in increased levels of (pro-) inflammatory molecules (Wang et al., 2015). Inflammation again might have deleterious effects on neurons and synapses and could affect their basic functions including long-term potentiation and synaptic plasticity (Wang et al., 2015). Ultimately, the damages to neurons and synapses that can arise due to the consumption of a Western diet can impair neuronal plasticity and potentially change memory and cognitive processes (Kanoski and Davidson, 2011;Bloom, 2014;Freeman et al., 2014). If these damages occur within the hippocampus, learning, memory, and pattern separation can be impaired (Morrison et al., 2010;Tucsek et al., 2014;Biessels and Reagan, 2015).

In contrast to the Western diet, the traditional Mediterranean diet represents a dietary pattern, which is of high value for cognitive health. Characterized by a high intake of beneficial components (e.g. fruit, vegetables, legumes, nuts, olive oil) and moderate consumption of fish, other meat, dairy products and red wine, the Mediterranean diet affects the same biochemical pathways as the Western diet does (Davis et al., 2015). However, due to various nutrients the direction is opposite and dominated by anti-oxidative and anti-inflammatory effects. Several potentially neuroprotective effects could help to maintain neuronal plasticity and might explain the positive influence of the Mediterranean diet on cognitive health (Psaltopoulou et al., 2013;Heneka et al., 2015;Aridi et al., 2017). A recent meta-analysis concluded that beneficial effects of the Mediterranean diet on cognition were especially reported in cohort studies conducted in the Mediterranean area or randomized controlled trials (Aridi et al., 2017). However, these studies also showed serious limitations and cognitive

measures were rather crude (Aridi et al., 2017). Nonetheless, the Mediterranean diet is a cost-effective and low-risk intervention that can be recommended for future trials investigating cognitive health (Psaltopoulou et al., 2013;Tuso et al., 2013).

Some studies also attempted to unveil the underlying mechanisms of the effects of whole dietary patterns and focused on single foods or even isolated nutrients (e.g. Krikorian et al., (2010), Brickman et al., (2014), Wightman et al., (2015)). Both, whole dietary pattern and single food/nutrient studies have their advantages and disadvantages. Challenges for the study design arise from the possibilities to change dosages of dietary components, to standardize the interventions, the occurrence of food matrix effects, the generalization to a daily diet, and the study duration.

In order to get a better understanding of single components of the Mediterranean diet, we conducted a literature review with a focus on the evidence linking polyunsaturated omega-3 fatty acids and polyphenols to cognition (see Publication 1, Huhn et al., (2015)). As a result, we identified the polyphenol resveratrol as one example of a single substance with promising effects on cognitive performance. It can be found in natural dietary sources (e.g. red grapes, cranberries, peanuts) and is generally safe and well tolerated (Rothwell et al., 2013;Cottart et al., 2014).

Various biochemical properties arise from resveratrol's ability to bind to a multitude of enzymes and other proteins (Britton et al., 2015). Antioxidant and anti-inflammatory properties have been established *in vitro* and were at least partly confirmed in human studies (Crichton et al., 2013;McAnulty et al., 2013;Poulsen et al., 2015). In addition, calorie-restriction mimicking effects have been described due to resveratrol's potential to activate the regulators of important biological pathways, so-called sirtuins (Kulkarni and Canto, 2015). This could result in enhanced DNA-repair, apoptosis, adult neurogenesis, and glucose-insulin homeostasis (Hubbard and Sinclair, 2014). As discussed before, hippocampal neurogenesis is a distinct process, as adult-born hippocampal neurons seem to be more excitable and build more synaptic connections compared to mature neurons (Ho et al., 2013). However, whether adult neurogenesis exists in humans is a matter of current debate (Snyder, 2018). Also related to the activation of sirtuins by resveratrol, a beneficial effect on blood glucose levels and insulin sensitivity has been described (Liu et al., 2014). Previously, already slight improvements in glucose levels (even within normal range) were shown to benefit memory performance and could therefore have implications for intervention studies (Kerti et al., 2013).

All the aforementioned properties – antioxidative, anti-inflammatory, and calorie-restriction mimicking, blood glucose influencing – could lead to beneficial changes on a biochemical level and thus contribute to maintaining functional hippocampal learning and memory (Ho et al., 2013;Biessels and Reagan, 2015). Yet, evidence from human trials is still scarce. Most studies investigating the effects of resveratrol used food-based approaches and concordantly reported beneficial effects on learning, memory performance, and pattern separation (Krikorian et al., 2010;Brickman et al., 2014;Small et al., 2014).

Yet, only a few studies investigated the impact of isolated resveratrol on cognition and underlying mechanisms. Acute effects on cerebral blood flow in a dose-dependent manner have been described, while no chronic changes of cerebral blood flow or cognition were reported so far (Kennedy et al., 2010;Wightman et al., 2014;Wightman et al., 2015). In addition, benefits of resveratrol for circulatory and cerebrovascular functions were shown, as well as improvements in overall cognition and verbal memory (Wong et al., 2016;Evans et al., 2017). Additionally, in a previous study half a year of resveratrol supplementation resulted in better memory performance, improved glucose metabolism, and higher functional connectivity of the hippocampus (Witte et al., 2014). However, the effects described above were mainly found in at-risk populations, such as overweight, obese, diabetic and post-menopausal subjects and might not translate to healthy individuals. Also, a recent meta-analysis concluded that resveratrol has no significant effect on memory and cognitive performance (Farzaei et al., 2017). However, doubts about the methodology and selection criteria of the meta-analysis were raised and available data remain inconsistent (Wong and Howe, 2018). To achieve more conclusive results, future clinical trials are encouraged to include advanced tests for cognition and memory functions as e.g. done in Publication 2 Huhn et al., (2018).

The “Resveratrol Study” (Publication 2) was conducted to investigate the effects of resveratrol on cognitive performance and elucidate possible underlying mechanisms. To optimize and improve the design compared to previous studies, we combined measures that were already described to be meaningful including blood parameters, anthropometry, neuropsychological tests, and neuroimaging. This was complemented with the assessment of various confounders. We thus hoped to more specifically assess the effects of 26 weeks with a daily dosage of 200 mg resveratrol on cognition in healthy, elderly individuals. For this purpose, we assessed

hippocampus-related cognitive performance with specific tests for learning, memory and pattern separation. The hippocampus itself was assessed with neuroimaging at higher field strength than in previous studies.

The results of the “Resveratrol Study” did not support the hypothesis that resveratrol improves memory performance in healthy, elderly individuals. Also, the blood glucose metabolism was not affected by resveratrol, and neither were hippocampus volume nor microstructure. With these findings, some open questions and suggestions for future studies arise. It will be important to address the implications of the study population for the experimental findings. Young individuals might be differently affected than old, and healthy differently than at-risk populations, such as overweight, obese and diabetic. Future studies could, therefore, investigate e.g. the effects of resveratrol on glucose metabolism in healthy participants as compared to at-risk groups. Furthermore, the comparability of resveratrol supplementation to whole food effects should be addressed. The administered dosages might play a crucial role, as well as synergistic effects within the food matrix. And in addition, the selection of outcome measures and thorough test instructions remain a crucial point also with regard to the minimization of missing data points. It is moreover important to take into account the time of year for longitudinal studies, to establish reliable compliance markers and to extensively assess confounding factors.

Summarizing, we could not confirm our hypothesis about improvements of cognitive performance after resveratrol. In view of the strength and details of our study design, resveratrol might in the end not have considerable effects on the assessed functions of the hippocampus, such as learning, memory and pattern separation. Future randomized controlled trials might, therefore, consider investigating the influence of the study population or using other components of the Mediterranean diet. This could help to substantiate further evidence for the impact of nutrition on hippocampal function.

Articles included in this thesis:

Huhn, S., Beyer, F., Zhang, R., Lampe, L., Grothe, J., Kratzsch, J., Willenberg, A., Breitfeld, J., Kovacs, P., Stumvoll, M., Trampel, R., Bazin, P.L., Villringer, A., and Witte, A.V. (2018). Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults - A randomized controlled trial. *Neuroimage* 174**,** 177-190. doi: 10.1016/j.neuroimage.2018.03.023.

Huhn, S., Kharabian Masouleh, S., Stumvoll, M., Villringer, A., and Witte, A.V. (2015). Components of a Mediterranean diet and their impact on cognitive functions in aging. *Front Aging Neurosci* 7**,** 132. doi: 10.3389/fnagi.2015.00132.

2. Deutsche Zusammenfassung

Ernährung, als beeinflussbarer Bestandteil des Lebensstils, besitzt ein großes Potenzial im Hinblick auf die Prävention von kognitivem Abbau und zugehörigen Krankheiten wie beispielsweise der Alzheimerschen Krankheit (e.g. Aridi et al., 2017;Barnard et al., 2014b). Eine Gehirnregion, die besonders früh von der Alzheimerschen Krankheit betroffen wird, ist der Hippocampus. Dieser scheint anfällig gegen altersbedingte Degeneration zu sein, aber gleichzeitig auch besonders sensibel auf (Lebensstil-) Interventionen anzusprechen (Wisse et al., 2015;Walhovd et al., 2015). Zu den herausstechenden Merkmalen des Hippocampus zählen seine vermeintliche Fähigkeit zur Neurogenese im Erwachsenenalter (der kontinuierlichen Entstehung von Nervenzellen), ein erhöhter Energiebedarf und lebenslange Plastizität (Walhovd et al., 2015). Auf funktioneller Ebene ist der Hippocampus vor allem an Lernen, Gedächtnis und der Erkennung von Mustern beteiligt (Brickman et al., 2014;Eichenbaum and Cohen, 2014). Diese Funktionen können mit spezifischen neuropsychologischen Tests gemessen werden, wohingegen man mit Magnetresonanztomographie die *in vivo* Anatomie und Mikrostruktur des Hippocampus darstellen kann.

Bisher gibt es keine Heilung für die Alzheimersche Krankheit und präventive Maßnahmen sind deshalb dringend erforderlich (Ising et al., 2015). Aus diesem Grund sollte das Potenzial des beeinflussbaren Lebensstils voll ausgeschöpft werden. Dazu zählt auch die Ernährung. Allerdings sind heutzutage Länder wie die USA, Australien und weite Teile Europas von einer Ernährung mit hohem Anteil an raffiniertem Zucker und (gesättigten bzw. trans-) Fettsäuren geprägt – der sogenannten “Western diet” (Cordain et al., 2005;Yeomans, 2017). Diese geht darüber hinaus noch mit einem überwiegend sesshaften Lebensstil einher (Carrera-Bastos et al., 2011). Daraus ergeben sich negative Auswirkungen auf die Biochemie des gesamten Körpers (Yeomans, 2017). Während des Stoffwechsels von Zucker und Fett werden erhöhte Spiegel von freien Radikalen und reaktiven Sauerstoffspezies freigesetzt, welche wichtige zelluläre Biomakromoleküle beschädigen können (Francis and Stevenson, 2013;Freeman et al., 2014). Dazu zählen Lipide, Zucker und Polynukleotide, es kann aber auch zur Konjugation und Funktionsbeeinträchtigung von Proteinen kommen (Andersen, 2004;Sayre et al., 2008). Dies kann auch zur Folge haben, dass Proteine schlechter abgebaut werden und sich dadurch potentiell toxische Stoffe anhäufen (beispielsweise Tau und

Amyloid-β in der Alzheimerschen Krankheit). Die genannten Toxine und zusätzlich auftretende Schäden können letztendlich zum Zelltod führen (Sayre et al., 2008;Bloom, 2014;Heneka et al., 2015;Ray et al., 2016). Besonders das Gehirn ist diesbezüglich anfällig, denn Neurone sind post-mitotische Zellen und häufen damit leichter DNA Schäden an (Andersen, 2004). Dazu kommt, dass die Stoffwechselrate besonders hoch ist im Vergleich zu anderen Organen (Andersen, 2004). Darüber hinaus kann oxidativer Stress zur Aktivierung von Mikroglia führen, was zusammen mit dem Stoffwechsel von Lipiden zu erhöhten Spiegeln (pro-) inflammatorischer Stoffe führt (Wang et al., 2015). Inflammation wiederum kann sich verheerend auf Neurone und Synapsen auswirken und deren Grundfunktionen inklusive Langzeitpotenzierung und synaptischer Plastizität beeinträchtigen (Wang et al., 2015). Letztendlich kann der Konsum einer „Western diet“ also zu Schäden an Neuronen und Synapsen führen und sich durch Beeinträchtigung der synaptischen Plastizität möglicherweise auf das Gedächtnis und weitere kognitive Prozesse auswirken (Kanoski und Davidson, 2011;Bloom, 2014;Freeman et al., 2014). Wenn solche Schäden Gehirnregionen wie den Hippocampus betreffen, können Lernen, Gedächtnis und die Erkennung von Mustern beeinträchtigt werden (Morrison et al., 2010;Tucsek et al., 2014;Biessels und Reagan, 2015).

Im Gegensatz zur „Western diet“ ist die mediterrane Diät ein Beispiel für eine Ernährungsweise mit positiver Wirkung auf die kognitive Gesundheit. Geprägt ist die mediterrane Ernährung von einem hohen Anteil förderlicher Bestandteile wie Obst, Gemüse, Hülsenfrüchte, Nüsse und Olivenöl in Kombination mit einem mäßigen Konsum an Fisch, anderem Fleisch, Milchprodukten und Rotwein. Dadurch werden die gleichen biochemischen Stoffwechselwege beeinflusst wie bei der „Western diet“ (Davis et al., 2015). Die Effektrichtung ist jedoch entgegengesetzt und verschiedene Nahrungsbestandteile fördern anti-oxidative und anti-inflammatorische Mechanismen. Verschiedene neuroprotektive Effekte könnten so zur Aufrechterhaltung von neuronaler Plastizität und kognitiver Funktionalität beitragen (Psaltopoulou et al., 2013;Heneka et al., 2015;Aridi et al., 2017). Eine kürzlich erschienene Meta-Analyse hat gefolgert, dass vorteilhafte Wirkungen der mediterranen Diät auf kognitive Funktionen vor allem in Studien berichtet wurden, die entweder in mediterranen Ländern durchgeführt wurden oder bei denen es sich um randomisierte Kontrollstudien handelte (Aridi et al., 2017). Allerdings wiesen diese Studien auch gravierende Schwächen auf und die herangezogenen Kognitionstests

waren sehr oberflächlich (Aridi et al., 2017). Nichtsdestotrotz stellt die mediterrane Diät eine kostengünstige und risikoarme Interventionsmaßnahme dar, die für zukünftige Studien empfohlen werden kann, die sich mit kognitiver Gesundheit beschäftigen (Psaltopoulou et al., 2013;Tuso et al., 2013).

Einige Studien versuchten bereits die zugrundeliegenden Mechanismen von Effekten der Ernährungsweise zu entschlüsseln und haben sich deswegen auf die Erforschung einzelner Lebensmittel oder sogar isolierter Nährstoffe konzentriert. Es gibt sowohl bei der Erforschung kompletter Ernährungsweisen als auch einzelner Lebensmittel oder Nährstoffe Vor- und Nachteile. Herausforderungen für das Studiendesign entstehen durch die Beeinflussbarkeit der Dosierung von Nahrungsbestandteilen, der Möglichkeit zur Standardisierung der Diäten/Interventionen, dem Auftreten von Lebensmittelmatrixeffekten, der Generalisierbarkeit zur täglichen Ernährung und der Studiendauer.

Um ein besseres Verständnis für die einzelnen Bestandteile der mediterranen Ernährung zu entwickeln, haben wir einen Review geschrieben, der die Evidenzlage zum Zusammenhang zwischen Kognition und mehrfach ungesättigten omega-3 Fettsäuren beziehungsweise Polyphenolen bewertet (Publikation 1 Huhn et al., (2015)). Als Ergebnis haben wir das Polyphenol Resveratrol als ein Beispiel für eine vielversprechende Substanz mit Einfluss auf die kognitive Leistungsfähigkeit herausgestellt. Resveratrol kommt natürlicherweise unter anderem in roten Trauben, Cranberries und Erdnüssen vor und gilt generell als sicher und gut verträglich (Rothwell et al., 2013;Cottart et al., 2014). Verschiedene biochemische Eigenschaften basieren auf der Fähigkeit Resveratrols an eine Vielzahl von Enzymen und anderer Proteine zu binden (Britton et al., 2015). *In vitro* wurden die anti-oxidativen und anti-inflammatorischen Wirkungen von Resveratrol etabliert und zumindest teilweise in Humanstudien bestätigt (Crichton et al., 2013;McAnulty et al., 2013;Poulson et al., 2015). Zusätzlich wurde Resveratrol als Mimetikum der Kalorienrestriktion beschrieben, was auf seiner Fähigkeit zur Aktivierung von Sirtuinen beruht (Kulkarni und Canto, 2015). Das könnte sich bemerkbar machen durch eine günstige Beeinflussung von DNA-Reparatur, Apoptose, adulter Neurogenese und der Glukose-Insulin Homöostase (Hubbard und Sinclair, 2014). Wie zuvor erwähnt, spielt die adulte Neurogenese im Hippocampus eine entscheidende Rolle, denn adult-geborene Neurone scheinen erregbarer zu sein und mehr synaptische Verbindung zu knüpfen als ausgereifte Neurone (Ho et al., 2013).

Allerdings ist gerade in letzter Zeit wieder sehr in Frage gestellt worden, ob es adulte Neurogenese im Menschen überhaupt gibt (Snyder, 2018).
Zusätzlich wurde in Bezug auf die Aktivierung von Sirtuinen durch Resveratrol auch ein günstiger Einfluss auf Blutglukosespiegel und Insulinsensitivität beschrieben (Liu et al., 2014). Denn eine frühere Studie zeigt, dass verbesserte Glukosespiegel selbst im Normbereich das Gedächtnis vorteilhaft beeinflussen (Kerti et al., 2013).
All die zuvor genannten Eigenschaften – anti-oxidativ, anti-inflammatorisch, die Kalorienrestriktion nachahmend und Blutglukose normalisierend – könnten zu günstigen Veränderungen auf biochemischer Ebene führen und somit zur Aufrechterhaltung der Funktionalität des Hippocampus führen (Ho et al., 2013, Biessels und Reagan, 2015). Bisher ist die Evidenz aus Humanstudien jedoch begrenzt. Die meisten Studien, die sich bisher mit dem Effekt von Resveratrol beschäftigt haben, wählten eine Nahrungsmittelintervention und haben übereinstimmend günstige Auswirkungen auf Verarbeitungsgeschwindigkeit, Lernen, Gedächtnis und Mustererkennung berichtet (Krikorian et al., 2010;Brickman et al., 2014;Small et al., 2014). Allerdings gab es nur wenige Studien, die sich mit der Auswirkung von isoliertem Resveratrol auf die Kognition und den zugrundeliegenden Mechanismen beschäftigten. Es wurden lediglich akute, dosisabhängige Wirkungen auf den zerebralen Blutfluss beschrieben, wohingegen über keine chronischen Veränderungen auf zerebralen Blutfluss oder Kognition berichtet wurde (Kennedy et al., 2010;Wightman et al., 2014;Wightman et al., 2015). Zusätzlich zeigten sich günstige Auswirkungen auf Kreislauf und zerebrovaskuläre Funktionen genauso wie Verbesserungen von genereller Kognition und Wortgedächtnis (Wong et al., 2016;Evans et al., 2017). Außerdem berichtete eine frühere Studie über eine Verbesserung von Gedächtnisleistung und Glukosestoffwechsel sowie eine höhere funktionelle Konnektivität des Hippocampus nach einem halben Jahr Supplementation mit Resveratrol (Witte et al., 2014). Allerdings wurden die bisher beschriebenen Effekte vor allem in Risikogruppen wie Übergewichtigen, Adipösen, Diabetikern und postmenopausalen Frauen beschrieben und könnten sich nicht auf gesunde Individuen übertragen lassen. Auch eine kürzlich erschienene Meta-Analyse hat gefolgert, dass Resveratrol keinen signifikanten Einfluss auf das Gedächtnis und die kognitive Leistungsfähigkeit hat (Farzaei et al., 2017). Allerdings wurden Zweifel an den Methoden und der Studienauswahl der Meta-Analyse erhoben, womit die Datenlage uneinheitlich bleibt (Wong und Howe, 2018). Für beweiskräftigere

Ergebnisse sollen zukünftige Studienleiter ermutigt werden, sensitive Tests für Kognition und Gedächtnis zu verwenden, wie beispielsweise erfolgt in Publikation 2 Huhn et al, (2018).

Die „Resveratrol Studie“ (Publikation 2) wurde durchgeführt, um die Effekte von Resveratrol auf die kognitive Leistungsfähigkeit und mögliche zugrundeliegende Mechanismen aufzuklären. Um das Design im Vergleich zu früheren Studien zu verbessern haben wir Erhebungsmethoden vereint, die sich zuvor bereits als zweckdienlich erwiesen haben. Dazu zählten Blutparameter, anthropometrische Messungen, Neuropsychologische Tests und Magnetresonanztomographie. Dies wurde ergänzt durch die Erhebung von verschiedenen Störgrößen („confoundern“). Dadurch erhofften wir uns die Auswirkungen von täglich 200 mg Resveratrol über 26 Wochen auf die Kognition von gesunden, älteren Probanden spezifischer erfassen zu können. Diesbezüglich haben wir, neben anderen Tests, die kognitive Leistungsfähigkeit des Hippocampus mit spezifischen Tests zu Lernen, Gedächtnis und der Erkennung von Mustern erfasst. Der Hippocampus selbst wurde *in vivo* mit verschiedenen Bildgebungsverfahren bei höherer Feldstärke als in früheren Studien dargestellt.

Es war mit den Ergebnissen der „Resveratrol Studie“ nicht möglich die Annahme zu erhärten, dass Resveratrol einen günstigen Effekt auf die Gedächtnisleistung gesunder, älterer Menschen hat. Zusätzlich wurden weder Blutglukosestoffwechsel, noch Hippocampusvolumen oder –mikrostruktur beeinflusst. Diesen Ergebnissen folgen sowohl offene Fragen als auch Empfehlungen für zukünftige Studien. So wird es von Bedeutung sein, die Studienpopulation sorgfältig auszuwählen. Junge Individuen könnten von Resveratrol unterschiedlich beeinflusst werden als Ältere und Gesunde wiederum anders als Risikogruppen wie Übergewichtige, Adipöse und Diabetiker. Als Beispiel einer Hypothese für zukünftige Studien, sei der Effekt genannt den Resveratrol auf den Glukosestoffwechsel in Gesunden im Vergleich zu Risikogruppen hat. Außerdem wäre es möglich die Supplementation von Resveratrol mit der Gabe von intakten Lebensmitteln zu vergleichen. Sowohl die verabreichte Dosierung als auch Lebensmittelmatrixeffekte spielen dabei möglicherweise eine Rolle. Des Weiteren ist die Auswahl von bedeutungsvollen Erhebungsmethoden ausschlaggebend für den Erfolg weiterer Studien. Auch die Jahreszeit, in der eine Längsschnittstudie durchgeführt wird, sollte beachtet werden, genauso wie die

Ermittlung verlässlicher „compliance marker“ und der ausführlichen Erfassung von Störgrößen.

Zusammenfassend konnten wir die Hypothese nicht bestätigen, dass sich Resveratrol günstig auf die kognitive Leistungsfähigkeit auswirkt. In Anbetracht der Stärken und Details unserer Studie ist es durchaus möglich, dass Resveratrol letztendlich keinen nennenswerten Effekt auf die erhobenen Funktionen des Hippocampus, wie Lernen, Gedächtnis und der Erkennung von Mustern, hat. Zukünftige randomisierte Kontrollstudien könnten sich demnach mit dem Einfluss der Studienpopulation befassen oder weitere Bestandteile der Mediterranen Diät erforschen. Dies könnte bedeutend zur Beweislage über den Einfluss von Ernährung auf die Funktionen des Hippocampus beitragen.

In diese Arbeit eingeschlossene Publikationen:

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Figures were designed by Tanja Bandhauer, t+c Werbeagentur, Karlstein (Main), Germany. Pictures were acquired from fotolia.com

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VI Appendix

A1 Supplementary information publication 2 Huhn et al. (2018)

Supplementary information

Magnetic Resonance Imaging (MRI) Acquisition and Analysis

Anatomical Imaging

Anatomical MRI for hippocampal volumetry was acquired at a Siemens Magnetom 7 T system (Siemens Healthcare, Erlangen, Germany) using a 32-channel head array coil (NOVA Medical Inc., Wilmington MA, USA). High-resolution T1-weighted images were acquired using a MP2RAGE (Marques et al., 2010) protocol (repetition time (TR) = 5000 ms; inversion time (TI) 1/2 = 900/2750 ms; echo time (TE) = 2.45 ms; image matrix: 320 x 320 x 240; voxel size 0.7 mm x 0.7 mm x 0.7 mm; flip angle 1/2 = 5°/3°; parallel imaging using GRAPPA (Griswold et al., 2002) with acceleration factor = 2). T2-weighted imaging slabs perpendicular to the anterior-posterior axis of the hippocampus were acquired using a Turbo-Spin Echo Sequence (TR = 13000ms; TE = 14ms; image matrix: 384 x 384; 50 slices; voxel size: 0.5 mm x 0.5 mm x 1mm; refocusing flip angle = 120°; turbo factor = 8; parallel imaging using GRAPPA with acceleration factor = 2).

The anatomical T1-weighted images were skull-stripped using CBS Tools (Bazin et al., 2014) and processed with the FreeSurfer image analysis suite version 6.0.0 (http://surfer.nmr.mgh.harvard.edu/) (Dale et al., 1999;Fischl et al., 2002). First, T1-weighted images (baseline, follow-up) were processed cross-sectionally, and then an unbiased template was created for each subject and used together with the data from each timepoint in the longitudinal stream (Reuter et al., 2012), which increases reliability and statistical power in longitudinal designs. T2-weighted imaging slabs were manually reoriented to overlap with each timepoint's normalized longitudinal T1-image (norm.mgz) in order to preserve the high in-plane resolution. Hippocampal subfield segmentation was performed using the T1- and T2-weighted images in a multimodal approach, which was initialized by the output of the longitudinal stream (Iglesias et al., 2015).

Out of 13 subfields and structures segmented by the algorithm, we considered six main subfields (Cornu Ammonis 1, 2/3, 4, Dentate Gyrus, Presubiculum and Subiculum) for further analysis (Erickson et al., 2011;Brickman et al., 2014). Volumes were extracted from the multimodal segmentation and the longitudinal segmentation stream (Iglesias et al., 2015) and averaged across hemispheres. See Figure S1 for an overview of intra- and inter-subject variability of hippocampus subfield volumes.

For the longitudinal anatomical analysis based on 7T MRI, 50 participants had complete data (n = 1 not MRI suitable at follow-up, n= 2 withdrew consent to undergo 7T MRI session at follow-up). Because of insufficient turbo spin echo (TSE) data quality at follow-up, another participant had to be excluded from the multimodal longitudinal subfield analysis (n (longitudinal) = 49; resveratrol n = 25, placebo n = 24).

As there is evidence for hippocampal asymmetry in both, healthy aging as well as neurodegenerative disease (Barnes et al., 2005;Shi et al., 2009), we checked for whole

hippocampus asymmetry and hemisphere-by-time interaction in our sample before averaging hippocampal subfield volumes across hemispheres. In line with the literature (Shi et al., 2009) we found the right hippocampus to be significantly larger than the left hippocampus (paired t-test at baseline: T= -4.9, p < 0.001 and follow-up: T= -4.0, p< 0.001) but did not find a significant hemisphere-by-time interaction (paired t-test between hemispheres of the pre-post differences: T=-0.94, p=0.35). For our longitudinal analysis we therefore decided to average across hemispheres for each timepoint separately.

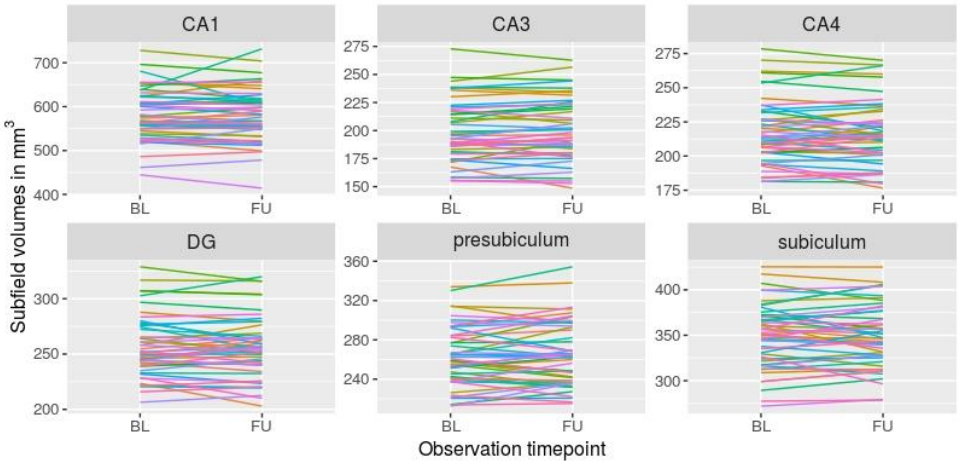


Figure S1: Hippocampus subfield volumes pre-and post intervention. Colors represent individual participants (n = 49).

Diffusion Weighted Imaging Analysis

Diffusion weighted images (DWI) were collected at 7T with a single shot echo planar imaging (EPI) sequence (TR = 6000 ms; TE = 62.8 ms; image matrix = 128 × 128, 60 slices; voxel size = 1.2 mm × 1.2 mm × 1.2 mm, 67 diffusion directions, b = 1000 s/mm2, parallel imaging using GRAPPA with acceleration factor = 2). The imaging slab was chosen to cover the bilateral hippocampus in all participants. In order to correct for image distortions, an additional volume with no diffusion weighting (b = 0) but with opposite phase-encoding direction was acquired.

Preprocessing of diffusion-weighted images was performed using FSL version 5.0.9. (Smith et al., 2004) and included estimation of susceptibility-induced off-resonance field based on b0—images in opposite phase-encode directions using TOPUP (Andersson et al., 2003) as implemented in FSL (Smith et al., 2004). The estimated susceptibility distortions were used to carry out corrections of susceptibility and eddy currents/movements with eddy tool (Andersson and Sotiropoulos, 2016). Then, a tensor model was fitted to the corrected data and MD was calculated from the estimated diffusion tensors at each voxel. The resulting MD imaging slabs were registered to the cross-sectional T1-weighted MP2RAGE image in two steps. First, scanner coordinates were used to achieve a rough co-registration using CBS

Tools (Bazin et al., 2014) as a plugin for MIPAV (McAuliffe et al., 2001) and JIST (Lucas et al., 2010) and then a non-linear registration of cerebral spinal fluid (CSF) masks from T1 and DWI was performed using ANTS's symmetric normalization (SyN) algorithm, initialized by rigid and affine transformations (Avants et al., 2011). CSF-masks were created from each subject's MD image by thresholding at MD > 0.002 mm^2/s and from T1-weighted images by using tissue segmentation implemented in FSL's FAST. The whole brain CSF image was cropped to the imaging slab of the DWI sequence before registration in order to improve the fit. The resulting transformation warp was then applied to the MD image. Hippocampal subfield labels based on the multimodal registration were brought from the longitudinal template space to the native space of each timepoint and then used to extract the median hemisphere-averaged MD of the six hippocampus subfields. MD values were restricted to be larger than zero and smaller than 0.002 mm^2/s to reduce the noise introduced by imperfect segmentation and low signal-to-noise ratio.

See Figure S2 for an overview of intra- and inter-subject variability of hippocampus subfield volumes.

Diffusion weighted imaging (DWI) data from one participant in the placebo group was further excluded based on an extreme mean diffusivity (MD) value (MD > 3rd percentile + 3 * interquartile range) in the presubiculum and generally poor data quality (n (DWI) = 48, resveratrol n = 25, placebo n = 23).

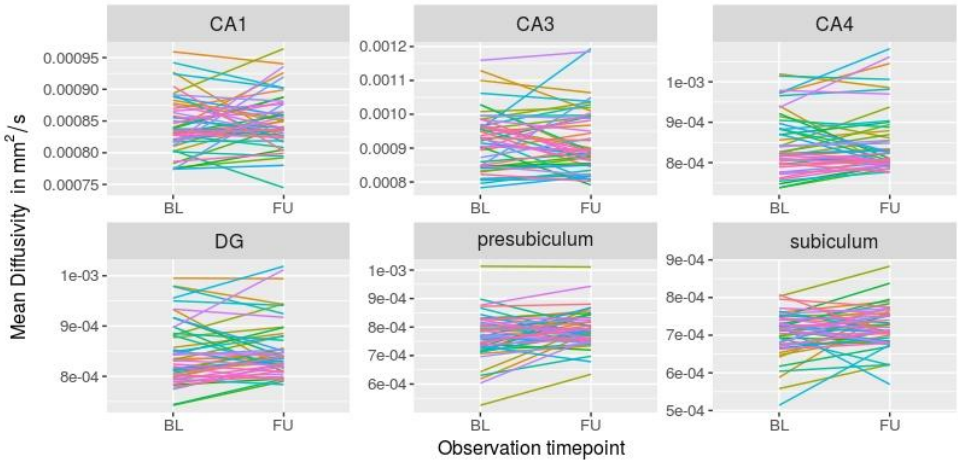


Figure S2: Mean Diffusivity of the subfields pre-and post intervention. Colors represent individual participants (n = 48).

Resting State Functional Connectivity

To achieve whole brain coverage resting state fMRI was performed on a 3T Siemens Verio Scanner with a 32 channel head coil. T1-weighted images were acquired using an MP-RAGE sequence and the Alzheimer's Disease Neuroimaging Initiative standard protocol

(TI = 900 ms; TR = 2300 ms; TE = 2.98 ms; image matrix = 256 × 240 x 176; voxel size = 1.0 mm × 1.0 mm × 1.0 mm; flip angle = 9°.

Cortical reconstruction and volumetric segmentation was performed with FreeSurfer version 5.3.0. (Dale et al., 1999;Fischl et al., 2002). FreeSurfer brain masks were corrected using gcut or manual edits in order to improve the co-registration to functional and MNI space when necessary. Anterior and posterior hippocampus segmentation was performed using PCA-based automated rotation separating head and posterior section at 41.7% of the length of the longitudinal axis (Lerma-Usabiaga et al., 2016). The resulting regions of interest were resampled to the resolution of the functional images (2 mm isotropic).

T2*-weighted functional images were acquired using a multi-band echo-planar-imaging sequence with the following parameters: TR = 1400 ms; TE = 30 ms; image matrix = 88 x 88; 64 slices; voxel size = 2.3 mm x 2.3 mm x 2.3 mm; flip angle = 69°; multiband factor = 4; 550 volumes; total acquisition time, 12:49 minutes. Preprocessing with FSL, FreeSurfer, ANTS and AFNI was implemented in a reproducible pipeline using Nipype (Gorgolewski et al., 2011), which is available to the public at https://github.com/fBeyer89/RSV_rsanalysis.

After removal of the first five volumes in order to allow the magnetization to reach steady state, rigid body and boundary-based co-registration with six degrees of freedom of the functional scan to the anatomical image was estimated. Motion and EPI distortion corrections were calculated and jointly applied in a subsequent step to each volume of the functional scan. Then (i) six components explaining the highest variance from a singular value decomposition of white matter and cerebrospinal fluid time series (CompCor)(Behzadi et al., 2007), (ii) 24 motion parameters (CPAC, (Friston et al., 1995)), (iii) motion and signal intensity spikes (Nipype rapidart), and (iv) linear and quadratic signal trends were regressed from the data and bandpass-filtering between 0.01 and 0.1 Hz was applied. Hippocampus functional connectivity was estimated by correlating the mean time series in left and right posterior and anterior hippocampus with all other voxels in the brain. Subsequently, Fisher's Z-transform was applied and the connectivity maps were transformed to MNI space by the non-linear transform from subject to MNI space previously derived using the ANTS's SyN algorithm (Avants et al., 2011). The connectivity maps were then 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) according to (Power et al., 2012). As motion is an important confounder in rs-fMRI we performed a sensitivity analysis following the approach described in Power et al. (2014). After the identification of motion-corrupted volumes (DVARS > 0.5 and FD > 0.5 mm) using a publicly available quality checking script (https://github.com/poldrack/fmriqa) we performed the scrubbing procedure (replacement of identified volumes with data of similar frequency content, filtering and removal of identified volumes) as described in Power et al. (2014). We then entered the scrubbed time series into the hippocampus connectivity pipeline. In total, 27 baseline and 21 follow-up scans were scrubbed, with a mean number of 15 respectively 20 scrubbed volumes in baseline and follow-up. All subjects had at least 8 minutes of resting state fMRI acquisition after removing volumes affected by motion.

To verify the selection of anterior and posterior hippocampus ROIs, we calculated the within-subject differences of anterior and posterior hippocampus connectivity for the right and left hippocampus separately using the baseline MRI data (n = 51 (one without MRI, six with strong head motion defined as mean framewise displacement (FD) > 0.5 mm or maximal FD > 3mm). For longitudinal analysis, n = 45 participants were eligible (one not MRI suitable at follow-up, three dropped out, two with strong head motion at follow-up). Out of these, 22 were in the resveratrol and 23 in the placebo group. There was no significant difference in mean or maximal FD between groups.

Supplementary Table S1: Changes in diet and physical activity dependent on group. Data is given as mean ± SD (range). Diet was evaluated according to the food frequency questionnaire and data is given in kilocalorie intake per day. Physical activity was estimated with the International Physical Activity Questionnaire and evaluated according to standard procedure. Data is given in metabolic equivalents (MET) per week.

	Resveratrol (n = 27)			**Placebo (n = 26)**		
	Pre	***Post***	***p, T(df) or p, Z***	***Pre***	***Post***	***p, T(df) or p, Z***
Diet (kilocalorie intake per day)	1969.7 ± 924.2 (474.02 – 5196.25)	2057.56 ± 933.38 (744.16 – 5301.82)	0.337, b	1996.24 ± 745.08 (985.19 – 4159.73)	1867.08 ± 801.98 (875.21 – 4410.72)	0.131, b
Physical Activity (MET/week) [e]	13802.70 ± 15526.72 (417 – 65382)	7445.43 ± 9539 (0 – 48443)	**0.002**, -3.027, b	9909.62 ± 6782.1 (1072 – 32874)	6858.58 ± 4224.84 (840 – 17856)	**0.015**, -2.426, b
Physical Activity (MET/week) [f]	8385.05 ± 5698.00 (417 – 22194)	4985.95 ± 3713.12 (0 – 12426.0)	**0.011**, -2.549, b	9335.02 ± 6834.06 (1072 – 32874)	6591.78 ± 4078.06 (840.00 – 17856.0)	0.052, -1.947, b

a) Dependent Sample T-Test, b) Wilcoxon Signed-Rank test. MET: metabolic equivalent.

Supplementary Table S2: Volume of whole hippocampus and subfields derived from the multimodal (T_1-and T_2-based) subfield segmentation before and after intervention/control period (n = 49). Data is given as mean ± SD.

Mean Volume (in mm^3)	Resveratrol (N = 25)			Placebo (N = 24)		
	Pre	***Post***	***p, T(df) or p, Z***	***Pre***	***Post***	***p, T(df) or p, Z***
Whole Hippocampus	2939.96 ± 320.48	2925.42 ± 309.25	0.35[a]	2937.43 ± 263.91	2948.83 ± 279.01	0.49[a]
Cornu Ammonis 1	581.25 ± 66.22	578.78 ± 65.37	0.61[a]	581.93 ± 48.10	581.60 ± 54.57	0.16[b]
Cornu Ammonis 2/3	198.30 ± 29.29	199.43 ± 30.15	0.55[a]	199.19 ± 24.50	200.52 ± 35.06	0.28[a]
Cornu Ammonis 4	218.65 ± 25.87	218.27 ± 25.10	0.82[a]	216.29 ± 20.25	215.31 ± 21.65	0.56[a]
Dentate Gyrus	258.23 ± 30.32	257.89 ± 29.08	0.88[a]	254.76 ± 24.88	254.04 ± 26.58	0.71[a]
Pre-Subiculum	259.49 ± 28.94	258.69 ± 30.02	0.74[a]	266.89 ± 31.47	271.10 ± 34.27	0.15[a]
Subiculum	348.58 ± 37.88	348.11 ± 35.67	0.86[a]	349.08 ± 31.76	350.59 ± 34.01	0.63[a]

a) Dependent Sample T-Test, b) Wilcoxon Signed-Rank test.

Supplementary Table S3: Mean diffusivity averaged over hemispheres of whole hippocampus and subfields before and after intervention/control period (n = 48). Data is given as mean ± SD.

Mean diffusivity (in 10^{-3} mm^2/s)	Resveratrol (N = 25)			Placebo (N = 23)		
	Pre	***Post***	***p, T(df) or p, Z***	***Pre***	***Post***	***p, T(df) or p, Z***
Whole Hippocampus	0.813 ± 0.039	0.826 ± 0.037	0.025 [b]	0.820 ± 0.041	0.833 ± 0.041	0.16[a]
Cornu Ammonis 1	0.840 ± 0.036	0.849 ± 0.041	0.24[b]	0.848 ± 0.052	0.853 ± 0.048	0.66[a]
Cornu Ammonis 2&3	0.915 ± 0.085	0.909 ± 0.069	0.63[a]	0.938 ± 0.090	0.936 ± 0.116	0.92[b]
Cornu Ammonis 4	0.836 ± 0.081	0.842 ± 0.068	0.2[b]	0.857 ± 0.066	0.866 ± 0.090	0.27[b]
Dentate Gyrus	0.838 ± 0.066	0.845 ± 0.047	0.43[a]	0.856 ± 0.058	0.865 ± 0.067	0.26[b]
Pre-Subiculum	0.782 ± 0.079	0.801 ± 0.068	0.098[a]	0.749 ± 0.084	0.781 ± 0.058	0.11[b]
Subiculum	0.709 ± 0.052	0.719 ± 0.056	0.3[b]	0.697 ± 0.067	0.722 ± 0.058	0.064[b]

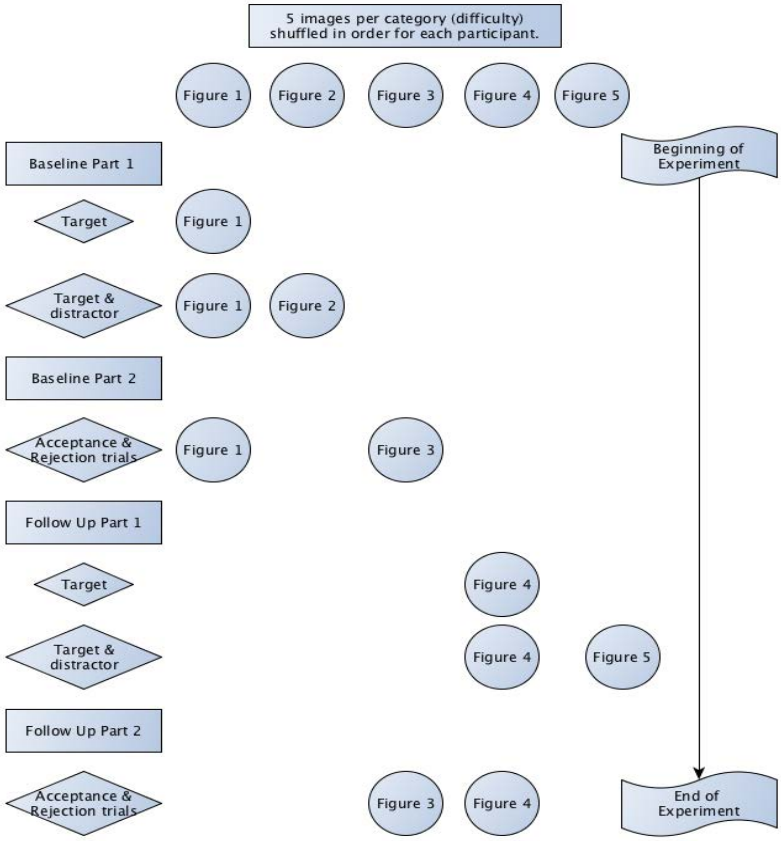
a) Dependent Sample T-Test, b) Wilcoxon Signed-Rank test.

Supplementary Information ModBent task

The ModBent task was carried out according to Brickman et al. (2014) without parallel versions as such (see Brickman et al. (2014), for details). Briefly, in part 1 of the task first a target figure is presented and after a short delay participants have to select the target, which is then presented together with a distractor figure. In part two of the ModBent participants see only one figure at a time and have to accept (picture was presented in part 1 of the task) or reject it (picture was not presented in part 1 = foil figure). Part 1 consists of 41 trials with different complexities (degrees of the used Lissajous figures) and part 2 consists of 82 trials (41 acceptances and 41 rejections).

The 41 trials in part 1 are based on different groups of pictures concerning the complexity of the Lissajous figures. For each level of complexity 5 pictures were available. The pictures were randomly shuffled for each participant. Then, in part 1 of the ModBent task at baseline, one picture was used as target image, while another one was selected as distractor and a third one as foil. In part 2 (at baseline) of the ModBent task always the target picture and the foil were shown.

For the follow up version of the ModBent a new target and a new distractor were chosen, while the foil remained the same as in part one. The 41 trials in part 1 occur in randomized order concerning their complexity. All participants had the same number of trials for each difficulty. Thus, the ModBent task does not have parallel versions, but each participant has his/her own version for baseline and follow up. Please also see the picture attached to clarify the order of figures.



Supplementary Table S4: Overview of baseline and follow up values of depressive symptoms, perceived stress, sleep quality and diet (measured in consumed kilocalories per day and Mediterranean diet score).

Parameter	Resveratrol (n = 27)			Placebo (n = 26)		
	Pre	***Post***	***p-value***	***Pre***	***Post***	***p-value***
Depressive symptoms[a]	4.67 ± 3.2	3.96 ± 3.5	0.29[e]	5.46 ± 4.8	5.15 ± 4.6	0.45[e]
Perceived stress[b]	8.93 ± 5.3	8.81 ± 5.4	0.84[e]	11.42 ± 6.2	10.78 ± 7.8	0.35[e]
Sleep quality[c]	5.19 ± 3.1	5.37 ± 3.1	0.61[f]	5.42 ± 2.9	5.80 ± 3.7	0.45[f]
Total kilocalories /day	1970 ± 924	2058 ± 933	0.34[f]	1996 ± 745	1867 ± 802	0.13[f]
Mediterranean diet Score[d]	4.6 ± 1.1	4.7 ± 1.4	0.91[e]	4.12 ± 1.6	4.19 ± 1.9	0.79[e]

a) according to Beck's Depression Inventory, b) according to Trierer Inventory for Chronic Stress (TICS), c) according to Pittsburgh Sleep Quality Index (PSQI), d) based on Trichopoulou et al. (1995) e) Dependent Samples T-test, f) Wilcoxon Sign-Rank test

Supplementary Information Antioxidant Score:

The "antioxidant-score" is similar to the Mediterranean diet score by Trichopoulou et al. (1995). The amount consumed of each food item in the respective groups was summed up (e.g. category 4: grams consumed of fresh fruit + grams consumed of processed fruit = grams consumed in category 4). Based on median splits for each category, participants were assigned 1 point, if they had a consumption above the median or 0, if below median. Then, the points for category 1 – 5 were summed up to receive the antioxidant-score. This approach did not reveal differences within or between groups in antioxidant/polyphenol content. Those conclusions were drawn within the limitations of our FFQ.

Abbreviations: BL = baseline; FU = follow up

Supplementary Table S5: Consumption in different composite food items measured with a semi-quantitative food frequency questionnaire.

Polyphenol/Antioxidant Score		Group	Quantity BL	Between-group differences BL	Quantity FU	Within-group differences BL-FU	Univariate analysis (time*group)
Category 1	**Fruit juices**	RSV	6159 ± 17290	P=0.65	5668 ± 13.238	P=0.84	P=0.77
	in ml/month	placebo	5098 ± 17.388	Z=-0.45	6255 ± 21.777	P=0.59	
	vegetable juices	RSV	559 ± 1478	P=0.74	146 ± 345	P=0.14	P=0.13
	in ml/month	placebo	340 ± 893	Z=-0.34	380 ± 1120	P=0.56	
	tea (black/green)	RSV	1500 ± 2493	P=0.77	1942 ± 3627	P=0.11	P=0.10
	in ml/month	placebo	3470 ± 10.267	Z=-0.30	2922 ± 7996	P=0.53	
	coffee	RSV	12.444 ± 5398	P=0.37	14.122 ± 6346	P=0.20	P=0.16
	in ml / month	placebo	13.523 ± 15.799	Z=-0.90	10.932 ± 7834	P=0.68	
Category 2	**beer**	RSV	2643 ± 5008	P=0.17	3315 ± 5947	P=0.84	P=0.15
	in ml/month	placebo	3119 ± 3829	Z=-1.36	2678 ± 4306	P=0.061	
Category 3	**Wine**	RSV	775 ± 566	P=0.55	938 ± 802	P=0.37	P=0.14
	in ml / month	placebo	1165 ± 1612	Z=-0.60	983 ± 1332	P=0.60	
	vegetables (fresh)	RSV	1758 ± 1297	P=0.63	1557 ± 1545	P=0.15	P=0.11
	in g/month	placebo	**1794 ± 1825**	Z=-0.48	**837 ± 707**	**P=0.020**	
	vegetables (processed)	RSV	1276 ± 1024	P=0.83	1545 ± 1301	P=0.27	P=0.46
	in g/month	placebo	1316 ± 1063	Z=-0.21	1364 ± 1059	P=0.53	
Category 4	**legumes**	RSV	305 ± 216	P=0.71	350 ± 245	P=0.29	P=0.69
	in g/month	placebo	304 ± 263	Z=-0.36	317 ± 257	P=0.84	
	fruit (fresh)	RSV	6266 ± 4382	P=0.65	6411 ± 5189	P=0.96	P=0.73
	in g/month	placebo	7090 ± 4820	Z=-0.46	6658 ± 5332	P=0.33	
Category 5	**fruit (processed)**	RSV	**465 ± 980**	P=0.91	**747 ± 1322**	P=0.054	**P=0.02**
	in g/month	placebo	**336 ± 521**	Z=-0.12	**211 ± 209**	P=0.39	
	chocolate	RSV	**131 ± 135**	P=0.23	**125 ± 133**	P=0.92	**P=0.046**
	in g/month	placebo	**389 ± 565**	Z=-1.21	**208 ± 305**	P=0.06	
	nuts	RSV	163 ± 273	P=0.42	159 ± 188	P=0.46	P=0.59
	in g/month	placebo	124 ± 177	Z=-0.80	163 ± 421	P=0.63	

Supplementary Table S6: Overview Antioxidants/Polyphenol-Score and comparison within and between groups

Antioxidant Score	Resveratrol (BL)	Resveratrol (FU)	Placebo (BL)	Placebo (FU)
Mean ± S.D (range)	2.41 ±1.2 (0-5)	2.59 ± 1.3 (0-5)	2.5 ±1.1 (0-4)	2.19 ±1.1 (0-4)
Wilcoxon Tests	Within resveratrol group: p=0.37, z=-0.89		Within placebo group: p=0.27, z=-1.09	
$ANOVA_{rm}$	Time*group: p=0.2, time-effect: p=0.7			

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A2 Author contributions to the publications

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)**, Anja Veronica Witte* **(AVW)**; Frontiers in Aging Neurosciences July 2015;
*Corresponding author

Principal investigator:	AV, MS, AVW
Conception and design of the paper:	SH, AVW
Literature review:	SH, SKM
Data interpretation:	SH, SKM, AVW
Figure creation	SH
Drafted manuscript	SH
Critical revision	SH, SKM, AV, AVW

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Leipzig, den 30.04.2018 Shahrzad Kharabian _____

Leipzig, den _____ Michael Stumvoll _____

Leipzig, den 11.5.18 Arno Villringer _____

Leipzig, den 3.5.2018 Veronica Witte _____

"Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults – a randomized controlled trial" by Sebastian Huhn **(SH)** Frauke Beyer **(FB)**, Rui Zhang **(RZ)**, Leonie Lampe **(LL)**, Jana Grothe **(JG)**, Jürgen Kratzsch **(JK)**, Anja Willenberg **(AW)**, Jana Breitfeld **(JB)**, Peter Kovacs **(PK)**, Michael Stumvoll **(MS)**, Robert Trampel **(RT)**, Pierre-Louis Bazin **(PLB)**, Arno Villringer **(AV)**, Anja Veronica Witte **(AVW)***;
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Principal investigator:	AV, MS, AVW
Conception and design of the study:	SH, FB, RZ, AVW
Data acquisition:	SH, FB, LL, JG
Data analysis:	SH, FB, RZ, LL, JG, JK, AW, JB, PK, RT, PLB
Data interpretation:	SH, FB, AVW
Figure creation:	SH, FB, AVW
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Continued signatures for "Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults – a randomized controlled trial"

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A3 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.

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Sebastian Huhn – Leipzig, _____ 2018

A4 Curriculum vitae

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EDUCATION

since 2015/02	PhD Student at the Max-Planck Institute for Human Cognitive and Brain Sciences, Leipzig Thesis: "The impact of nutrition on hippocampal function - Results of a literature review and a randomized controlled trial" (Prof. Dr. Arno Villringer & Dr. Anja Veronica Witte)
2012/10 – 2014/10	Master of Science Nutritional Science (graded 1.4) Martin-Luther University Halle-Wittenberg Thesis (graded 1.1): How a Mediterranean-like Diet affects Hippocampal Volume in Elderly People (Prof. Dr. Arno Villringer & Dr. Anja Veronica Witte)
2009/10 – 2012/09	Bachelor of Science Nutritional Science (graded 1.7) Martin-Luther University Halle-Wittenberg Thesis (graded 1.3): Optimizing a HPLC-based method to analyze branched-chain amino acids in tissue samples, and elucidating their role in hunger and satiety (Prof. Dr. Gabriele Stangl)
2000/09 – 2009/07	Abitur/secondary school: Spessart-Gymnasium Alzenau (grade 1.9)

WORK EXPERIENCE

2018/03	Research stay at the Ben Gurion University of the Negev, Beer Sheva, Israel, (Prof. PhD Iris Shai); Neuroimaging collaboration for the DIRECT PLUS trial
2014/05 – 2014/12	Student Assistant at the Max-Planck Institute for Human Cognitive and Brain Sciences, Department of Neurology, Group 'Aging and Obesity' (Dr. Veronica Witte)

SCHOLARSHIPS & AWARDS

2016/07	Free Registration for the Alzheimer's Association International Conference (AAIC) 2017; awarded for winning the 5K Fun Run during AAIC 2016.
2015/02 – 2018/07	Max Planck International Network on Aging: stipend for PhD students

RESEARCH SCHOOLS

2015/02 - 2015/07	MaxNetAging Research School, Max Planck Institute for Demography Research, Rostock, Germany
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MEMBERSHIPS

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A5 List of publications

Paper:

Huhn, S., Beyer, F., Zhang, R., Lampe, L., Grothe, J., Kratzsch, J., Willenberg, A., Breitfeld, J., Kovacs, P., Stumvoll, M., Trampel, R., Bazin, P.L., Villringer, A., and Witte, A.V. (2018). Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults - A randomized controlled trial. *Neuroimage* 174**,** 177-190. doi: 10.1016/j.neuroimage.2018.03.023.

Huhn, S., Kharabian Masouleh, S., Stumvoll, M., Villringer, A., and Witte, A.V. (2015). Components of a Mediterranean diet and their impact on cognitive functions in aging. *Front Aging Neurosci* 7**,** 132. doi: 10.3389/fnagi.2015.00132.

Book Chapter:

Huhn, S. and Witte A.V. (2017) Effects of Resveratrol on Cognitive Functions. Chapter 24 in “Nutrition and Functional Foods for Healthy Aging”, Elsevier Inc., Academic Press, Editor: Ronald Ross Watson. DOI: http://dx.doi.org/10.1016/B978-0-12-805376-8.00024-1.

A6 Conference contributions

2018/07 Huhn, S., Beyer, F., Zhang, R., Lampe, L., Luck, T., Riedel-Heller, S.G., Kratzsch, J., Schroeter, M.L., Loeffler, M., Stumvoll, M., Villringer, A., Witte, A. V. “Effects of Leptin on Hippocampus Volume and Cognitive Performance” Poster to be presented at the Alzheimer’s Association International Conference 2018, Chicago, Illinois, USA

2017/07 Huhn, S., Beyer, F., Zhang, R., Stumvoll, M., Villringer, A. Witte, A. V. „Effects of six month resveratrol supplementation on verbal memory performance in healthy elderly adults – a randomized controlled trial“ Poster presented at the International Congress on Nutrition 2017, Buenos Aires, Argentina

2017/07 Huhn, S., Zhang, R., Beyer, F., Lampe, L., Luck, T., Riedel-Heller, S.G., Schroeter, M.L., Loeffler, M., Stumvoll, M., Villringer, A., Witte, A. V. “Association of Hippocampal Volumes with Cognitive Tasks in a Large Population-Based Cohort” Poster presented at the Alzheimer’s Association International Conference 2017, London, England

2017/05 Huhn, S., Beyer, F., Zhang, R., Stumvoll, M., Villringer, A. Witte, A. V. „The Resveratrol Study – Study Conception and Preliminary Results“ Poster presented at the MaxNetAging Conference, Rostock, Germany

2016/07 Huhn, S., Zhang, R., Beyer, F., Kharabian Masouleh, S., Lampe, L., Luck, T., Riedel-Heller, S., Schroeter, M., Loeffler, M., Stumvoll, M., Scholz, M., Burkhardt, R., Villringer, A., Witte, A.V. “FTO is not related to Imaging Parameters of the Hippocampus -A Volumetric and Diffusion Tensor Imaging Study”. Poster presented at the Alzheimer’s Association International Conference, Toronto, Canada

2015/09 Huhn, S. “Einfluss der Ernährung auf das Gehirn im Alter” (The impact of Nutrition on the Aging Brain). Symposium talk at the 30th Annual Meeting of the Society for Neuropsychology (GNP), Lübeck, Germany

2015/06 Huhn, S., Kharabian Masouleh, S., Riedel-Heller, S., Schroeter, M., Thiery, J., Löffler, M., Villringer, A., Witte, A.V. “Volumetric Changes of Hippocampal Subfields over Age in a Large Cohort of Healthy Older Adults”. Poster presented at the Organization for Human Brain Mapping Conference, Honolulu, Hawaii, USA

A7 Acknowledgements

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Magnetresonanztomographische Untersuchungen über die anatomische Variabilität des Frontallappens des menschlichen Großhirns

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Electrophysiological markers of rhythmic processing in infants with and without risk for Specific Language Impairment (SLI)

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Temporospatial characteristics of error correction

55 Britta Stolterfoht
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Mechanisms of auditory sentence comprehension in first and second language: An electrophysiological miniature grammar study

59 Franziska Biedermann
Auditorische Diskriminationsleistungen nach unilateralen Läsionen im Di- und Telenzephalon

60 Shirley-Ann Rüschemeyer
The Processing of Lexical Semantic and Syntactic Information in Spoken Sentences: Neuroimaging and Behavioral Studies of Native and Non-Native Speakers

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The Development of Argument Processing Mechanisms in German. An Electrophysiological Investigation with School-Aged Children and Adults

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Bestimmung der Lateralisierung von Sprachprozessen unter besondere Berücksichtigung des temporalen Cortex, gemessen mit fMRT

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Prosodische Informationsverarbeitung bei normalsprachlichem und deviantem Satzmaterial: Untersuchungen mit ereigniskorrelierten Hirnpotentialen

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Functional specialization in the lateral frontal cortex: The role of the inferior frontal junction in cognitive control

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The cognitive representation of tasks – Exploring the role of response modalities using the task-switching paradigm

66 Ulrike Toepel
Contrastive Topic and Focus Information in Discourse – Prosodic Realisation and Electrophysiological Brain Correlates

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Die Anwendung von Spektral- und Waveletanalyse zur Untersuchung der Dynamik von BOLD-Zeitreihen verschiedener Hirnareale

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The role of the basal ganglia in auditory language processing: Evidence from ERP lesion studies and functional neuroimaging

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