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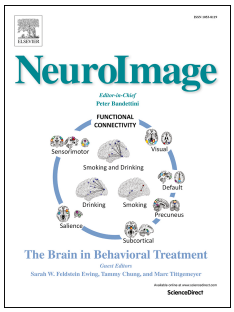

Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults – A randomized controlled trial

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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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General Theme:
Cognition and Aging

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 Tesla, 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.

Keywords (maximum 6):
7 Tesla MRI, subjective cognitive decline, resting-state fMRI, mean diffusivity, hippocampus subfields, polyphenol

Abbreviations:

ASAT/ALAT	Alanine/Aspartate Aminotransferase
ApoE	Apolipoprotein E
BDI	Beck's Depression Inventory
BMI	Body Mass Index
CVLT	California Verbal Learning Task
FC	Functional Connectivity
HbA1c	Glycated hemoglobin
HDL/LDL	High/Low Density Lipoprotein
MRI	Magnetic Resonance Imaging
MD	Mean Diffusivity
MMST	Mini Mental Status Test
RCT	Randomized Controlled Trial
SCD	Subjective Cognitive Decline
TMT	Trail Making Task
UHF	Ultra-High Field
WHR	Waist Hip Ratio

1. 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 (reviewed in (Davinelli et al., 2012; Huhn et al., 2015; Tellone et al., 2015; Wong and Howe, 2017).

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 in part explained 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 six 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.

2. Material and Methods

2.1. 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 grams 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.

2.2. 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 six 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).

2.3. 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 x 100 mg = 200 mg resveratrol (3,5,4'-trihydroxy-trans-stilbene) and 2 x 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 anamneses 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.

========== *FIGURE 1 about here* ==========

2.4. *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 am and 4 pm and at follow up between 9 am and 4 pm.

========== *FIGURE 2 about here* ==========

2.5. Neuropsychological testing

We assessed verbal memory performance**, i.e. learning ability, delayed recall,retention of words/rate of forgetting 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 minutes 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*" 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.**

2.6. 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 seconds. A Lissajous-figure is a sinusoidal curve derived in a mathematically controlled manner and figures differ in order, e.g. number of horizontal and vertical nodes (see Figure 2). After a delay of one second 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.

2.7. 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). Il-6 measures below detection limit were set to 1.50 pg/ml (lowest value). 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/m²) 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).

2.8. 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)).

2.9. Magnetic Resonance Imaging (MRI) Acquisition and Analysis

2.9.1. 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 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 = 13000 ms; TE = 14 ms; image matrix: 384 x 384; 50 slices; voxel size: 0.5 mm x 0.5 mm x 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). Three 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), leaving n = 50 for analysis (n = 26 resveratrol, n = 24 placebo). One additional participant had to be excluded based on poor data quality, leaving 49 for analysis (n = 25 resveratrol, n = 24 placebo).

2.9.2. 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 S**upplementary 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).

2.9.3. 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 x 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 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.

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 ROIs, 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 3mm). For longitudinal analysis, another 6 participants were excluded from analysis due to motion, leaving 45 for analysis (n = 22 resveratrol, n = 23 placebo).

2.10. Statistical Analysis

2.10.1. 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-sum, 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.

2.10.2. 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).

2.10.3. 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 (TFCE, 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 $|T| > 3$.

3. Results

3.1. 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.**

========== *TABLE 1 about here* ==========

3.2. 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 minute workout had been: hours = 1, minutes = 30. However, during data curation we noticed that participants, who engaged in an activity for 3 hours 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 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.

3.3. Memory Performance

There was no significant time by 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 ($F_{(4, 48)} = 1.39$, $p = 0.25$). 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*order, $F_{(4, 48)} = 3.2$, $p = 0.021$). 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.

========== FIGURE 3 about here ==========
========== TABLE 2 about here ==========

With regard to pattern recognition memory performance measured using the ModBent task, we observed a trend for a time by 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).

========== FIGURE 4 about here ==========

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-group interactions: all p > 0.4). Correcting for age and sex did not change these results.

3.4. Fasting blood levels and anthropometric measures

For details on blood measures, see **Table 2**. There was no significant time by 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 group-by-time 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^{-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 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).

========== *FIGURE 5 about here* ==========

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 pressure in the placebo group (Z = 2.061, p = 0.039). No further changes were observed.

========== *TABLE 2 about here* ==========

3.5. Hippocampus subfield measures

$MANOVA_{RM}$ revealed no significant time ($F_{(6, 42)}$ = 0.96, p = 0.46) or time by 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 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.

========== *FIGURE 6 about here* ==========

3.6. 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.

========== *FIGURE 7 about here* ==========

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.

4. 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.

4.1. Resveratrol and memory performance

Considering verbal memory performance, we could not replicate previous results of a significant improvement after resveratrol compared to placebo (Witte 2014; Evans 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 effects of time on learning, delayed recall and forgetting rate can be simple learning sequence effects 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.

4.2. 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/m², 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.

4.3. 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 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, Koebe 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 x 2 mm x 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.

4.4. 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.

4.5. 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.

4.6. 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.

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6. Declarations and Conflict of Interests

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7. Figure Legends

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 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 Tesla n = 26/24.

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.

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 group interaction. Triangles represent mean, error bars represent standard error (S.E.).

Figure 4: Pattern recognition memory performance, measured using the ModBent task, at baseline and after 6 months of either resveratrol (n =22) or placebo intake (n = 22), showing a trend for a time*group interaction for sensitivity index d' (ANOVARM 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.).

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.

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 (ROIs, delineated in bold) derived from the mean of all individual hippocampus masks transformed to MNI space and thresholded at 0.2.

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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]

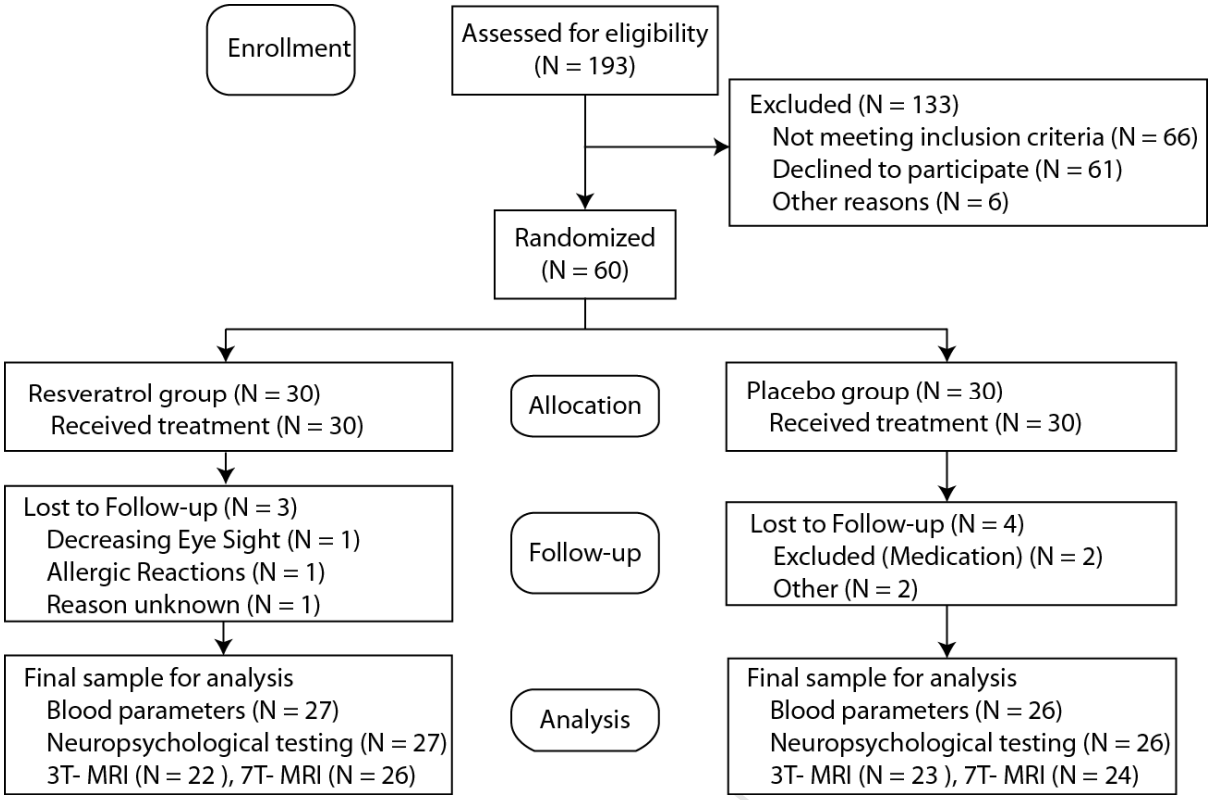
Trail Making Task A (in seconds)	43 ± 13 (28 – 84)	41 ± 14 (16 – 79)	0.66[g]
Trail Making Task B (in seconds)	89 ± 29 (45 – 161)	80 ± 25 (40 – 127)	0.33[g]

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 (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	5.61 ± 0.30	0.202 [c]	5.55 ± 0.23	5.56 ± 0.2	0.564, c
Glucose (mmol/L)	5.17 ± 0.45	5.45 ± 0.66	**0.008**, -2.67 [d]	5.36 ± 0.57	5.55 ± 0.57	**0.029**, -2.19, d
Insulin (pmol/L) [b]	61.5 ± 39.41	72.6 ± 50.11	0.3 [d]	65.36 ± 56.67	59.68 ± 39.41	0.53, d
Total Cholesterol (mmol/L)	5.74 ± 0.92	6.02 ± 1.07	**0.006**, -3.0 (26) [c]	5.99 ± 0.93	5.70 ± 0.83	0.087, c
LDL/HDL-ratio	2.5 ± 1.2	2.6 ±1.2	0.7 [c]	2.3 ± 0.79	2.1 ± 0.7	0.1, c
Triacylglycerides (mmol/L)	1.22 ± 0.71	1.37 ± 0.87	0.77 [d]	1.16 ± 0.61	1.15 ± 0.45	0.35, d
Interleukin-6 (pg/ml)	2.25 ± 1.35	3.5 ± 2.16	**0.002**, -3.10 [c]	2.53 ± 3.25	3.12 ± 4.2	**0.032**, -2.143, d
HsCRP (mg/L)	2.33 ± 2.22	4.9 ± 8.02	**0.022**, -2.28 [c]	1.93 ± 2.39	3.82 ± 11.28	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	96.2 ± 24.9	**0.001**, 3.7 (26) [c]	107.9 ± 34.5	103.3 ± 32.5	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	4.90 ± 5.57	**0.001, -3.92** [d]	0.16 ± 0.81	0.0 ± 0.0 [e]	0.32, -1.00 [d]
Glucuronated Resveratrol (ng/mL)	0.26 ± 0.50	894.19 ± 593.07	**0.001, -4.52** [d]	0.21 ± 0.50	0.73 ± 0.65	**0.007, -2.69** [d]
Sulfated Resveratrol (ng/mL)	0.39 ± 0.79	336.16 ± 217.91	**0.001, -4.52** [d]	0.12 ± 0.36	0.08 ± 0.29	0.50, -0.67 [d]
Sulfo-Glucuronated Resveratrol (ng/mL)	0.59 ± 0.99	155.85 ± 168.25	**0.001, -4.35** [d]	0.32 ± 0.49	0.21 ± 0.33	0.49, -0.686 [d]

Weight (kg)	75.54 ± 14.29	76.35 ± 14.61	0.062, -1.95 [c]	76.02 ± 17.32	76.29 ± 17.36	0.279, d
Body-Mass Index (kg/m^2)	26.76 ± 3.91	26.95 ± 4.06	0.113 [d]	26.94 ± 4.46	27.01 ± 4.28	0.424, d
Body fat (%)	29.07 ± 8.07	29.71 ± 7.97	**0.028**, -2.20 [d]	30.43 ± 8.47	30.87 ± 8.31	0.073, -1.791 d
Systolic Blood Pressure (mm Hg)	139.10 ± 16.17	139.95 ± 17.49	0.748 [c]	141.35 ± 17.63	138.08 ± 15.76	0.174, d
Diastolic Blood Pressure (mm Hg)	86.30 ± 8.86	85.89 ± 7.65	0.773, c	89.92 ± 11.40	87.38 ± 10.86	**0.039**, -2.061 [d]
Trail Making Task A (sec)	42.65 ± 12.83	38.96 ± 17.83	0.15, d	40.66 ± 13.72	35.90 ± 19.18	0.16, d
Trail Making Task B (sec)	88.56 ± 28.88	86.08 ± 28.06	0.52, d	80.32 ± 24.91	75.47 ± 25.38	0.27, d
Trail Making Task B/A	2.16 ± 0.73	2.43 ± 0.93	0.12, d	2.06 ± 0.53	2.14 ± 0.58	0.79, d

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



a) Eligibility for Participation

- ✓ Age > 60 years
- ✓ 22 < BMI < 40 kg/m²
- ✓ MMST > 26
- ✓ No history of stroke, diabetes mellitus, psychiatric or severe internal disease
- ✓ No intake of centrally active medication or antioxidative supplements
- ✓ No MRI contraindications
- ✓ Daily consumption of less than 50g alcohol/ 6 cups of coffee/10 cigarettes

b) Study Design

Baseline → Follow-up

Resveratrol N = 30

26 weeks of Resveratrol supplementation 100 mg twice/day

N = 27 completed

Randomization Stratified by Sex & Age groups

Assessments at Baseline and Follow-up
- ✓ Neuropsychological testing (primary outcome)
- ✓ ModBent-Task
- ✓ Blood parameters
- ✓ Anthropometry
- ✓ Structural and functional Neuroimaging at 3T & 7T

Placebo N = 30

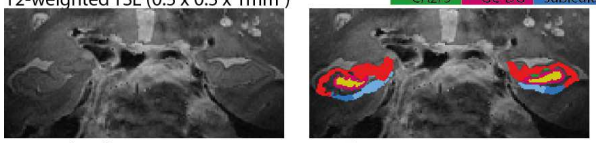
26 weeks of Placebo supplementation

N = 26 completed

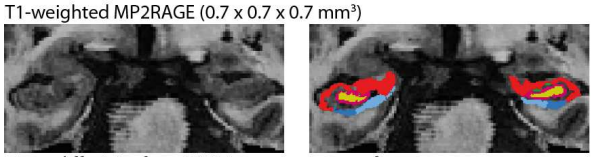
c) Hippocampal subfield segmentation (7T)

CA1 CA4 presubiculum CA2/3 GC-DG subiculum

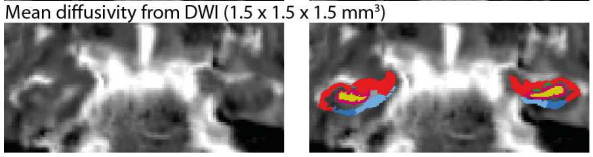
T2-weighted TSE (0.5 x 0.5 x 1mm³)



T1-weighted MP2RAGE (0.7 x 0.7 x 0.7 mm³)

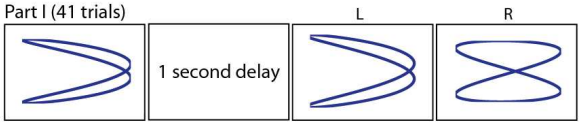


Mean diffusivity from DWI (1.5 x 1.5 x 1.5 mm³)



d) ModBent-Task

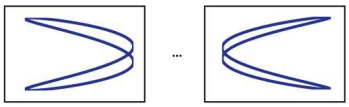
Part I (41 trials)



Presentation of target picture

Choice of previously shown target

Part II: Selection of previously shown targets (82 trials)



yes

no

Outcome measures:
- ✓ Sensitivity index (d')
- ✓ reaction times in correct rejection trials