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A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease

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ABSTRACT

Objective: A randomized, placebo-controlled, double-blind, multicenter 52-week phase 2 trial of resveratrol in individuals with mild to moderate Alzheimer disease (AD) examined its safety and tolerability and effects on biomarker (plasma Aβ40 and Aβ42, CSF Aβ40, Aβ42, tau, and phospho-tau 181) and volumetric MRI outcomes (primary outcomes) and clinical outcomes (secondary outcomes).

Methods: Participants (n = 119) were randomized to placebo or resveratrol 500 mg orally once daily (with dose escalation by 500-mg increments every 13 weeks, ending with 1,000 mg twice daily). Brain MRI and CSF collection were performed at baseline and after completion of treatment. Detailed pharmacokinetics were performed on a subset (n = 15) at baseline and weeks 13, 26, 39, and 52.

Results: Resveratrol and its major metabolites were measurable in plasma and CSF. The most common adverse events were nausea, diarrhea, and weight loss. CSF Aβ40 and plasma Aβ40 levels declined more in the placebo group than the resveratrol-treated group, resulting in a significant difference at week 52. Brain volume loss was increased by resveratrol treatment compared to placebo.

Conclusions: Resveratrol was safe and well-tolerated. Resveratrol and its major metabolites penetrated the blood–brain barrier to have CNS effects. Further studies are required to interpret the biomarker changes associated with resveratrol treatment.

Classification of evidence: This study provides Class II evidence that for patients with AD resveratrol is safe, well-tolerated, and alters some AD biomarker trajectories. The study is rated Class II because more than 2 primary outcomes were designated. *Neurology*® 2015;85:1–9

GLOSSARY

3G-RES = 3-O-glucuronidated-resveratrol; **4G-RES** = 4-O-glucuronidated-resveratrol; **AD** = Alzheimer disease; **ADAS-cog** = Alzheimer's Disease Assessment Scale–cognitive; **ADCS** = Alzheimer's Disease Cooperative Study; **ADCS-ADL** = Alzheimer's Disease Cooperative Study Activities of Daily Living Scale; **AE** = adverse event; **BMI** = body mass index; **CDR-SOB** = Clinical Dementia Rating-sum of boxes; $\mathbf{C_{max}}$ = maximal plasma concentration; **DMSO** = dimethyl sulfoxide; **ITT** = intention-to-treat; **MMRM** = mixed-model repeated-measures; **MMSE** = Mini-Mental State Examination; **NPI** = Neuropsychiatric Inventory; **S-RES** = 3-sulfated-resveratrol; **SAE** = serious adverse event.

Caloric restriction prevents aging-dependent phenotypes[1] and activates sirtuins (including SIRT1), a highly conserved family of deacetylases that are regulated by NAD+/NADH and thus link energy metabolism to gene expression.[2] SIRT1 substrates include FOXO and PGC-1α.[3] A screen of SIRT1 activators identified resveratrol (trans-3,4′,5-trihydroxystilbene) as a potent compound.[4] Similar to caloric restriction,[5,6] resveratrol decreases aging-dependent cognitive decline and pathology in Alzheimer disease (AD) animal models.[7,8] Xenohormesis is the ability to transmit resilience to stress from one species to another—for example, via

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consumption of resveratrol-containing foods.[9] Resveratrol is under investigation to prevent age-related disorders including cancer, diabetes mellitus, and neurodegeneration.[4,9–12] Due to its low bioavailability but high bioactivity,[13,14] we increased the dose to the maximal amount considered safe and well-tolerated for this study.[15]

We conducted a randomized, placebo-controlled, double-blind, multicenter 52-week phase 2 trial of resveratrol in individuals with mild to moderate AD. The primary objectives were to (1) assess the safety and tolerability of resveratrol; (2) assess effect on plasma and CSF Aβ42 and Aβ40, CSF tau and phospho-tau 181, and volumetric MRI; and (3) examine pharmacokinetics. The secondary objectives were to (1) explore the effects of resveratrol on cognitive, functional, and behavioral outcomes; (2) examine the influence of *APOE* genotype; and (3) determine whether resveratrol affects insulin and glucose metabolism. We hypothesized that resveratrol would alter AD biomarker trajectories.

METHODS

Classification of evidence. This study provides Class II evidence that for patients with AD resveratrol is safe, well-tolerated, and alters some AD biomarker trajectories. The study is rated Class II because more than 2 primary outcomes were designated.

Study design. A multicenter, double-blind, placebo-controlled trial was conducted June 2012–March 2014 with participants recruited from 26 US academic clinics affiliated with the Alzheimer's Disease Cooperative Study (ADCS). The enrollment target was 120 (60 per group) randomized to drug or placebo. Actual enrollment was 119. A subgroup of 15 participants enrolled in a randomized 4:1 (n = 15, 12 treated plus 3 placebo) study for 24-hour pharmacokinetics at selected sites. For these individuals, blood samples were collected at times 0, 0.17, 0.33, 0.5, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours. Measurements included resveratrol, 3-O-glucuronidated-resveratrol (3G-RES), 4-O-glucuronidated-resveratrol (4G-RES), and 3-sulfated-resveratrol (S-RES). These participants completed 24-hour pharmacokinetics at each dosage: after the first dose following baseline, after the first dose with each increment (weeks 13, 26, and 39), and after the final dose (week 52). The afternoon dose of resveratrol was withheld during the 24-hour blood sampling.

Standard protocol approvals, registrations, and patient consents. This study was conducted in accordance with Good Clinical Practice guidelines. Informed consent was obtained from participants and study partners. The study was conducted under local institutional review board supervision, under Food and Drug Administration IND 104205, and registered at ClinicalTrials.gov (NCT01504854).

Study visits. Visits occurred at screening, baseline, and weeks 6, 13, 19, 26, 32, 39, 45, and 52. Visits included concomitant medications and adverse events (AEs) review, physical and neurologic examination, urinalysis, pill count, and venipuncture for laboratory tests, pharmacokinetics, and biomarker analyses. Brain MRIs were obtained at baseline, week 13, and week 52. ECGs and CSF collections were performed at baseline and week 52. Oral glucose tolerance tests, with peripheral blood mononuclear cell collections at 0 and 120 minutes, were performed at screening and week 52 (except in participants enrolled in the 24-hour pharmacokinetics substudy).

Participants and randomization. Inclusion criteria for enrollment included age >49 years, fluent in English or Spanish, diagnosis of probable AD by National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria,[16] Mini-Mental State Examination (MMSE) score[17] 14–26 at screening, modified Hachinski Score[18] <5, normal laboratory values, stable medications for 4 months, and stable use of cholinesterase inhibitors or memantine. Exclusion criteria included non-AD dementia, Down syndrome, sensory impairments precluding participation, pregnancy, contraindication to lumbar puncture or MRI, >4 microhemorrhages on a recent MRI, treated diabetes mellitus, use of resveratrol-containing supplements, and unsuitable disorder or laboratory finding. Participants were assigned to resveratrol or placebo using a stratified permuted block method with an allocation ratio of 1:1. Assignment to groups was stratified by site. After participants signed informed consent and eligibility was confirmed, study sites received randomization numbers from the Informatics Core. A subgroup (n = 15) was randomized 4:1 (12 treated, 3 placebo) for 24-hour pharmacokinetics. Sample sizes (60 per group) were determined from power analyses utilizing published data on CSF biomarkers in AD trials, and a predicted 20% dropout rate.

Study medication. Aptuit Laurus, Inc. (Kansas City, MO, now Catalent, Inc., Somerset, NJ) synthesized and encapsulated resveratrol (trans-3,5,4′-trihydroxystilbene) and provided identical placebo, according to current Good Manufacturing Practices. The dose escalation was in 500-mg increments every 13 weeks as follows: 500 mg QAM, 500 mg BID, 1,000 mg QAM and 500 mg QPM, and 1,000 mg BID.

Outcomes. Primary outcomes were levels of plasma Aβ40 and Aβ42, CSF Aβ40, Aβ42, tau, and phospho-tau 181, and volumetric MRI (rate of whole brain volume change, rate of ventricular volume change, rate of hippocampal volume change, and rate of entorhinal cortex volume change). Additional outcomes included safety and tolerability (AEs, physical examinations, neurologic examinations, clinical laboratory results) and pharmacokinetics. Secondary outcomes included scores on the MMSE,[17] Alzheimer's Disease Assessment Scale–cognitive (ADAS-cog),[19] ADCS Activities of Daily Living Scale (ADCS-ADL),[20] Clinical Dementia Rating–sum of boxes (CDR-SOB),[21] and Neuropsychiatric Inventory (NPI),[22] *APOE* genotype, and insulin and glucose metabolism (including oral glucose tolerance tests).

Safety assessments. Participants received physical and neurologic examinations and vital signs at each visit. Site investigators classified AEs by severity and causality. If a participant withdrew, an early termination visit similar to a baseline visit was scheduled. An independent Data and Safety Monitoring Board reviewed data quarterly.

Liquid chromatography–mass spectrometry conditions for resveratrol plasma and CSF pharmacokinetics. Stock solutions of resveratrol, hexestrol (Sigma Aldrich, St. Louis, MO), trans-resveratrol-3-O-β-D-glucuronide, trans-resveratrol-4-O-β-D-glucuronide, and trans-resveratrol-3-sulfate sodium

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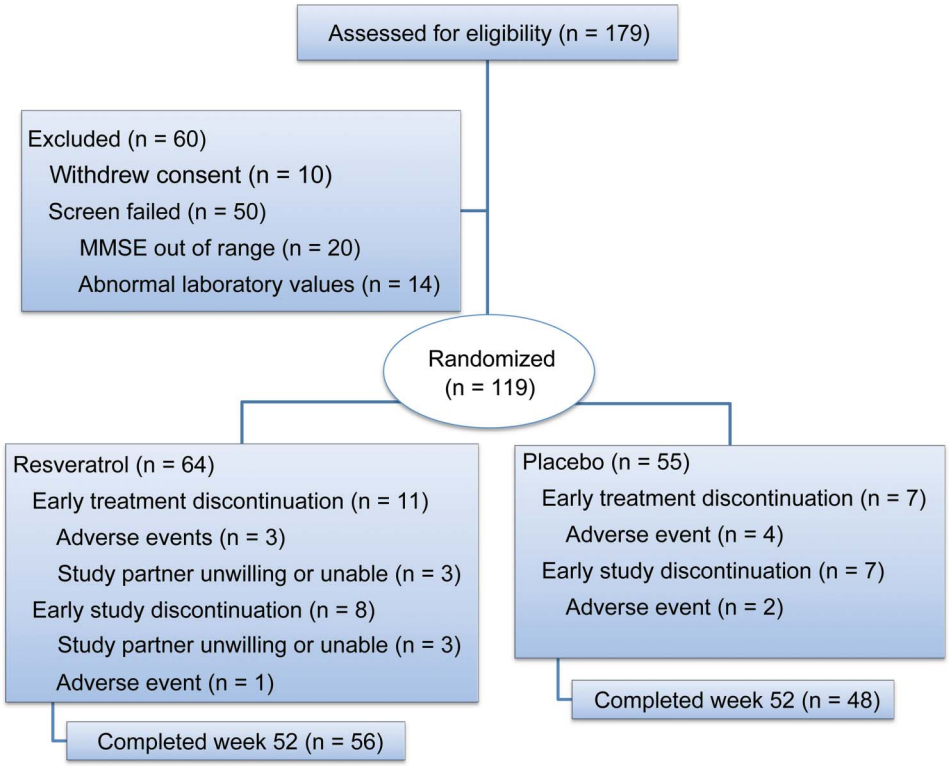
salt (Toronto Research Chemicals Inc., Canada) were suspended to 2 mg/mL in dimethyl sulfoxide (DMSO). Working stocks of 0.1 mg/mL were made in DMSO. Calibrator standards and quality controls were made in human pooled plasma (Gemini Bioproducts, Sacramento, CA). Plasma samples were mixed with acetonitrile with 0.1% formic acid and centrifuged for 2 minutes to remove precipitate. Pharmacokinetic analysis was performed using an AB Sciex (Framingham, MA) QTRAP 5500 and Shimadzu UFLC XR rack changer liquid chromatography–mass spectrometry system. Data acquisition and analysis was made using Analyst 1.6 (AB Sciex). Quantification was done in Analyst (AB Sciex) by using linear regression with a $1/x^2$ weighing factor based on goodness-of-fit criteria and coefficient of determination (r^2). The CSF conditions were the same as plasma except the injection volume was 20 μL. Calibrator standards were made in 50:50 (v/v) acetonitrile and water with 0.1% formic acid.

Bioassays. The Biomarker Core assayed Aβ40 and Aβ42 in plasma and CSF and tau and phospho-tau 181 in CSF.[23] Validated assay platforms from Meso Scale Discovery (Rockville, MD) were used to detect Aβ isoforms and total tau. Innotest pTau181 was used for phospho-tau 181. Internal standards were used to adjust for plate-to-plate variation and assess freezer storage effects.[23] The Biomarker Core performed *APOE* genotyping using real-time PCR restriction fragment length polymorphism analysis. Genomic DNA from blood was extracted using QIAamp DNA blood maxi kit (Qiagen, Venlo, Netherlands) and *APOE* genotyping performed using Applied Biosystems (Foster City, CA) TaqMan SNP Genotyping Assay. The assay was run on a Bio-Rad (Hercules, CA) CFX96.

MRI methods. MRIs at baseline, 3 months, and 12 months were acquired using GE (Cleveland, OH), Philips (Best, the Netherlands), or Siemens (Munich, Germany) 1.5 and 3.0 T scanners. Site personnel scanned participants longitudinally on the same scanner using a consistent protocol and quality checks confirmed that parameters held constant. Volumetric MRI analysis was performed on 3D T1-weighted volumes acquired sagittally with imaging parameters modeled on the nonaccelerated T1-weighted sequence from the Alzheimer's Disease Neuroimaging Initiative. NeuroQuant (CorTechs Labs, San Diego, CA) image preprocessing and automated segmentation was used to measure whole-brain, hippocampus, and entorhinal volumes[24–26] and other methods were as described.[27–32] Regional deformation was quantified and averaged within all segmented areas; however, to reduce multiple comparisons for primary analysis, only ventricular volume change was assessed using longitudinal registration.

Statistical analyses. Mixed-model repeated-measures (MMRM) analyses were used to assess between-group differences in change scores from baseline to week 52. The dependent variable in each MMRM analysis was change from baseline. Fixed effects included baseline scores on outcome measures, age at baseline, group assignment, study visit, and treatment-by-visit interaction. Additionally, covariates that were significantly associated with the response measure ($p < 0.15$) and were out of balance at baseline ($p < 0.2$) were included as fixed effects. Study visit was modeled as a categorical variable; an autoregressive (order 1) covariance structure was specified. Variables considered as potential covariates in each model included biomarkers CSF total tau, CSF phospho-tau 181, CSF Aβ40, CSF Aβ42, plasma Aβ40, plasma Aβ42, brain volume, ventricular volume, hippocampal volume, and entorhinal thickness; insulin and glucose; *APOE*; clinical measures: baseline ADAS-cog, baseline MMSE, and baseline CDR-SOB.

Figure 1 Flow diagram and disposition of the treatment groups



MMSE = Mini-Mental State Examination.

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The key efficacy analysis was based on a modified intention-to-treat (ITT) population, which included all randomly assigned participants with at least one postbaseline observation. Secondary analyses were performed for completers, for *APOE4* carriers and noncarriers, and for mild AD (MMSE > 19). The baseline characteristics of the study groups were compared with the use of Fisher exact test for categorical variables and a Wilcoxon signed-rank test for continuous variables. Safety analyses were based on summary listings of AEs, with Fisher exact test used for pairwise comparisons. Safety analyses were based on the full ITT population. *R* version 3.1 (www.r-project.org) was used for all analyses. A p value <0.05 indicated significance. All testing was 2-sided. Blinding of investigators was maintained until outcomes were determined. Results for testing several endpoints were not adjusted.

RESULTS A total of 179 participants were screened, of whom 60 were not randomized (50 screen-failed and 10 withdrew consent). Participants (119) were randomized as shown (figure 1). A total of 104 completed the study (12.6% dropout), and 77 completed 2 CSF collections (34% dropout). Eighteen participants discontinued treatment early and 15 discontinued the study. The population was English-speaking, 57% female, and 91% Caucasian.

Table 1 Baseline characteristics of the resveratrol and placebo groups

	Resveratrol (n = 64)[a]	Placebo (n = 55)[a]	p Value
Female, n (%)	40 (62.5)	28 (50.9)	0.27[b]
Caucasian, n (%)	57 (89.1)	51 (92.7)	0.81[b]
Age, y, mean (SD)	69.8 (7.7)	73 (8.2)	0.07[c]
Education, y, mean (SD)	15.5 (3.0)	14.6 (2.9)	0.11[c]
AD duration (from year of symptom onset), y, mean (SD)	3.9 (2.3)	5.5 (2.6)	<0.001
Weight, kg, mean (SD)	71.2 (15.2)	71.2 (13.6)	0.80[c]
BMI, mean (SD)	25.8 (4.3)	25.5 (4.0)	0.83[c]
MMSE, mean (SD)	20.2 (4.4)	20.7 (4.3)	0.57[c]
CDR-SOB, mean (SD)	5.1 (2.4)	5.4 (2.3)	0.43[c]
ADCS-ADL, mean (SD)	63.7 (10.8)	60.5 (10.7)	0.08[c]
NPI, mean (SD)[d]	7.5 (7.9)	11.1 (11.6)	0.10[c]
ADAS-cog, mean (SD)	25.3 (10.1)	23.7 (8.6)	0.50[c]
Brain volume, mL, mean (SD)	865.9 (84.5)	850.5 (98.9)	0.38[c]
Ventricular volume, mL, mean (SD)	54.5 (23.8)	55.6 (19.2)	0.32[c]
CSF Aβ40, ng/mL, mean (SD)[e]	6,574 (2,346)	6,560 (2,190)	0.77[c]
Plasma Aβ40, ng/mL, mean (SD)[f]	163.0 (58.2)	165.3 (55.4)	0.64[c]

Abbreviations: AD = Alzheimer disease; ADAS-cog = Alzheimer's Disease Assessment Scale–cognitive; ADCS-ADL = Alzheimer's Disease Cooperative Study Activities of Daily Living Scale; BMI = body mass index; CDR-SOB = Clinical Dementia Rating-sum of boxes; MMSE = Mini-Mental State Examination; NPI = Neuropsychiatric Inventory.
[a] Except as indicated.
[b] Fisher exact test.
[c] Wilcoxon rank-sum test.
[d] Resveratrol (n = 63).
[e] Resveratrol (n = 51), placebo (n = 51).
[f] Resveratrol (n = 53).

The baseline characteristics revealed that the placebo group had a longer AD duration (measured from year of symptom onset) (table 1). Results must be interpreted with caution given this caveat. However, a post hoc exploratory analysis found no difference between groups when AD duration was measured in years from diagnosis, and a post hoc re-analysis adjusting for age and AD duration in the MMRM model did not alter results, with the exception that difference in ventricular volume became nonsignificant ($p = 0.10$) (appendix e-1 on the *Neurology*® Web site at Neurology.org).

Safety and tolerability. No differences between the resveratrol and placebo-treated groups were found on vital signs, physical examinations, or neurologic examinations. Routine laboratory tests were normal. A total of 657 AEs (490 mild, 139 moderate, 28 severe) were reported (355 on drug, 302 on placebo) (table 2). A total of 113 out of 119 (95%) participants reported at least 1 AE. The most common AEs were nausea and diarrhea (in 42% of individuals with drug vs 33% with placebo, $p = 0.35$). Few participants reported nausea and diarrhea—the most likely drug-related AE—that led to treatment discontinuation, a treatment plateau at a lower dosage, or study discontinuation (figure 1). The placebo group gained 0.54 ± 3.2 kg body weight, while the treated group lost 0.92 ± 4.9 kg (mean ± SD, $p = 0.038$) resulting in a difference in body mass index (BMI). The treated group's BMI was 25.4 ± 4.0 vs the placebo group's 26.1 ± 4.1 at week 52 (mean ± SD, $p = 0.047$).

Thirty-six serious AEs (SAEs) were reported (19 on drug, 17 on placebo) including 27 hospitalizations (14 on drug, 13 on placebo) and 3 deaths (1 on drug, 2 on placebo)—none study drug-related. There were no differences in participants who experienced at least one SAE (20.3% on drug, 18.2% on placebo), at least one hospitalization (18.8% drug, 16.4% placebo), or died (1.6% drug, 3.6% placebo). Seven new neoplasms were reported (1 on drug, 6 on placebo, $p < 0.048$) (table 2). Retrospective review of the brain MRIs of a placebo-enrolled participant with malignant glioma, which resulted in death, revealed that the tumor was present at screening. Two participant deaths were due to lung melanoma (placebo group) and drowning (drug group).

Pharmacokinetics. Metabolites of resveratrol include 3G-RES, 4G-RES, and S-RES. A 24-hour pharmacokinetic substudy determined the maximal plasma concentrations (C_{max}) of resveratrol and its metabolites at baseline and weeks 13, 26, 39, and 52. The C_{max}, t_{max}, and half-life of resveratrol and its metabolites at week 52 are shown in table e-1. Resveratrol pharmacokinetics in plasma from the

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Table 2 Participants with adverse events by system

System	Resveratrol (n = 64), n (%)	Placebo (n = 55), n (%)	p Value[a]
Infections and infestations	27 (42.2)	23 (41.8)	>0.999
Nervous system disorders[b]	25 (39.1)	21 (38.2)	>0.999
Gastrointestinal disorders[c]	27 (42.2)	18 (32.7)	0.345
Psychiatric disorders	23 (35.9)	18 (32.7)	0.847
Injury, poisoning, or procedural complications[d]	22 (34.4)	16 (29.1)	0.561
Investigations[e]	25 (39.1)	12 (21.8)	0.049
Musculoskeletal and connective tissue disorders[f]	15 (23.4)	20 (36.4)	0.158
General disorders and administrative site conditions	12 (18.8)	12 (21.8)	0.819
Respiratory, thoracic, and mediastinal disorders	13 (20.3)	10 (18.2)	0.819
Skin and subcutaneous tissue disorders[g]	7 (10.9)	12 (21.8)	0.134
Renal and urinary disorders	7 (10.9)	9 (16.4)	0.429
Vascular disorders	8 (12.5)	5 (9.1)	0.769
Cardiac disorders	5 (7.8)	6 (10.9)	0.753
Eye disorders	5 (7.8)	6 (10.9)	0.753
Metabolism and nutrition disorders	5 (7.8)	6 (10.9)	0.753
Neoplasms benign, malignant, and unspecified[h]	1 (1.6)	6 (10.9)	0.048

[a] Fisher exact test.
The most frequent events by system and by participant were [b] headache (11 on drug, 6 on placebo); [c] diarrhea (26 on drug, 7 on placebo), nausea (14 on drug, 5 on placebo); [d] fall (22 on drug, 14 on placebo); [e] weight decrease (11 on drug, 0 on placebo); [f] back pain (7 on drug, 15 on placebo), arthralgia (1 on drug, 7 on placebo); [g] rash (1 on drug, 7 on placebo); [h] one bladder cancer on drug and 7 cancers in 6 participants on placebo—3 malignant melanoma, 2 squamous cell carcinoma, 1 basal cell carcinoma, and 1 malignant glioma.

entire study population are shown in figure e-1 (A–D) and week 52 CSF levels are shown in figure e-2 (A–D). These pharmacokinetic results confirmed compliance in both groups.

Outcomes. At week 52, the treated group's CSF Aβ40 declined from 6,574 ± 2,346 to 6,513 ± 2,279 ng/mL and from 6,560 ± 2,190 to 5,622 ± 1,736 ng/mL with placebo, resulting in a difference at week 52 (mean ± SD, $p = 0.002$) (figure 2A). This difference was also found in secondary analyses of study completers ($p = 0.002$), in the mild dementia subgroup ($p = 0.01$), and in *APOE*4 carriers ($p = 0.05$) and noncarriers ($p = 0.01$) (table e-2). During the study, the treated group's plasma Aβ40 (figure 2B) declined from 163 ± 58 to 153 ± 54 ng/mL and from 165 ± 55 to 132 ± 54 ng/mL with placebo (mean ± SD, $p = 0.024$). Secondary analyses by *APOE*4 genotype revealed an effect of treatment on plasma Aβ40 in *APOE*4 carriers ($p = 0.04$) but not noncarriers (table e-2). There were no effects on CSF Aβ42 or plasma Aβ42 (figure 2, C and D), although trends were similar to Aβ40. There was no difference in CSF tau and a trend toward an increase in CSF phospho-tau 181 with treatment ($p = 0.08$), and in a secondary analysis of mild dementia ($p = 0.047$) (data not shown).

Volumetric MRIs revealed that brain volume (excluding CSF, brainstem, and cerebellum) declined more in the treatment group ($p = 0.025$) with an increase in ventricular volume ($p = 0.05$) at week 52 (figure 3, A and B). In the treatment group, brain volume decreased from 866 ± 84 to 839 ± 85 mL and ventricular volume increased from 55 ± 24 to 81 ± 24 mL (mean ± SD). With placebo, brain volume decreased from 850 ± 99 to 840 ± 93 mL and ventricular volume increased from 56 ± 19 to 76 ± 25 mL (mean ± SD). Secondary analyses revealed that brain volume declined with treatment in *APOE*4 carriers ($p = 0.02$) but not noncarriers (table e-2). Similar results were found with ventricular volume, which increased with treatment in *APOE*4 carriers ($p = 0.05$) but not noncarriers.

This phase 2 trial (underpowered to detect differences in clinical outcomes) found no significant effects on CDR-SOB, ADAS-cog, MMSE, or NPI. The drug-treated group's ADCS-ADL declined from 63.7 ± 10.8 to 57.4 ± 12.3 and from 60.5 ± 10.7 to 51.3 ± 14.5 in the placebo group (mean ± SD, $p = 0.03$), indicating less decline with treatment. No drug effects were found with plasma glucose or insulin metabolism (data not shown). We also analyzed (post hoc) the subset of individuals with CSF Aβ42 <600 ng/mL at baseline as a proxy of AD amyloid pathology. At week 52, differences between treatment groups persisted for CSF Aβ40 ($p = 0.001$, total n = 70) and plasma Aβ40 ($p = 0.02$, n = 83). In this analysis, we also found a treatment effect on CSF Aβ42 ($p = 0.02$, n = 70) but lost significance in brain volume loss ($p = 0.06$, n = 83) and ADCS-ADL ($p = 0.055$, n = 88).

DISCUSSION High-dose oral resveratrol is safe and well-tolerated. The most common AEs were nausea and diarrhea, but results were similar to placebo. Weight and fat loss with resveratrol are reported in some preclinical studies,[4] but human studies are scarce and of shorter duration. A decrease in body fat and a trend toward weight loss were reported in a 26-week trial with 200 mg/day resveratrol in healthy older participants.[33] Weight and fat loss may be related to enhanced mitochondrial biogenesis mediated by SIRT1 activation of PCG-1α.[4,10,11]

Aβ levels declined as dementia advanced. The altered CSF Aβ40 trajectory suggests that the drug penetrated the blood–brain barrier to have central effects. At week 52, the mean CSF levels of resveratrol, 3G-RES, 4G-RES, and S-RES were 3.3%, 0.4%, 0.4%, and 0.3%, respectively, of plasma levels at the same study visit. At the highest dosage, low μM

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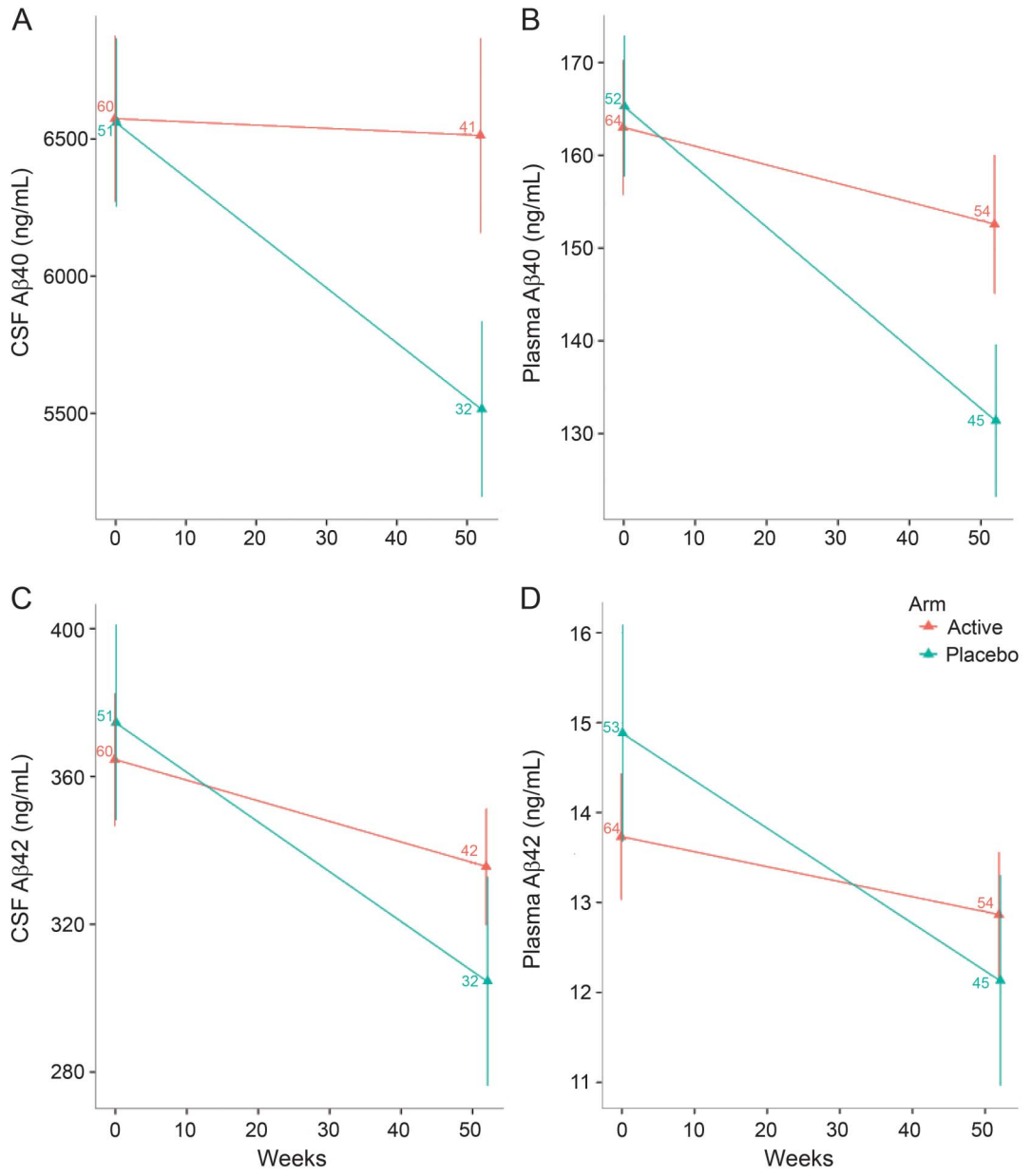
Figure 2 **Effects of resveratrol on Aβ levels**



Resveratrol altered levels of CSF Aβ40 (A) and plasma Aβ40 (B) (ng/mL, mean ± SE). Similar but nonsignificant trends were found for CSF Aβ42 (C) and plasma Aβ42 (D) (ng/mL, mean ± SE). Note difference in scales. Sample sizes are indicated.

levels of resveratrol and its metabolites were measured in plasma, with corresponding low nM levels found in CSF. Resveratrol has many targets, with some engaged at uM concentrations.[4] These findings suggest that a central molecular target may be engaged at nM concentrations. In addition to anti-inflammatory, antioxidant, and anti-Aβ aggregation, putative targets include sirtuin activation with enhanced α-cleavage of amyloid precursor protein[34] and promotion of autophagy.[35] Further studies of banked CSF, plasma, pellets, DNA, and blood mononuclear cells from participants will examine mechanisms.

Resveratrol treatment increased brain volume loss. This finding persisted when participants with weight loss (table 2) were excluded (data not shown). The etiology and interpretation of brain volume loss observed here and in other studies are unclear, but they are not associated with cognitive or functional decline. In the first human active Aβ immunization trial, antibody responders had greater brain volume loss, and greater volumetric changes were associated with higher antibody titers.[36] In the phase 2 bapineuzumab trial, treatment resulted in greater ventricular enlargement, but only in *APOE4* carriers.[37] In the phase 3 bapineuzumab *APOE4* carrier trial and the high-dose noncarrier study, treatment resulted in a trend toward greater brain atrophy.[38] Since this phase 2 study lacks consistent changes in clinical outcomes,

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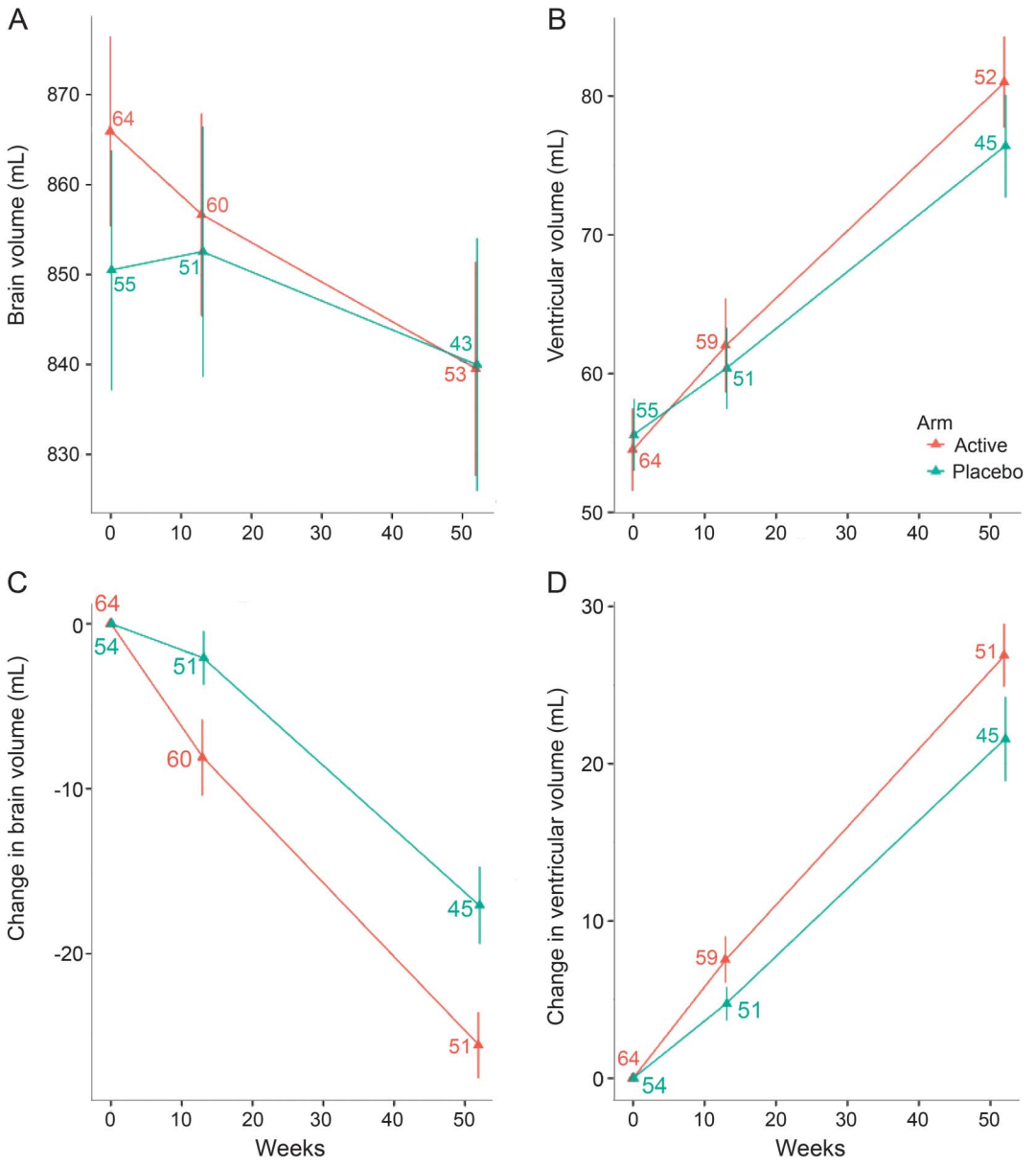
Figure 3 Effects of resveratrol on brain volume



Resveratrol increased brain volume loss (A, C) (mL, mean ± SE) with a corresponding increase in ventricular volume (B, D) (mL, mean ± SE). Sample sizes are indicated.

interpretation of the effects on trajectories for plasma and CSF Aβ40, and brain and ventricular volume, remain uncertain.

This phase 2 study has limitations. It was designed to determine the safety and tolerability of resveratrol and to examine pharmacokinetics. Although some biomarker trajectories were altered, we found no effects of drug treatment on plasma Aβ42, CSF Aβ42, CSF tau, CSF phospho-tau 181, hippocampal volume, entorhinal cortex thickness, MMSE, CDR, ADAS-cog, NPI, or glucose or insulin metabolism. The altered biomarker trajectories must be interpreted with caution. Although they suggest CNS effects, they do not indicate benefit. A larger study is required to determine whether resveratrol may be beneficial. More potent and bioavailable SIRT1 activators are also in development.[39,40]

AUTHOR CONTRIBUTIONS

R.S. Turner conceived and designed the study with P.S. Aisen and S. Craft. R.G. Thomas and R. Raman performed data analyses. C.H. van Dyck., J. Mintzer, and B.A. Reynolds were the leading site PIs—at Yale University, the Medical University of South Carolina, and Georgetown University, respectively—in recruitment of participants and study partners. J.B. Brewer performed volumetric MRI analyses and R.A. Rissman performed biomarker and pharmacokinetic assays. R.S. Turner performed data analysis, prepared the figures, and drafted the manuscript, which was edited and approved by all authors, members of the Data Safety and Monitoring Board, and the Publications Committee of the ADCS.

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