Effect of a 2-year diet intervention with walnuts on cognitive decline. The Walnuts And Healthy Aging (WAHA) study: a randomized controlled trial

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ABSTRACT

Background: Walnut consumption counteracts oxidative stress and inflammation, 2 drivers of cognitive decline. Clinical data concerning effects on cognition are lacking.

Objectives: The Walnuts And Healthy Aging study is a 2-center (Barcelona, Spain; Loma Linda, CA) randomized controlled trial examining the cognitive effects of a 2-y walnut intervention in cognitively healthy elders.

Methods: We randomly allocated 708 free-living elders (63–79 y, 68% women) to a diet enriched with walnuts at ∼15% energy (30–60 g/d) or a control diet (abstention from walnuts). We administered a comprehensive neurocognitive test battery at baseline and 2 y. Change in the global cognition composite was the primary outcome. We performed repeated structural and functional brain MRI in 108 Barcelona participants.

Results: A total of 636 participants completed the intervention. Besides differences in nutrient intake, participants from Barcelona smoked more, were less educated, and had lower baseline neuropsychological test scores than those from Loma Linda. Walnuts were well tolerated and compliance was good. Modified intention-to-treat analyses ($n = 657$) uncovered no between-group differences in the global cognitive composite, with mean changes of −0.072 (95% CI: −0.100, −0.043) in the walnut diet group and −0.086 (95% CI: −0.115, −0.057) in the control diet group ($P = 0.491$). Post hoc analyses revealed significant differences in the Barcelona cohort, with unadjusted changes of −0.037 (95% CI: −0.077, 0.002) in the walnut group and −0.097 (95% CI: −0.137, −0.057) in controls ($P = 0.040$). Results of brain fMRI in a subset of Barcelona participants indicated greater functional network recruitment in a working memory task in controls.

Conclusions: Walnut supplementation for 2 y had no effect on cognition in healthy elders. However, brain fMRI and post hoc analyses by site suggest that walnuts might delay cognitive decline in subgroups at higher risk. These encouraging but inconclusive results warrant further investigation, particularly targeting disadvantaged populations, in whom greatest benefit could be expected. This trial was registered at clinicaltrials.gov as NCT01634841. *Am J Clin Nutr* 2020;00:1–11.

Keywords: α-linolenic acid, cognition, neuroimaging, nuts, omega-3

Introduction

The steady increase of longevity coupled with population aging has led to a global rise in the prevalence of dementia, which imposes a huge socioeconomic burden (1). Because there are no available pharmacological agents to cure dementia, identifying interventions to prevent or at least delay its onset is a major public health issue. Oxidative stress has long been linked to age-related cognitive decline (2), the harbinger of dementia. Results of epidemiologic studies relating increased adherence to antioxidant-rich dietary patterns to lower rates of cognitive impairment (3) provide supportive evidence for the oxidative hypothesis of cognitive decline. A clinical trial in elders at high vascular risk disclosed better cognitive performance in participants randomly assigned to a Mediterranean diet supplemented with either extra-virgin olive oil or mixed nuts than in those assigned a control low-fat diet (4). Similar results were observed in a trial of advice to increase consumption of plant foods as part of a multidomain intervention in elders at risk of dementia compared with a control group given general health advice (5). Epidemiologic observations have also suggested that

intake of the marine omega-3 fatty acid DHA (22:6n–3) or increased fish consumption is associated with a lower risk of dementia and Alzheimer disease (6, 7). In contrast to these findings, no cognitive benefits were documented in large trials of DHA-rich fish oil administered for ≥2 y, given either alone to cognitively healthy elders (8, 9) or as part of a multidomain intervention to elderly adults with memory complaints (10). Possible explanations for the lack of cognitive effects of the marine ω-3 fatty acid trials relate to methodological issues (mostly dose and duration, and the concomitant intervention), to cognitive status at the time of the supplementation, and to the baseline ω-3 fatty acid intake.

Walnuts are a whole food rich in α-linolenic acid (18:3n–3, ALA, the plant ω-3 fatty acid); they also possess more polyphenols than any other nut type (11). Both ω-3 fatty acids and polyphenols are considered critical brain foods (3, 12), hence walnuts could be predicted to beneficially influence cognition. Indeed, several experimental studies have investigated the effects of walnuts on brain health, consistently describing positive results (13–17). Two cross-sectional studies found that walnut consumption was directly associated with cognitive function (18, 19). Clinical trial evidence is limited to a small 8-wk study in college students wherein walnut consumption at 60 g/d improved inferential verbal reasoning compared with a control diet (20). Thus, there is abundant experimental evidence but little clinical data on cognitive outcomes ensuing from walnut consumption. To fill this gap, we investigated the effects of daily ingestion of walnuts for 2 y on cognitive function in a large sample of cognitively healthy elders in the Walnuts And Healthy Aging (WAHA) trial (NCT01634841).

Methods

Study design and participants

The WAHA study is a 2-y parallel-group, observer-blinded, randomized controlled trial examining the effects of a diet enriched with walnuts at 15% of energy compared with a diet without walnuts in cognitively healthy elders. The 2-y intervention period was defined as the minimum period to be able to appreciate changes of cognitive function and the maximum period that older individuals would accept for consuming walnuts daily, i.e., without compromising adherence. The daily amount of walnuts (15% of energy) was chosen because they contain 654 kcal/100 g, that is, 294 kcal/45 g (1.5 servings), which is ∼15% of energy for a standard 2000-kcal diet, and these doses are suggested to be cardioprotective (21) and therefore could also benefit brain health. The study was conducted in 2 centers: Loma Linda University, CA, USA (Loma Linda) and Hospital Clínic, Barcelona, Spain (Barcelona). An abbreviated version of the protocol has been published (22). The WAHA study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of each center. All participants gave written informed consent before enrolment.

Participants were recruited between May 2012 and May 2014. Eligible candidates were men and women aged 63–79 y. Exclusion criteria were inability to undergo neuropsychological testing; previously diagnosed neurodegenerative disease; prior stroke, significant head trauma, or brain surgery; relevant psychiatric illness; major depression; morbid obesity; uncontrolled diabetes; uncontrolled hypertension; prior chemotherapy; allergy to walnuts; habitual consumption of tree nuts (>2 servings/wk); or customary use of fish oil, flaxseed oil, and/or soy lecithin.

Randomization and masking

We randomly assigned participants to either the walnut group (consuming ∼15% of daily energy intake as walnuts) or the control group (abstention from walnuts) using a computerized, web-based, random number table with stratification by gender and age range in a 1:1 ratio in each center. Pairs of individuals (i.e., couples, household members, partners) entering the study were allocated to the same group using the same stratification criteria. We advised participants not to discuss the intervention during testing sessions, restricting between-group interactions. All study clinicians and researchers were blind to participants' intervention group, except for the dietitians in charge of dietary assessment and walnut supply. All data obtained during the study were recorded in a dedicated online database developed by Costaisa SA. An independent Data and Safety Monitoring Board blinded to subject allocation periodically reviewed and evaluated the accumulated study data during the trial's progress.

Procedures

All eligible candidates met the study clinician of each site at screening for a detailed medical history and physical examination. Clinical data were obtained again at the end of the trial. We considered participants as diabetic, hyperlipidemic, or hypertensive if they had a previous diagnosis of these conditions, and/or they were treated with antidiabetic, cholesterol-lowering, or antihypertensive agents, respectively. We categorized smoking status into never, current, or past smoking according to self-reports.

At baseline, in a face-to-face visit, a study dietitian measured height, weight, and waist circumference by standard methods. In the same visit, participants also completed a validated short version of the Minnesota physical activity questionnaire (23)

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Supplemental Methods 1 and 2, Supplemental Tables 1–7, and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Data described in the article, code book, and analytic code will be made available upon request pending application and approval.

AS-V and CV-P contributed equally to this work.

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Abbreviations used: ALA, α-linolenic acid; RAVLT, Rey Auditory Verbal Learning Test; WAHA, Walnuts And Healthy Aging; WAIS-III, Wechsler Adult Intelligence Scale-III.

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and a 3-d food record. We estimated energy requirements of each participant by using the WHO formula for energy needs for adults >60 y old (24). The estimated amount of walnuts ranged from 30 to 60 g/d. We instructed participants in the walnut group to eat walnuts daily, preferably raw, either as a snack or by incorporating them into dishes, and to finish their daily dose either in 1 sitting or spread throughout the day. We offered coffee grinders at no cost to participants with dental problems who reported difficulty chewing and advised them to eat the ground walnuts mixed with a soft meal such as yogurt. Individuals allocated to the control group were instructed to abstain from walnuts, and to avoid other nuts at doses >2 servings/wk for the duration of the study.

We scheduled participants for a visit with the dietitians every 2 mo, aimed at assessing compliance, increasing retention, collecting data on tolerance, and delivering walnuts when appropriate. For participants in the walnut group, dietitians provided 8-wk allotments of pieced walnuts (in sachets for daily consumption) at each visit. Dietitians obtained follow-up data on adiposity and physical activity from all participants at the 12- and 24-mo visits. In addition, they monitored food consumption through 3-d food records at the 6-, 12-, and 24-mo visits (Barcelona) or via 5 unannounced 24-h diet recalls over the duration of the trial (Loma Linda). We calculated the nutrient composition of the diets with Food Processor Plus software (ESHA Research), adapted to nutrient databases of local foods when appropriate.

At baseline and the end of intervention, we obtained blood samples after an overnight fast. We kept aliquots of EDTA-plasma, serum, and whole blood at −80°C until analyses. Except for *APOE* genotyping and routine chemistry for safety assessment, we performed all assays at the end of the study in the same laboratory to control for between-assay variability. We extracted genomic DNA from blood samples with the Puregene blood kit (Gentra Systems). The Genetic and Molecular Epidemiology Unit (University of Valencia, Spain) genotyped the APOE-rs429358 (apoE112; $\varepsilon 4$ variant allele) and APOE-rs7412 (apoE158; $\varepsilon 2$ variant allele) on a 7900HT Sequence Detection System (Applied Biosystems), and we converted the genotype outputs from the 2 *APOE* single nucleotide polymorphisms into the conventional 6 *APOE* genotypes (2/2, 2/3, 3/3, 3/4, 2/4, and 4/4) based on 3 alleles (*APOE* $\varepsilon 2$, *APOE* $\varepsilon 3$, and *APOE* $\varepsilon 4$). Finally, in a random subsample of 430 participants ($n = 213$ in the control group and $n = 217$ in the walnut group) we objectively assessed compliance by measuring changes in the ALA proportion of RBC membranes, as described elsewhere (25).

Outcomes

The primary study outcome was the mean 2-y change from baseline in a global cognitive composite score. As secondary outcomes, we analyzed the mean change from baseline in composites for the cognitive domains memory, language, perception, and frontal function. **Supplemental Method 1** contains detailed bibliographic information on the administered tests. In brief, the memory composite included the mean standardized individual change scores of the Rey Auditory Verbal Learning Test (RAVLT) (immediate and delayed recall) and the immediate recall of the Rey-Osterrieth Complex Figure. The language composite included scores from Semantic Fluency (Animals) and the Boston Naming Test. The perception composite comprised scores from the Visual Object and Space Perception battery (number location and incomplete letters) and the Block Design subtest from the Wechsler Adult Intelligence Scale-III (WAIS-III) battery. Finally, the frontal function composite included scores from the Trail Making Test (parts A and B), Phonemic Fluency, Stroop Color Word Test, Symbol Digit Modalities Test, Digit Span forward and backward from the WAIS-III, and the Conners Continuous Performance Test-II. We also assessed cognitive reserve and mood by using a cognitive reserve questionnaire (26) and the Hamilton Depression Rating Scale (27), respectively. We assessed premorbid intelligence with the American National Adult Reading Test (28) at Loma Linda and the Word Accentuation Test (29) at Barcelona. Participants who dropped out of the study for whatever reason were asked to attend a final clinical visit and repeat cognitive tests at 2 y.

In addition, to investigate the effect of long-term walnut consumption on brain structure, resting state connectivity, blood flow, and the expression of functional brain networks during cognitive demands, we conducted brain MRI studies in a subset of 120 participants (number limited by budgetary reasons) at the Barcelona center, where an MRI facility was available. Changes in brain MRI were a prespecified secondary outcome. At the start of the trial, the procedure was offered to consecutively randomly assigned participants until the available slots were completed. At baseline and end of intervention, participants underwent a scanning session in a 3T MRI to acquire T1-weighted structural images (cortical thickness, voxel-based morphometry, white matter hyperintensity volumes, and perfusion arterial spin labeling), in addition to fMRI. In fMRI, we measured activity within the working memory network of all participants while they were performing the task. Briefly, for each subject and time point we created load-dependent maps of brain activity by comparing activity at the highest cognitive demand (namely, 3-Back) with that at the lowest demand (0-Back). Furthermore, we recorded the task performance individual scores during scanning. Detailed information on MRI methods can be found in **Supplemental Method 2**.

Statistical analyses

We calculated the study sample size based on previous results from the PREDIMED trial (4), using 2-y unadjusted change in RAVLT (total learning) scores in the Mediterranean diet supplemented with nuts compared with the control diet. To have a 90% power and a type 1 error of 5% to detect differences in the contrast with the null hypothesis, assuming mean changes of 1.05 and 2.10 points in the control and intervention groups, respectively, with an SD of 4.00, the total number of participants required was 308 per group. Considering an estimated dropout rate of 10%, we needed to include a total of 686 participants.

We standardized participants' raw test scores to z scores to generate a global cognition composite, obtained by computing the mean standardized changes of all neuropsychological tests. According to a predefined statistical analysis plan, we carried out primary and secondary outcome analyses on a modified intention-to-treat basis, thus including both participants who completed the

2-y intervention and dropouts who returned for the final cognitive assessment.

We assessed differences between participants who withdrew from the study and those who remained by 1-factor ANOVA, the Kruskal–Wallis test, or the chi-square test, as appropriate. We examined between-center baseline differences in demographics, clinical characteristics, scores of neuropsychological tests, and energy and nutrient intake by 1-factor ANOVA. We assessed between-group differences in 2-y changes in energy and nutrient intake, ALA proportion of RBC membranes, body weight, blood pressure, neuropsychological tests, and cognitive composites by 1-factor ANOVA. Changes in cognitive variables were further assessed by ANCOVA adjusted by gender, years of education, *APOE* ε4 carriers (yes/no), smoking status (ever smoker/never smoker), baseline age, BMI, physical activity, diabetes (yes/no), dyslipidemia (yes/no), hypertension (yes/no), Hamilton Depression Rating Scale score, and center.

We conducted subgroup analyses according to these variables, besides cluster randomization (entering the study with a partner) and multivitamin use, and effect modification was analyzed by using repeated-measures ANCOVA with 3 factors: time (baseline compared with 2 y) as repeated measure, group (control compared with walnut), and each variable of interest (dichotomous), and their interactions, adjusting for the variables previously listed. The *P* value for the interaction between center and intervention when exploring composite scores for global cognition ($P = 0.023$) prompted us to conduct center-specific analyses. Therefore, we also analyzed the 2 centers separately as a post hoc analysis. To this end, we calculated center-specific *z* scores.

Supplemental Method 2 provides detailed information on statistical methods applied to the MRI procedures. For fMRI maps, we used voxel-wise statistics to compare the activity within the working memory network between groups and time points. We calculated group and time differences as well as group × time interactions. All analyses were considered significant at a family-wise corrected level of $P < 0.05$.

Statistical significance was set at the <0.05 level. We performed analyses using SPSS software, release 19.0 (IBM Corp.).

Results

A total of 1452 potential candidates were prescreened by completing short questionnaires and 352 were excluded for not meeting eligibility criteria. Of the remaining 1100 candidates formally assessed for eligibility, 392 were further excluded after a clinical visit and physical examination. This resulted in 708 recruited subjects randomly assigned to 1 of the 2 interventions. The study ended in May 2016 with 636 participants completing the 2-y intervention (90% retention rate). We found no significant differences between completers and dropouts for baseline demographic and clinical characteristics, except for a significantly higher percentage of dyslipidemia in dropouts ($P = 0.027$ compared with completers). Six completers did not undergo cognitive assessment at the end of the intervention, whereas 27 dropouts returned for a second cognitive test battery. Therefore, we included in the modified intention-to-treat analyses 657 participants ($n = 336$ in the walnut diet and $n = 321$ in the control diet). **Figure 1** depicts the flowchart of the study. **Table 1** presents the baseline characteristics of participants included in the modified intention-to treat analysis by group allocation. **Supplemental Table 1** depicts a comparison of baseline features of Barcelona and Loma Linda participants; participants from Barcelona were younger, less educated, smoked more, and displayed lower scores in the Hamilton Depression Rating Scale than those from Loma Linda. In addition, the Barcelona cohort had a lower prevalence of *APOE* ε4 carriers.

Nutrient intake

Table 2 presents baseline and 2-y changes in energy and nutrient intakes and nut consumption in completers by intervention group. As per the trial's design, participants from both intervention groups reported negligible intake of walnuts and other nuts at baseline. No significant in-trial differences in walnut consumption were observed in the control group, whereas the walnut group increased consumption, as planned. At the end of the trial, participants in the walnut diet arm increased dietary energy and total fat and reciprocally decreased carbohydrate, translating into significant differences with changes observed in the control diet. Two-year increases in fiber, linoleic acid (18:2n−6), and ALA intakes in the walnut group were also significantly higher than those observed in the control group, reflecting the nutrient composition of walnuts. In line with this, whereas there were no significant between-group differences in RBC ALA at baseline ($P = 0.793$), the 2-y change in the walnut group was significantly higher than that observed in the control group ($P < 0.001$) (**Supplemental Figure 1**), confirming good adherence to the intervention.

Baseline nutrient intake was dissimilar between the 2 sites, reflecting customary dietary practices in Barcelona (Mediterranean diet) and Loma Linda (healthy American diet) (**Supplemental Table 2**). Of note, Loma Linda participants reported a significantly higher intake of ALA and, accordingly, they displayed a significantly higher mean RBC ALA proportion at baseline than the Barcelona cohort (0.31%; 95% CI: 0.29%, 0.32% compared with 0.12%; 95% CI: 0.12%, 0.13%; $P < 0.001$).

Tolerance and side effects

In-trial clinical events were similarly distributed between the 2 groups (**Supplemental Table 3**). Data on digestive tolerance to walnuts at 2 y were available in 358 participants from the walnut arm. There were 13 dropouts due to severe dyspepsia attributed to walnuts, whereas 74 participants had milder dyspepsia, which was solved by temporarily reducing walnut doses. In addition, 57 participants required grinding the walnuts because of difficulty chewing due to bad dentures. Information on bowel habits was obtained in 694 trial participants. Of 355 participants in the walnut diet arm, 200 (56.3%), 131 (36.9%), and 24 (6.8%) reported no change, improvement (softening of previously hard stools), or worsening (harder or inconveniently soft stools), respectively. The respective values in 339 controls were 320 (94.4%), 11 (3.2%), and 8 (2.4%). Body weight was stable throughout the 2 dietary periods, with mean changes at 2 y of

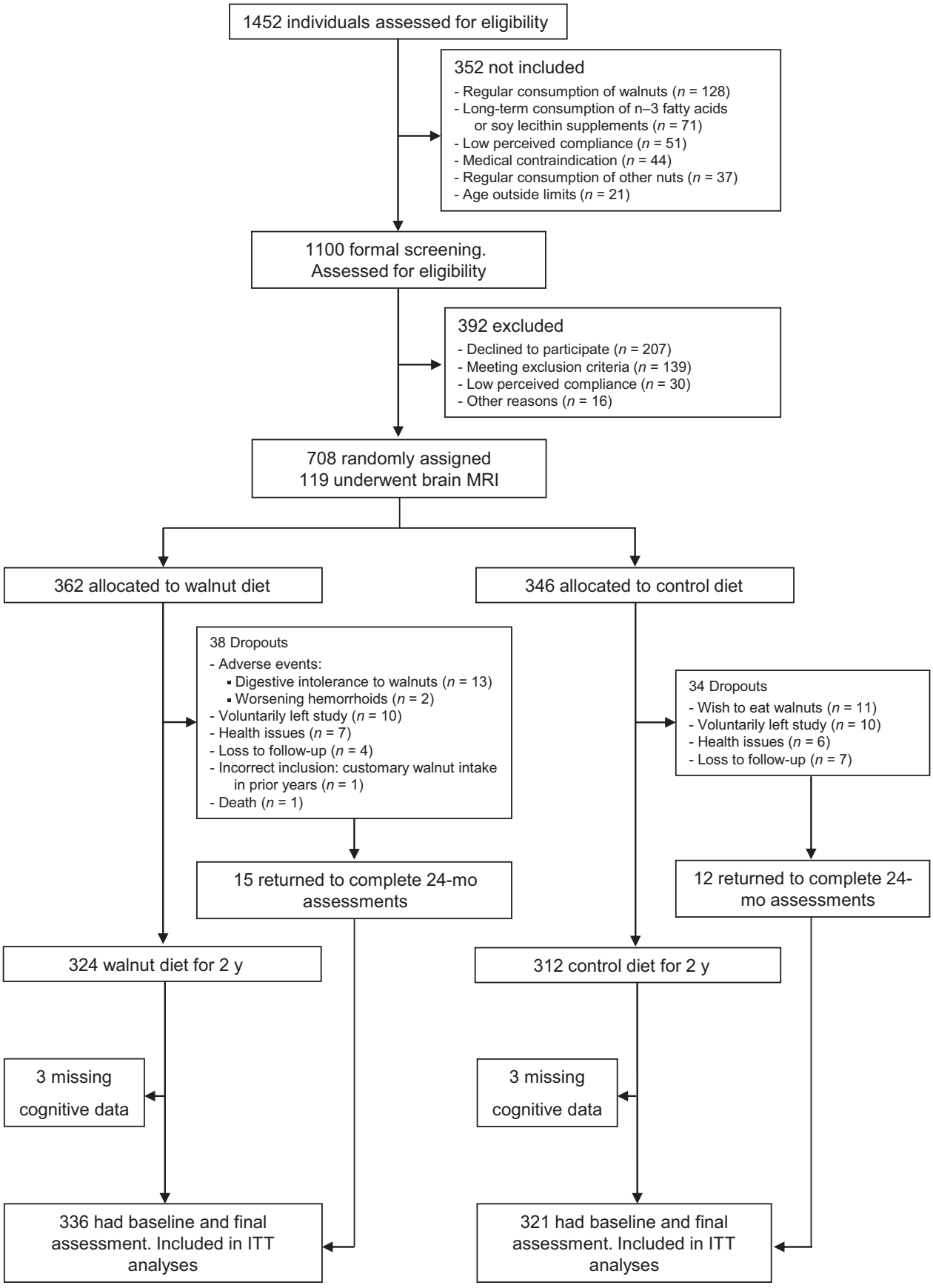

FIGURE 1 Flowchart of the study. ITT, intention-to-treat.

TABLE 1 Baseline characteristics of the modified intention-to-treat population by intervention group[1]

Characteristics	Walnut diet ($n = 336$)	Control diet ($n = 321$)
Enter with partner	79 (24)	78 (24)
Women	223 (66)	216 (67)
Age, y	69.4 (69.0, 69.8)	68.9 (68.5, 69.3)
Smoking		
Never smoker	280 (83)	269 (84)
Former smoker	41 (12)	48 (15)
Current smoker	15 (5)	4 (1)
Education		
Basic (0–4 y)	11 (3)	7 (2)
Elementary (5–8 y)	59 (18)	64 (20)
Secondary (9–12 y)	63 (19)	65 (20)
Postsecondary (>12 y)	203 (60)	185 (58)
Multivitamin use	77 (23)	72 (22)
Height, cm	164.2 (163.2, 165.2)	163.3 (162.3, 164.3)
Weight, kg	73.5 (71.9, 75.2)	73.4 (71.8, 75.0)
BMI, kg/m^2	27.1 (26.7, 27.6)	27.4 (27.0, 27.9)
Hypertension	174 (52)	169 (53)
Type 2 diabetes	35 (10)	28 (9)
Dyslipidemia	186 (55)	169 (53)
Physical activity[2]	2438 [1223–3727]	2441 [1408–4216]
APOE ε4 carriers [3]	80 (24)	71 (22)
Hamilton Depression Rating Scale	2 [0–4]	2 [0–4]

[1] Values are *n* (%) or means (95% CIs), except for Physical activity and Hamilton Depression Rating Scale score, which are medians [IQRs].

[2] Physical activity is expressed in MET-min/d, i.e., minutes/day at a given metabolic equivalent level (units of energy expenditure in physical activity; 1 MET-min is roughly equivalent to 1 kcal).

[3] Data from 655 participants (2 missing *APOE* genotype).

0.05 kg (95% CI: −0.3, 0.4 kg) in the walnut diet and −0.5 kg (95% CI: −0.9, −0.1 kg) in controls.

Cognitive test battery

No participants developed clinically significant cognitive impairment during follow-up. **Supplemental Table 4** depicts baseline scores, unadjusted changes, and adjusted changes for individual tests by intervention group, whereas **Table 3** displays baseline composite scores for global cognition and the specific cognitive domains investigated, as well as unadjusted and adjusted 2-y changes. Unadjusted mean changes in the global cognition score were −0.072 (95% CI: −0.100, −0.043) in the walnut diet group and −0.086 (95% CI: −0.115, −0.057) in the control diet group ($P = 0.491$). Likewise, no differences for the secondary outcomes memory, language, perception, and frontal function were observed. **Supplemental Table 5** shows sensitivity analyses under various assumptions. Besides the described interaction by center, there was effect modification by dyslipidemia (better cognitive response to walnuts when present).

Post hoc analyses revealed significant differences between the walnut diet and control diet groups at 2 y only at the Barcelona site (**Table 4**), wherein unadjusted changes in the global cognition composite were −0.037 (95% CI: −0.077, 0.002) in the walnut diet arm and −0.097 (95% CI: −0.137, −0.057) in controls ($P = 0.040$). The mean difference in changes between groups was 0.060 (95% CI: 0.005, 0.115). To help interpret this mean difference, we derived the effect of aging from the cognitive trajectory of controls. In this sample, 1 y of aging was associated with a mean decline of 0.049 (95% CI: 0.029, 0.069) in the global composite score. Hence, the observed difference is roughly equivalent to 1.24 y of cognitive aging. Among specific cognitive domains, only the perception score improved significantly in the walnut diet group compared with the control diet group ($P = 0.011$). We found no significant effect of the intervention on the primary or secondary outcomes at the Loma Linda site (Table 4).

Neuroimaging

After exclusions due to artifacts, the MRI sample included 108 subjects, distributed into $n = 58$ in the walnut group and $n = 50$ in the control group. We observed no significant differences between the participants undergoing MRI and the whole sample from the Barcelona site in terms of demographics or risk factors, including *APOE* ε4 status (data not shown).

Regarding brain structure, we found similar rates of atrophy (measured by cortical thickness and brain volumes), white matter hyperintensity ratings, and brain perfusion with time for the 2 groups (**Supplemental Table 6**). Regarding fMRI (N-back task, **Supplemental Table 7**), we found no between-group differences or group × time interactions. Nevertheless, compared with baseline, the control group exhibited an increased activation for the contrast of interest (3-back > 0-back) at 2 y, which was not observed in the walnut diet group. The cluster result area encompassed lateral occipital and temporal regions from the right hemisphere, areas outside the task-related regions (**Figure 2**). In addition, and based on the performance scores, for 0-back and 1-back N-back (the lowest loads), we detected a group × time interaction for the reaction time scoring (0-back: $F = 7.186$, $P = 0.009$; 1-back: $F = 4.431$, $P = 0.038$), with the control group exhibiting a significant increase after 2 y (0-back: $t = 3.349$, $P = 0.002$; 1-back: $t = 2.440$, $P = 0.018$) and the walnut group remaining unchanged (0-back: $t = 0.292$, $P = 0.771$; 1-back: $t = 0.373$, $P = 0.711$) (**Supplemental Figure 2**).

Discussion

In this 2-site randomized feeding trial, we found that walnut supplementation at ∼15% of daily energy intake for 2 y did not delay cognitive decline in community-dwelling elderly but cognitively healthy men and women. However, post hoc analyses showed that participants from the Barcelona site in the walnut diet arm, but not those from Loma Linda, improved global cognition and perception scores compared with controls. A benefit from the intervention in the Barcelona cohort was also suggested by findings from brain fMRI performed in a subset of participants.

To our knowledge, WAHA is the first completed, long-term, randomized clinical trial testing a single whole food for cognitive outcomes in older adults (12, 30). The WAHA study was conceived to provide high-level evidence of the brain benefits of walnut consumption, adding to experimental and epidemiologic findings (13–19). The optimal composition of walnuts in terms of bioactive micronutrients, including sizable amounts of ALA, melatonin, and polyphenols (11), was hypothesized to be influential in reducing the potency of vascular

TABLE 2 Baseline and 2-y changes in energy and nutrient intakes and nut consumption in completers by intervention group[1]

Variable	Walnut diet ($n = 321$)	Control diet ($n = 309$)	P value[2]
Energy, kcal/d			
Baseline	1669 (1617, 1721)	1592 (1541, 1643)	
Change	177 (115, 239)	44 (1, 87)	0.001
Protein, % energy			
Baseline	17.4 (16.9, 17.8)	17.7 (17.2, 18.2)	
Change	− 0.8 (−1.3, −0.3)	− 0.2 (−0.6, 0.3)	0.063
Carbohydrate, % energy			
Baseline	44.1 (43.0, 45.1)	44.0 (42.9, 45.0)	
Change	− 3.9 (−5.0, −2.7)	1.3 (0.4, 2.2)	<0.001
Total fat, % energy			
Baseline	36.6 (35.7, 37.5)	37.0 (36.1, 37.9)	
Change	6.3 (5.4, 7.3)	− 1.0 (−1.8, −0.1)	<0.001
SFA, % energy			
Baseline	10.6 (10.2, 11.0)	10.4 (10.0, 10.8)	
Change	− 0.8 (−1.2, −0.3)	− 0.1 (−0.5, 0.2)	0.029
MUFA, % energy			
Baseline	16.5 (15.8, 17.1)	17.0 (16.3, 17.6)	
Change	− 1.1 (−1.7, −0.6)	− 0.7 (−1.2, −0.2)	0.227
LA, % energy			
Baseline	5.3 (5.0, 5.6)	5.6 (5.3, 5.9)	
Change	7.4 (6.9, 7.8)	− 0.1 (−0.4, 0.1)	<0.001
ALA, % energy			
Baseline	0.57 (0.53, 0.61)	0.63 (0.58, 0.68)	
Change	2.14 (2.02, 2.26)	0.03 (−0.02, 0.08)	<0.001
EPA + DHA, % energy			
Baseline	0.23 (0.19, 0.27)	0.23 (0.19, 0.28)	
Change	− 0.03 (−0.07, 0.02)	0.00 (−0.04, 0.05)	0.343
Fiber, g/d			
Baseline	19.5 (18.6, 20.5)	18.3 (17.5, 19.1)	
Change	2.5 (1.3, 3.7)	0.8 (0.0, 1.7)	0.023
Cholesterol, mg/d			
Baseline	232 (218, 246)	234 (218, 250)	
Change	− 6.4 (−22.4, 9.5)	− 4.1 (−19.8, 11.6)	0.838
Walnuts, g/d			
Baseline	0.43 (0.21, 0.65)	0.75 (0.53, 0.97)	
Change	40.6 (39.7, 41.5)	− 0.42 (−1.30, 0.47)	<0.001
Other nuts, g/d			
Baseline	2.0 (1.3, 2.8)	2.5 (1.7, 3.2)	
Change	− 1.4 (−2.1, −0.7)	− 0.91 (−1.62, −0.20)	0.364

[1] Values are means (95% CIs). ALA, α-linolenic acid; LA, linoleic acid.
[2] Obtained by 1-factor ANOVA.

risk factors and curbing inflammation and oxidation in the brain, thus delaying the onset of age-related cognitive impairment. To further the trial's validity, we recruited participants from 2 clinical centers located in different geographical areas and with noticeable background differences in diet and risk factors for cognitive decline.

Similarly to previous studies on fish oil–derived ω-3 fatty acids, we failed to demonstrate a significant effect on cognition after 2 y of walnut supplementation. The neutral findings may have been due to experimental design issues, namely insufficient duration of the intervention and too healthy a study population. In this regard, it is noticeable that no participants developed clinically significant cognitive impairment during follow-up. The results were null in the whole WAHA sample but in the Barcelona cohort walnut consumption positively influenced global cognitive performance, and the difference with the cognitive trajectory of the control group was equivalent to ∼1.3 y of cognitive aging. This finding should be considered exploratory and needs to be confirmed, because the trial was not originally designed to examine outcomes in subgroups. Such different outcomes by center might rely on the background differences between the 2 cohorts. Of note, Barcelona participants disclosed less education (a variable directly related to the outcome) and a lower baseline status of ALA (a variable directly related to the intervention). In trials on lifestyle and cognition, sample characteristics largely influence the final outcome. In recent multidomain trials, cognitive benefits were observed in studies conducted in healthy elders at high vascular risk (4) or at risk of dementia (5), but not in older people from general practices (31) or in elders who either were frail or had subjective memory complaints (10). Interestingly, the latter study reported statistically significant effects of the intervention in certain high-risk subgroups, namely individuals with untreated hypertension (10) or those with a Cardiovascular Risk Factors, Aging, and

TABLE 3 Cognitive composites (*z* scores) of the modified intention-to-treat population by intervention group[1]

Cognitive composites	Walnut diet[2] ($n = 336$)	Control diet ($n = 321$)	*P* value[3]
Global cognition			
Baseline	0.010 (−0.048, 0.067)	−0.013 (−0.071, 0.045)	
2 y	−0.062 (−0.126, 0.002)	−0.099 (−0.162, −0.036)	
Unadjusted change	−0.072 (−0.100, −0.043)	−0.086 (−0.115, −0.057)	0.491
Adjusted change	−0.069 (−0.097, −0.040)	−0.089 (−0.118, −0.060)	0.334
Memory			
Baseline	0.039 (−0.045, 0.123)	−0.041 (−0.126, 0.044)	
2 y	−0.022 (−0.120, 0.076)	−0.095 (−0.187, −0.004)	
Unadjusted change	−0.061 (−0.121, −0.001)	−0.055 (−0.114, 0.005)	0.885
Adjusted change	−0.057 (−0.116, 0.002)	−0.059 (−0.119, 0.001)	0.954
Language			
Baseline	0.055 (−0.038, 0.148)	−0.057 (−0.147, 0.033)	
2 y	0.010 (−0.083, 0.104)	−0.087 (−0.178, 0.004)	
Unadjusted change	−0.044 (−0.098, 0.009)	−0.030 (−0.091, 0.031)	0.722
Adjusted change	−0.042 (−0.099, 0.014)	−0.032 (−0.090, 0.026)	0.805
Perception			
Baseline	0.008 (−0.066, 0.081)	−0.008 (−0.077, 0.061)	
2 y	−0.096 (−0.174, −0.018)	−0.153 (−0.239, −0.066)	
Unadjusted change	−0.104 (−0.174, −0.034)	−0.145 (−0.218, −0.071)	0.430
Adjusted change	−0.103 (−0.174, −0.032)	−0.145 (−0.218, −0.072)	0.418
Frontal function			
Baseline	−0.007 (−0.070, 0.055)	0.002 (−0.064, 0.069)	
2 y	−0.036 (−0.104, 0.032)	−0.033 (−0.101, 0.035)	
Unadjusted change	−0.029 (−0.061, 0.003)	−0.035 (−0.069, 0.000)	0.795
Adjusted change	−0.025 (−0.058, 0.008)	−0.037 (−0.071, −0.004)	0.609

[1] Values are means (95% CIs).

[2] $n = 334$ in adjusted model (2 missing *APOE* genotype).

[3] For "unadjusted change," *P* obtained by 1-factor ANOVA; for "adjusted change," *P* obtained by ANCOVA adjusted for gender, education years, *APOE* ε4 carriers, ever smoking, baseline age, BMI, diabetes, dyslipidemia, hypertension, physical activity, Hamilton Depression Rating Scale score, and center.

Incidence of Dementia Risk Score ≥ 6 (31). Therefore, further trials should test whether individuals who are cognitively healthy but at higher risk (i.e., due to lower education, suboptimal nutrition, and/or higher potency of vascular risk factors) are those who should be targeted for preventive interventions because they might obtain the largest benefit (32).

Interestingly, in the Barcelona cohort we also observed a benefit of walnuts on brain fMRI examination in the absence of structural changes. After 2 y, participants allocated to the control arm increased activity in brain regions outside the original task-related areas, a common fMRI finding associated with response to cerebral damage in aging (33). Even though both groups achieved equal working memory task scores, participants in the walnut group did not show such increased functional recruitment, suggesting greater brain efficiency. This finding, along with data from reaction time task measurement during the lower loads, suggests that walnut supplementation was associated with an attenuation of the age-related decline in working memory efficiency networks. The results of the fMRI concur with previous studies in patients with early Alzheimer disease, in whom more stable patterns of brain activity during cognitive demands after therapeutic interventions were observed while brain structure remained essentially unchanged (26).

Our study has limitations. First, a 2-y follow-up may be insufficient to detect cognitive changes after a dietary intervention, albeit a longer follow-up might compromise compliance. Second, participants were not blinded to the intervention, because it consisted of a whole food. Third, we did not prespecify the post hoc analyses to examine effects separately by site. Fourth, we planned a study in free-living individuals, although we acknowledge that an isoenergetic intervention in a controlled feeding study setting would have counteracted the long-term cumulative increase in energy intake in the walnut group, which might also have a deleterious effect on cognition. Finally, the 2 locations used different protocols to obtain 2-y dietary data, although both of them had proven validity and provided unbiased estimates. The WAHA study also has strengths. First, it was a dual-center, observer-blinded, randomized controlled trial. Second, there was an excellent retention rate for a 2-y trial in elders (90%) and also there was good compliance, as attested by changes in RBC ALA, an objective biomarker of walnut consumption. Third, the WAHA study used a comprehensive neuropsychological battery assessing the different cognitive domains with construction of composite scores, which is considered to be a more robust method of assessing domain cognitive changes than the use of single test scores (34). Fourth, the prespecified inclusion of brain MRI imaging studies in a subset of the study population allowed detecting subtle brain activity changes during a demanding working memory task. Finally, we analyzed the data with a modified intention-to-treat analysis.

In conclusion, dietary supplementation with walnuts at ∼15% of daily energy for 2 y did not affect cognitive function in cognitively healthy elders compared with a control diet (abstention from walnuts). However, brain fMRI and post hoc analyses revealed the effectiveness of the intervention in the Barcelona site participants, who displayed lower education and

TABLE 4 Cognitive composites (z scores) of the modified intention-to-treat population from the Barcelona and Loma Linda sites by intervention group[1]

	Barcelona			Loma Linda		
Cognitive composites	Walnut diet ($n = 174$)	Control diet ($n = 170$)	P value[2]	Walnut diet ($n = 162$)[3]	Control diet ($n = 151$)	P value[2]
Global cognition						
Baseline	0.011 (−0.079, 0.101)	− 0.016 (−0.102, 0.069)		0.009 (−0.062, 0.080)	− 0.010 (−0.087, 0.068)	
Unadjusted change	− 0.037 (−0.077, 0.002)	− 0.097 (−0.137, −0.057)	0.040	− 0.108 (−0.149, −0.068)	− 0.074 (−0.116, −0.032)	0.240
Adjusted change	− 0.032 (−0.072, 0.008)	− 0.102 (−0.143, −0.062)	0.016	− 0.105 (−0.146, −0.065)	− 0.076 (−0.118, −0.035)	0.323
Memory						
Baseline	0.031 (−0.091, 0.152)	− 0.031 (−0.151, 0.088)		0.047 (−0.069, 0.164)	− 0.051 (−0.172, 0.071)	
Unadjusted change	0.005 (−0.080, 0.089)	− 0.039 (−0.121, 0.043)	0.469	− 0.131 (−0.215, −0.047)	− 0.073 (−0.160, 0.015)	0.341
Adjusted change	0.015 (−0.068, 0.098)	− 0.049 (−0.133, 0.035)	0.292	− 0.131 (−0.215, −0.046)	− 0.075 (−0.162, 0.012)	0.366
Language						
Baseline	0.067 (−0.062, 0.197)	− 0.069 (−0.199, 0.061)		0.041 (−0.093, 0.176)	− 0.044 (−0.169, 0.080)	
Unadjusted change	− 0.091 (−0.163, −0.019)	− 0.044 (−0.113, 0.026)	0.351	0.006 (−0.074, 0.085)	− 0.014 (−0.118, 0.089)	0.761
Adjusted change	− 0.085 (−0.156, −0.015)	− 0.049 (−0.121, 0.022)	0.485	0.004 (−0.087, 0.095)	− 0.011 (−0.105, 0.082)	0.821
Perception						
Baseline	− 0.031 (−0.134, 0.072)	0.031 (−0.063, 0.126)		0.049 (−0.057, 0.156)	− 0.053 (−0.155, 0.049)	
Unadjusted change	− 0.069 (−0.168, 0.030)	− 0.249 (−0.348, −0.151)	0.011	− 0.141 (−0.241, −0.042)	− 0.027 (−0.135, 0.081)	0.124
Adjusted change	− 0.059 (−0.157, 0.039)	− 0.260 (−0.359, −0.161)	0.005	− 0.141 (−0.245, −0.037)	− 0.028 (−0.135, 0.080)	0.139
Frontal function						
Baseline	0.007 (−0.090, 0.105)	− 0.019 (−0.114, 0.076)		− 0.023 (−0.101, 0.054)	0.026 (−0.068, 0.119)	
Unadjusted change	− 0.001 (−0.045, 0.042)	− 0.038 (−0.085, 0.009)	0.256	− 0.058 (−0.105, −0.011)	− 0.031 (−0.083, 0.020)	0.450
Adjusted change	0.000 (−0.045, 0.045)	− 0.039 (−0.085, 0.006)	0.235	− 0.054 (−0.102, −0.005)	− 0.034 (−0.084, 0.016)	0.575

[1]Values are means (95% CIs).

[2]Comparison between walnut diet and control diet. For "unadjusted change," P value obtained by 1-factor ANOVA; for "adjusted change," P value obtained by ANCOVA adjusted for gender, education years, APOE $\varepsilon 4$ carrier, ever smoking, baseline age, BMI, diabetes, dyslipidemia, hypertension, physical activity, and Hamilton Depression Rating Scale score.

[3]$n = 160$ in adjusted model (2 missing *APOE* genotype).

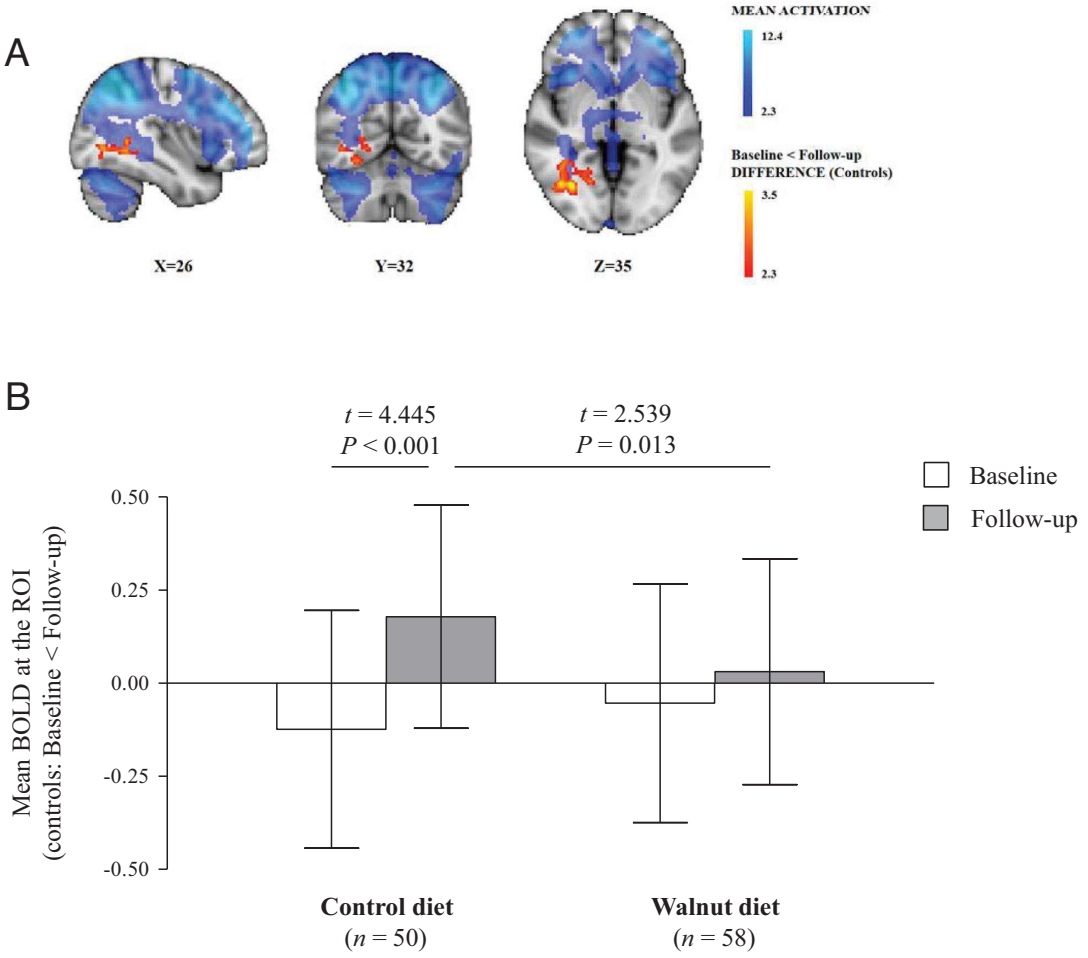

FIGURE 2 Results of fMRI using a general linear model design to evaluate the BOLD activity in the highest load compared with the lowest load (3-back > 0-back). (A) Significant activity maps, where the mean activation for the overall sample in this cognitive contrast is represented in blue, and the pre–post difference is shown in red–yellow. The control group exhibits increases in brain activity at the second time point compared with baseline that were not observed in the walnut group. (B) BOLD signal values at the ROI in the control and walnut groups were extracted in order to obtain summary statistics separated by baseline and follow-up activations. The ROI included mainly the following areas on the right hemisphere: lateral occipital cortex, occipital fusiform gyrus, temporal occipital fusiform cortex, and lingual gyrus. Differences between groups were found at follow-up, but only controls showed significant differences between the first and second time points. In addition, there was a significant group × time interaction ($F = 6.412$, $P = 0.013$). Error bar: ±1 SEM. ROI, region of interest.

a lower background status of dietary ALA (the ω-3 supplied by walnuts) than the Loma Linda cohort. The present results are encouraging but not conclusive for an effect of walnuts on brain health, and further investigation is warranted, in particular directed to disadvantaged populations in whom greatest profit could be expected.

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