

Longer-term soy nut consumption improves cerebral blood flow and psychomotor speed: results of a randomized, controlled crossover trial in older men and women

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ABSTRACT

Background: Effects of soy foods on cerebral blood flow (CBF)—a marker of cerebrovascular function—may contribute to the beneficial effects of plant-based diets on cognitive performance.

Objectives: We aimed to investigate longer-term effects of soy nut consumption on CBF in older adults. Changes in 3 different domains of cognitive performance were also studied.

Methods: Twenty-three healthy participants (age: 60–70 y; BMI: 20–30 kg/m²) participated in a randomized, controlled, single-blinded crossover trial with an intervention (67 g/d of soy nuts providing ~25.5 g protein and 174 mg isoflavones) and control period (no nuts) of 16 wk, separated by an 8-wk washout period. Adults followed the Dutch food-based dietary guidelines. At the end of each period, CBF was assessed with arterial spin labeling MRI. Psychomotor speed, executive function, and memory were assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB).

Results: No serious adverse events were reported, and soy nut intake was well tolerated. Body weights remained stable during the study. Serum isoflavone concentrations increased (daidzein mean difference ± SD: 128 ± 113 ng/mL, *P* < 0.001; genistein: 454 ± 256 ng/mL, *P* < 0.001), indicating excellent compliance. Regional CBF increased in 4 brain clusters located in the left occipital and temporal lobes (mean ± SD increase: 11.1 ± 12.4 mL · 100 g⁻¹ · min⁻¹, volume: 11,296 mm³, *P* < 0.001), bilateral occipital lobe (12.1 ± 15.0 mL · 100 g⁻¹ · min⁻¹, volume: 2632 mm³, *P* = 0.002), right occipital and parietal lobes (12.7 ± 14.3 mL · 100 g⁻¹ · min⁻¹, volume: 2280 mm³, *P* = 0.005), and left frontal lobe (12.4 ± 14.5 mL · 100 g⁻¹ · min⁻¹, volume: 2120 mm³, *P* = 0.009) which is part of the ventral network. These 4 regions are involved in psychomotor speed performance, which improved as the movement time reduced by (mean ± SD) 20 ± 37 ms (*P* = 0.005). Executive function and memory did not change.

Conclusions: Longer-term soy nut consumption may improve cerebrovascular function of older adults, because regional CBF increased. Effects may underlie observed improvements in psychomotor speed. This trial was registered at clinicaltrials.gov as NCT03627637. *Am J Clin Nutr* 2021;114:2097–2106.

Keywords: soy nuts, aging, arterial spin labeling, cerebral blood flow, cerebrovascular function, cognitive performance, psychomotor speed, older males and females

Introduction

Aging-related health conditions, such as impaired cognitive performance and cardiovascular diseases (CVDs), are among the most prevalent disorders in the world (1, 2). Effective intervention strategies are therefore much needed to prevent or reduce the burden of these conditions (1). Although less extensively studied than the potentially beneficial effects on CVD risk, consumption of plant-based diets has also been associated with improvements

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Supplemental Figures 1 and 2 and Supplemental Tables 1–3 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/>.

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Abbreviations used: ALA, α -linolenic acid; ASL, arterial spin labeling; CANTAB, Cambridge Neuropsychological Test Automated Battery; CBF, cerebral blood flow; CGM, continuous glucose monitoring; CVD, cardiovascular disease; DMS, delayed matching to sample; FU, follow-up day; HIRI, hepatic insulin resistance index; MISI, muscle insulin resistance index; MNI, Montreal Neurological Institute; MPRAGE, magnetization-prepared rapid acquisition with gradient echo; MT, movement time; MTT, multitasking test; net iAUC, net incremental area under the curve; NIRS, near-infrared spectroscopy; OGTT, oral-glucose-tolerance test; PAL, paired associates learning; RCT, randomized controlled trial; RT, reaction time; RTI, reaction time task; SSP, spatial span; TE, total number of errors.

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in cognitive performance across different cognitive domains (2, 3). Consequently, studies on the health effects of specific plant-based foods such as soy are gaining increasing attention. Soy is rich in phytoestrogens (isoflavones), *cis*-PUFAs, and high-quality plant proteins, which may all improve cognitive performance (4–7). Effects of soy-rich foods on cerebrovascular function are of major interest. In fact, an impaired vascular function in the brain may precede the age-related decline in cognitive performance, and several reviews have already concluded that diet-induced improvements in cerebrovascular function contribute to the beneficial effects observed on cognitive performance (8–10).

The consumption of specific substances that are also present in soy may improve cerebral blood flow (CBF) (8, 11), but effects of soy products on this physiological marker of cerebrovascular function (11) have not been reported before, to our knowledge. Moreover, glucose metabolism may play an important role because beneficial effects of interventions on CBF may be partly mediated by improvements in glucose metabolism (12–15). This randomized, controlled, crossover trial investigated the effects of longer-term soy nut consumption on CBF, which was the primary outcome of the study, and cognitive performance in older men and women. These participants are expected to have decreased CBF and are also at increased risk of cognitive impairment (16). The noninvasive MRI perfusion method arterial spin labeling (ASL) was used as the primary outcome to quantify CBF, whereas cognitive performance was assessed as the secondary outcome using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Our focus was on 3 main domains of cognitive performance (i.e., psychomotor speed, executive function, and memory). Effects on glucose metabolism were also investigated by a 7-point oral-glucose-tolerance test (OGTT) and by monitoring glucose concentrations continuously during daily life.

Methods

Study participants

Healthy older men and postmenopausal women were recruited through advertisements in local newspapers; flyers in the university, the hospital, and public buildings in Maastricht; and among people who had participated in earlier studies. They were invited for a screening visit when they were aged between 60 and 70 y and had a BMI (in kg/m²) between 20 and 30. During a screening visit, anthropometrics and blood pressure were measured, and a fasting blood sample was drawn. Participants were included if they met the following criteria: stable body weight (<3 kg body weight gain or loss in the past 3 mo); systolic blood pressure < 160 mm Hg and diastolic blood pressure < 100 mm Hg; fasting plasma glucose < 7.0 mmol/L, fasting serum total cholesterol < 8.0 mmol/L, and fasting serum triacylglycerol < 4.5 mmol/L. Participants were excluded when having an allergy or intolerance to soy; when they were smoking, or quit smoking <12 mo before starting the study; taking dietary supplements known to interfere with the main study outcomes; taking medication known to affect blood pressure, lipid metabolism, or glucose metabolism; and having specific contraindications for MRI (e.g., permanent make-up, surgical clips, or claustrophobia). In addition, volunteers suffering from

severe medical conditions, including CVD (e.g., congestive heart failure or any other CVD event in the past), diabetes mellitus, familial hypercholesterolemia, epilepsy, asthma, kidney failure, chronic obstructive pulmonary disease, inflammatory bowel diseases, autoinflammatory diseases, and rheumatoid arthritis, were not allowed to participate. The study was approved by the medical ethics committee of Maastricht University Medical Center (METC-183017). All study participants gave written informed consent before the start of the intervention trial. This study was registered at clinicaltrials.gov (NCT03627637) on 13 August, 2018, and performed between August 2018 and December 2019 in Maastricht, Netherlands.

Study design

This randomized, controlled, crossover trial consisted of a 16-wk intervention period and a 16-wk control period, separated by a washout period of 6–12 wk (median: 8 wk) (**Supplemental Figure 1**). During the soy nut intervention period, participants received unsalted soy nuts (Knusperkerne; Hensel, SALUS Haus), which provided ~25.5 g soy protein daily and 174 mg of isoflavones. **Supplemental Table 1** shows the nutrient composition of the product. Compliance to the intervention was checked by measuring serum daidzein and genistein concentrations as described (LGC Limited) (17). The daidzein metabolite equol was also determined to identify equol producers (18). During the intervention and control periods, participants had to adhere to the 2015 Dutch food-based dietary guidelines, for which they received instructions at baseline and throughout the study from our research assistant. Volunteers were not allowed to use other soy products or dietary supplements known to interfere with the outcomes during the whole study. Participants could consume the soy nuts at any time of the day. A validated FFQ was completed at the end of both periods to assess energy and nutrient intakes over the past 4 wk, which were calculated using the Dutch food composition table (NEVO table) (19). Participants were requested to record in diaries any protocol deviations or health problems, medication use, and alcohol intake during the whole study period. Except for the dietary changes, participants were asked not to change their habitual lifestyles during the entire study.

Allocation to treatment order was determined using a randomized block design (block size: 2 or 4) with stratification for gender. The aim was to recruit an equal number of male and female participants. However, proportions between 40% and 60% were considered to be acceptable. Except for the research assistant, all researchers were blinded to the intervention. However, owing to the nature of the trial, participants could not be blinded. Measurements were performed at the start of the control and intervention periods (baseline), halfway after 8 wk, and during 2 follow-up days (FU1 and FU2) at the end of each period. On the days preceding measurements, participants were requested to have a regular meal and to abstain from alcohol and heavy exercise. They arrived after an overnight fast (no food or drink after 20:00, except for water) by car or public transport at our Metabolic Research Unit Maastricht.

MRI acquisition and processing

Scans were performed at FU1 during the intervention and control periods at the Scannexus research facilities in Maastricht

on a 3T MAGNETOM Prisma Fit MRI system using a 64-channel head/neck coil (Siemens Healthcare). Details about the MRI acquisition and processing have been published before (20). In brief, 1 high-resolution anatomical 3-dimensional magnetization-prepared rapid acquisition with gradient echo (MPRAGE) scan was acquired (repetition time 2400 ms, echo time 2.18 ms, inversion time 1040 ms, 1.0 mm isotropic resolution, 8° flip angle, and 160 sagittal slices). Thereafter, pseudo-continuous ASL was performed with background-suppressed segmented 3-dimensional gradient and spin echo readouts. The sequence parameters were repetition time 4050 ms, echo time 13.6 ms, GeneRALized Autocalibrating Partially Parallel Acquisitions (GRAPPA) 2, labeling duration 1750 ms, postlabeling delay 2000 ms, segmentation factor 6, 10 label-control repetitions with 19 slices, and a voxel resolution of 3.0 mm isotropic.

Before quantification, individual images were distortion-corrected with FSL TopUp using M_0 images with opposite phase-encoding direction and a readout time of 75 ms. Quantification followed the recommendations of the ASL White Paper (21) and was performed using FSL version 6.0 (Analysis Group, FMRIB, Oxford, UK) and the FSL BASIL toolbox (version 4.0.15). Images were voxel-wise calibrated using the M_0 image and with a repetition time of 20 s (22, 23). The used labeling efficiency was 0.64 (4 background suppression pulses; 0.93^4), the T_1 of gray matter was 1330 ms, and bolus arrival time was 1300 ms; images were also corrected for hemoglobin concentration (24). The volBrain online web interface (<http://volbrain.upv.es>) was used to perform brain extraction, along with tissue segmentation for the anatomical MPRAGE image (25). CBF images were co-registered to the anatomic resolution using Boundary-Based Registration. Thereafter, mean CBF values were calculated for global brain, gray matter, and both hemispheres based on the high-resolution anatomical scan. Voxel-wise comparison was performed after co-registration to the Montreal Neurological Institute (MNI) (2 mm) using a repeated-measures mixed-effects analysis with a general linear model with a single-group paired difference (FMRIB's Local Analysis of Mixed Effects (FLAME) stages 1 and 2), and a Z-threshold of 2.3 ($P < 0.05$). Thereafter, family-wise error correction was performed based on smoothness estimates. Atlasquery was used to determine the location of significant clusters in the MNI structural and the Harvard-Oxford (sub)cortical structural atlas.

Cognitive performance

Standardized cognitive performance tests were taken on FU2 using the computerized and fully automated CANTAB cognitive research software. These tests were related to 3 main cognitive domains which are known to be affected by aging: psychomotor speed, executive function, and memory (26). Participants were first familiarized with the digital tablet (iPad, 5th generation; Apple) based touchscreen test method using the motor screening task (MOT). Thereafter, psychomotor speed was assessed using the reaction time task (RTI), during which reaction time (RT) and movement time (MT) were measured. The multitasking test (MTT) was used to assess executive function. The variables used for the MTT were incongruency cost, multitasking cost, median latency, and the total number of errors (TE). Cognitive tests to evaluate memory included spatial span (SSP), delayed matching to sample (DMS), and paired associates learning (PAL). Parallel tests including different patterns were used with high test-retest

repeatability to increase the sensitivity to longitudinal changes by minimizing practice effects (27). For SSP, the maximal completed span length variable was used. The percentage of correctly answered trials for all delays was used for DMS, whereas for PAL the first attempt memory score and TE were used. **Supplemental Table 2** shows a summary of the cognitive tests and reported outcomes. The cognitive tests are described in detail on the CANTAB website (26).

Blood sampling and glucose metabolism

Fasting blood samples were taken by venipuncture from a forearm vein at baseline, week 8, and FU1. At FU2, blood samples were obtained using an intravenous catheter at baseline ($T = 0$), and 15, 30, 45, 60, 90, and 120 min after ingestion of a drink containing 75 g glucose (Novolab) during a 7-point OGTT. Glucose concentrations (Horiba ABX) were determined in plasma samples obtained at all time points using NaF-containing vacutainer tubes (Becton, Dickson and Company). These tubes were placed on ice immediately after sampling and centrifuged within 30 min at $1300 \times g$ for 15 min at 4°C. Insulin concentrations were determined in serum samples (RIA, Millipore), which were obtained at all time points using vacutainer serum tubes (Becton, Dickson and Company). These tubes were first allowed to clot for ≥ 60 min at 21°C and centrifuged at $1300 \times g$ for 15 min at 21°C. Obtained samples were immediately portioned into aliquots, frozen in liquid nitrogen, and stored at -80°C until analysis at the end of the study.

Fasting glucose and insulin concentrations were used to calculate the HOMA-IR as a measure of insulin resistance. The postload glucose and insulin concentrations from the OGTT were used to calculate the Matsuda index and net incremental area under the curve (net iAUC) using GraphPad (GraphPad Prism 8 Software). Also, muscle and hepatic insulin resistance indexes (MISI and HIRI) were derived from the OGTT. The MISI was calculated using the product of total AUC for plasma glucose and insulin concentrations during the first 30 min of the OGTT, whereas the rate of decay of plasma glucose concentration from its peak value to its nadir was divided by the mean plasma insulin concentration for the HIRI (28). Finally, continuous glucose monitoring (CGM) (Freestyle Libre Pro, Abbott) was performed between FU1 and FU2. A sensor was placed at the back of the upper arm and measured the glucose concentration every 15 min for 96 h. The AUC and net iAUC were calculated for the CGM using GraphPad Prism 8. For every 24 h the minimal 1-h value was calculated and averaged, which was used as the baseline to calculate the net iAUC.

Statistical analyses

Results are shown as means \pm SDs, unless otherwise indicated. Based on our previous study on the effects of a lifestyle intervention on CBF (20), it was determined before the start of the study that 23 participants would be needed to detect a 0.8-SD unit change in CBF with 80% power and a 2-sided α of 0.05. A 0.8-SD unit change in CBF can be expected after dietary interventions and corresponds to a change of $\sim 10\%$ – 15% (8, 20), which is clinically relevant (29).

All variables were normally distributed based on the Shapiro-Wilk test. First, a repeated-measures ANOVA with period, gender, and order as between-subject factors was performed. Order

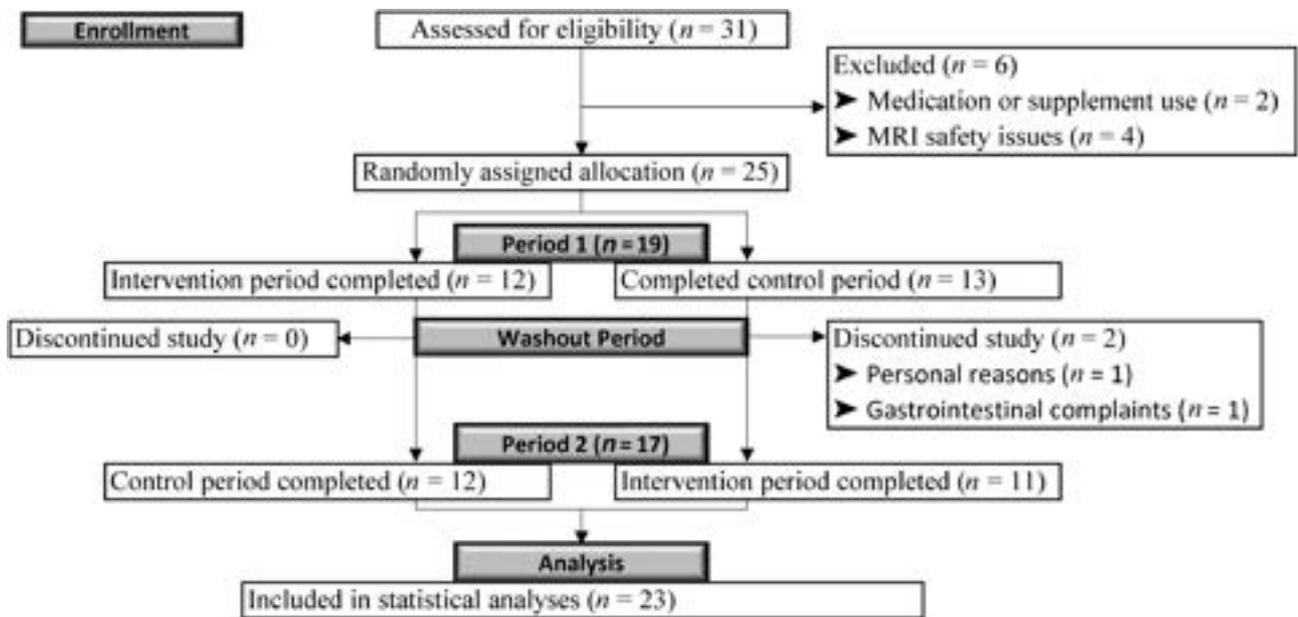


FIGURE 1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram showing the progress of older men and women through the phases of this randomized, controlled crossover study.

effects were not observed and were therefore excluded from the final model to test for differences between treatments. Linear mixed models were performed for anthropometrics and fasting glucose and fasting insulin concentrations to test for differences between treatments over time. Time, treatment, period, gender, and time \times treatment interaction were used as fixed factors, and participant and intercept as random factors. The interaction term was omitted from the model if it was not significant. Best model fit was obtained with an autoregressive covariance structure based on the chi-square statistic with log-likelihood values ($P < 0.05$), and the Akaike information criterion. The postload glucose and insulin concentrations during the OGTT were analyzed using a Toeplitz covariance structure. Pearson correlations were determined between the percentage change in CBF clusters that changed significantly and changes in cognitive performance variables. SPSS was used to perform all statistical analyses (IBM SPSS Statistics version 26). Differences with a $P < 0.05$ using 2-tailed tests were considered to be statistically significant.

Results

Study participants

Figure 1 shows a Consolidated Standards of Reporting Trials (CONSORT) flow diagram. Twenty-five older men and women

were eligible and started the study. Two women dropped out during the soy nut intervention: 1 woman owing to personal reasons and 1 woman owing to mild gastrointestinal discomfort. A total of 23 participants (11 men and 12 women) completed the study and were included in the statistical analyses. Participants had a mean age of 64 ± 3 y, and the mean BMI was 26.8 ± 2.8 for men and 25.0 ± 2.3 for women. No serious adverse events or protocol deviations were reported in the diaries and the soy nut regime was well tolerated. Overall, compliance was excellent based on returned empty sachets or unused study products and based on increased serum isoflavone concentrations. Specifically, serum daidzein concentrations increased by 128 ± 113 ng/mL ($P < 0.001$) and those of genistein by 454 ± 256 ng/mL ($P < 0.001$). Six participants (24%) could be classified as equol producers and their serum equol concentrations increased by 190 ± 102 ng/mL ($P = 0.020$) after the soy nut intervention (see **Table 1**).

As expected, food-frequency data indicated a higher protein ($\Delta 3.1 \pm 2.0$ En%; $P < 0.001$) and a lower carbohydrate intake ($\Delta -2.0 \pm 3.7$ En%; $P = 0.008$) during the soy intervention period (**Supplemental Table 3**). Total fat intake was not changed ($\Delta -1.1 \pm 3.4$ En%; $P = 0.123$). However, lower intake of SFAs ($\Delta -1.3 \pm 1.6$ En%; $P = 0.001$) and *cis*-MUFAs ($\Delta -1.5 \pm 1.9$ En%; $P = 0.001$) was observed, whereas the consumption of *cis*-PUFAs ($\Delta 1.9 \pm 1.4$ En%; $P < 0.001$) was higher during the

TABLE 1 Serum isoflavone concentrations at the end of the 16-wk soy nut and control periods¹

	Intervention period	Control period	Mean difference	$F(1, 19)$	MSE	P value ²
Daidzein, ng/mL	134 ± 114	3 ± 2	128 ± 113	25.38	5482.3	<0.001
Genistein, ng/mL	459 ± 416	5 ± 7	454 ± 256	28.06	83310	<0.001
Equol, ³ ng/mL	190 ± 105	0 ± 0	190 ± 102	20.89	4316.6	0.020

¹ $n = 23$. Values are means \pm SDs. MSE, mean square error.

²Repeated-measures ANOVA with period and gender as between-subject factors.

³Equol producers ($n = 6$). df (1, 3).

TABLE 2 Anthropometrics during the soy nut and control intervention throughout the intervention trial¹

	Intervention period			Control period			<i>P</i> value ²	
	Baseline	Midterm	Follow-up	Baseline	Midterm	Follow-up	Time × treatment	Treatment
Weight, kg	74.6 ± 10.4	74.5 ± 10.5	74.4 ± 10.5	74.4 ± 10.0	74.2 ± 10.1	74.0 ± 9.9	0.931	0.533
BMI, kg/m ²	25.5 ± 2.7	25.5 ± 2.8	25.4 ± 2.6	25.5 ± 2.5	25.5 ± 2.5	25.4 ± 2.5	0.916	0.860
WC, cm	86.2 ± 7.8	86.8 ± 9.1	86.0 ± 8.5	85.7 ± 9.1	85.4 ± 9.1	86.4 ± 8.5	0.117	0.475
W-H ratio	0.84 ± 0.07	0.84 ± 0.08	0.83 ± 0.08	0.84 ± 0.07	0.84 ± 0.08	0.85 ± 0.08	0.045	—

¹*n* = 23. Values are means ± SDs. WC, waist circumference; W-H ratio, waist-to-hip ratio.

²Linear mixed models were performed for anthropometrics to test for differences between treatments over time. Time, treatment, period, gender, and time × treatment interaction were used as fixed factors, and participant and intercept as random factors. An autoregressive covariance structure was used. When the interaction term (time × treatment) did not reach statistical significance (*P* > 0.05), it was removed from the model to calculate the treatment effect.

soy nut intervention. In addition, the intake of cholesterol was reduced by 4 ± 6 mg/MJ (*P* = 0.002), whereas the intake of dietary fibers was higher ($\Delta 8.8 \pm 3.5$ g/d; *P* < 0.001), after soy nut intake. Although total energy intake tended to be higher during the soy nut period ($\Delta 111 \pm 283$ kcal/d; *P* = 0.066), body weight, BMI, and body fat percentages did not differ. However, the waist-to-hip ratio was 0.02 lower at follow-up after the soy nut intervention (time × treatment; *P* = 0.045) (Table 2).

CBF

Compared with the control period, global and gray matter CBF, and the CBF in the left and right hemispheres, were not different (Figure 2, Table 3). Regional blood flow, however, significantly increased in 4 clusters after the soy nut intervention (Figure 3, Table 3). CBF in the largest cluster increased by 11.1 ± 12.4 mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 36\%$; *P* < 0.001). Cluster 1 had a volume of 11,296 mm³ and the average probability of the location based on the MNI structural atlas was in the occipital lobe (40%) and temporal lobe (16%). The specific location based on the Harvard-Oxford atlas was 13% in the left occipital pole, 7% in the temporal fusiform cortex, 5% in the lateral occipital cortex, and 4% in the temporal occipital fusiform cortex. In

cluster 2 (bilateral occipital lobe, 59%), blood flow increased by 12.1 ± 15.0 mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 32\%$; *P* = 0.002). The volume of that cluster was 2632 mm³ and the specific average probability of the location was 24% in the lingual gyrus, 14% in the occipital pole, 7% in the intracalcarine cortex, and 7% in the cuneal cortex. CBF increased by 12.7 ± 14.3 mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 47\%$; *P* = 0.005) in cluster 3 (right occipital, 30%; and parietal lobe, 11%), which was 2280 mm³ in volume, and the average probability of the location was 22% in the right lateral occipital cortex and 6% in the right intracalcarine cortex. Finally, blood flow also increased in cluster 4 (left frontal lobe, 18%) by 12.4 ± 14.5 mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 43\%$; *P* = 0.009). The average probability of the location of that cluster, which had a total cluster volume of 2120 mm³, was 10% in the left middle frontal gyrus and 9% in the left inferior frontal gyrus.

Cognitive performance

The MT during the RTI was reduced by 20 ± 37 ms ($\Delta 7\%$; *P* = 0.005) from 295 ± 68 ms after the control period to 275 ± 49 ms after the soy nut intervention. This suggests that cognitive performance in the domain of psychomotor speed was improved, whereas the RT did not change ($\Delta 0 \pm 24$ ms;

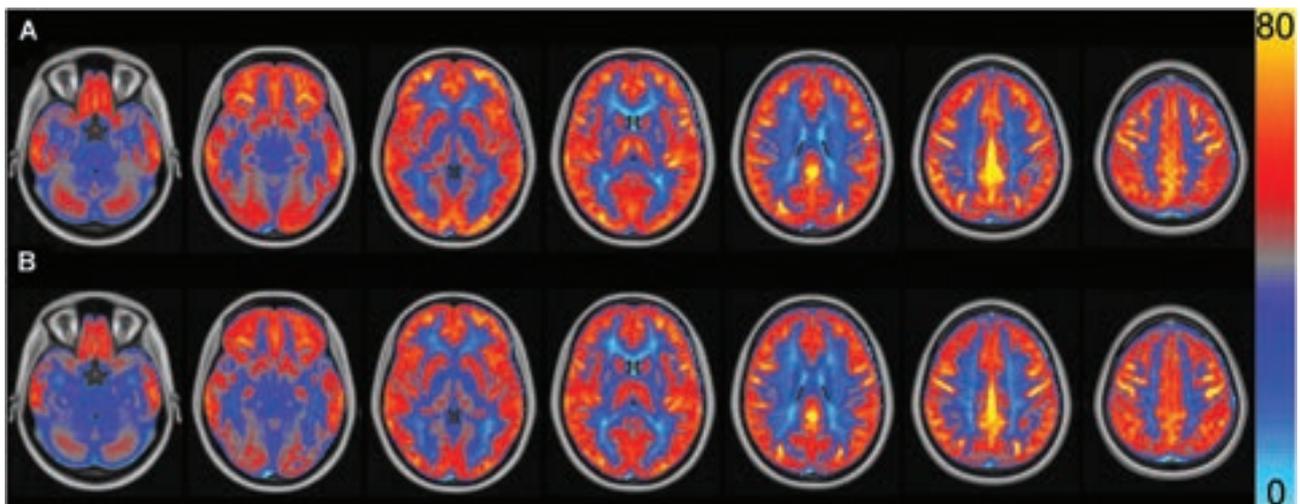


FIGURE 2 Mean CBF maps from a randomized, controlled crossover study in older adults (*n* = 23) after nonlinear co-registration to the Montreal Neurological Institute template, after soy nut intake (A) and the control period (B). The images show the CBF in mL · 100 g tissue⁻¹ · min⁻¹ (scale shown by color bar). No differences were observed between periods in global CBF (*P* = 0.567), gray matter CBF (*P* = 0.593), and CBF in the left (*P* = 0.570) and right (*P* = 0.542) hemispheres. CBF, cerebral blood flow.

TABLE 3 CBF after a soy intervention and control period in a randomized, controlled crossover study with older men and women¹

Outcome	Intervention period, mL · 100 g ⁻¹ · min ⁻¹	Control period, mL · 100 g ⁻¹ · min ⁻¹	Mean difference, mL · 100 g ⁻¹ · min ⁻¹	<i>F</i> (1, 19)	MSE	<i>P</i> value ²
Global CBF	40.6 ± 8.7	41.2 ± 9.5	-0.6 ± 5.2	0.38	13.81	0.567
Gray matter CBF	48.5 ± 10.3	49.2 ± 10.9	-0.6 ± 6.0	0.33	14.11	0.593
Left hemi CBF	42.5 ± 8.9	43.1 ± 9.3	-0.6 ± 5.6	0.30	16.14	0.570
Right hemi CBF	42.2 ± 9.3	42.9 ± 10.2	-0.7 ± 5.6	0.34	12.71	0.542
Cluster 1 CBF	41.9 ± 9.1	30.8 ± 7.4	11.1 ± 12.4			<0.001
Cluster 2 CBF	49.6 ± 12.4	37.6 ± 6.8	12.1 ± 15.0			0.002
Cluster 3 CBF	39.6 ± 11.3	26.9 ± 6.8	12.7 ± 14.3			0.005
Cluster 4 CBF	41.0 ± 10.1	28.6 ± 8.3	12.4 ± 14.5			0.009

¹*n* = 23. Values are means ± SDs. CBF, cerebral blood flow; hemi, hemisphere; MSE, mean square error.

²Repeated-measures ANOVA with period and gender as between-subject factors and participant and treatment as fixed factors. Clusters were the result of a voxel-wise analysis within FSL applying a repeated-measures mixed-effects analysis using a general linear model with a single-group paired difference [FMRIB's Local Analysis of Mixed Effects (FLAME) stage 1 and 2], and a Z-threshold of 2.3 (*P* < 0.05). Family-wise error correction was performed based on smoothness estimates.

P = 0.926) (Table 4). After excluding 1 participant with extreme responses, a significant inverse correlation was observed between the percentage changes in CBF in cluster 2 (*r* = -0.45, *P* = 0.036) and cluster 4 (*r* = -0.46, *P* = 0.031), and the change in RTI MT (see Supplemental Figure 2). Correlations with changes in cluster 1 (*r* = -0.36, *P* = 0.101) and cluster 3 (*r* = -0.38, *P* = 0.084) were also negative, but did not reach statistical significance. No treatment effects were observed for the executive function tests MTT and SSP, and the memory tests DMS and PAL (see Table 4).

Glucose metabolism

The time × treatment interactions for fasting glucose (*P* = 0.745) and insulin (*P* = 0.206) concentrations, and the HOMA-IR (*P* = 0.425), were not statistically significant. After the interaction term was omitted from the model, no significant treatment effects were observed (glucose: *P* = 0.643; insulin: *P* = 0.398; HOMA-IR: *P* = 0.150). In addition, no differences

were observed in postload glucose (time × treatment: *P* = 0.952; treatment: *P* = 0.950) and insulin (time × treatment: *P* = 0.738; treatment: *P* = 0.737) concentrations during the OGTT (Figure 4A, B). Also, the net iAUC did not differ between treatments for glucose ($\Delta -8 \pm 117$ mmol/L × h; *P* = 0.746) and insulin ($\Delta -147 \pm 1614$ μU/L × h; *P* = 0.657). The MISI ($\Delta -0.032 \pm 0.163$ arbitrary units; *P* = 0.405) and HIRI ($\Delta 2.264 \pm 105.303$ arbitrary units; *P* = 0.922) also did not change. Finally, the continuous glucose concentrations over 96 h did not differ as indicated by the AUC ($\Delta -20 \pm 49$ mmol/L × h; *P* = 0.079) and the iAUC ($\Delta -4 \pm 32$ mmol/L × h; *P* = 0.583) (see Figure 4C).

Discussion

In this randomized, controlled crossover trial with older men and women, longer-term soy nut consumption increased regional CBF in 4 brain clusters. Three clusters were located in the bilateral occipital and parietal lobes, although the largest cluster

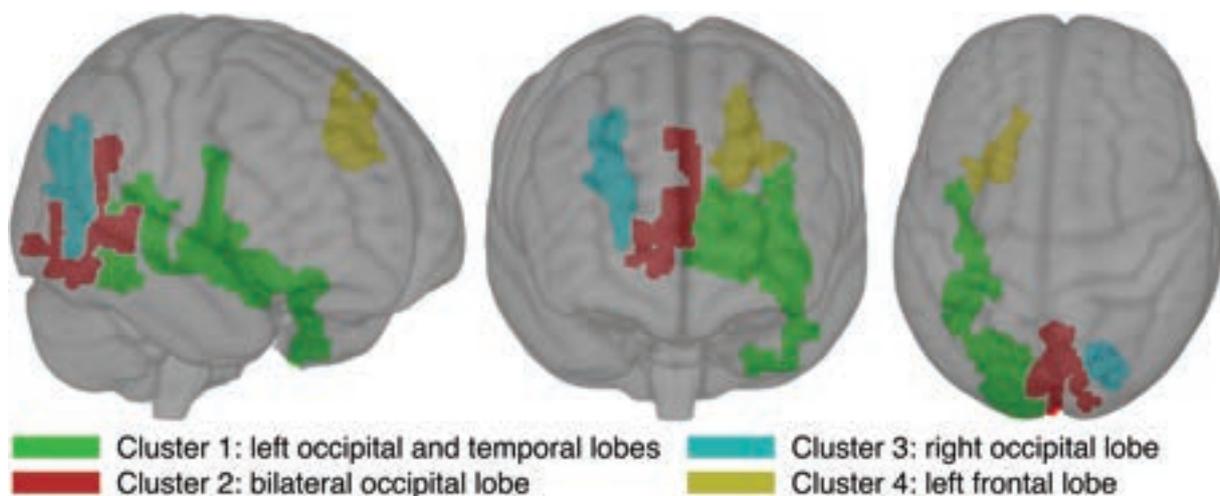


FIGURE 3 Results of voxel-wise comparisons including all acquired CBF data from a randomized, controlled crossover study in older adults (*n* = 23) in the 3-dimensional Montreal Neurological Institute template. CBF increased in 4 clusters after soy nut intake as compared with the control period (family-wise error corrected). Cluster 1: left occipital and temporal lobes, mean ± SD $\Delta 11.1 \pm 12.4$ mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 36\%$), volume 11,296 mm³, *P* < 0.001; cluster 2: bilateral occipital lobe, $\Delta 12.1 \pm 15.0$ mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 32\%$), volume 2632 mm³, *P* = 0.002; cluster 3: right occipital and parietal lobes, $\Delta 12.7 \pm 14.3$ mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 47\%$), volume 2280 mm³, *P* = 0.005; cluster 4: left frontal lobe, $\Delta 12.4 \pm 14.5$ mL · 100 g tissue⁻¹ · min⁻¹ ($\Delta 43\%$), volume 2120 mm³, *P* = 0.009. CBF, cerebral blood flow.

TABLE 4 Outcomes of cognitive tests after a soy intervention and control period in a randomized, controlled crossover study with older men and women¹

Outcome	Intervention period	Control period	Mean difference	<i>F</i> (1, 19)	MSE	<i>P</i> value ²
RTI MT, ms	275 ± 49	295 ± 68	−20 ± 37	9.92	474.32	0.005
RTI RT, ms	395 ± 43	395 ± 41	0 ± 24	0.01	310.93	0.926
MTT IC, ms	114 ± 42	99 ± 56	15 ± 64	1.12	310.93	0.303
MTT MTC, ms	311 ± 100	292 ± 102	19 ± 165	0.03	13,923	0.584
MTT ML, ms	749 ± 158	746 ± 169	3 ± 96	0.02	4929.3	0.901
MTT TE	5 ± 6	8 ± 12	−3 ± 12	1.90	66.97	0.183
SSP SL	6 ± 1	6 ± 1	0 ± 1	0.25	0.71	0.622
DMS TC, %	85 ± 11	84 ± 12	−1 ± 15	0.01	110.01	0.934
PAL FAMS	12 ± 4	12 ± 3	0 ± 3	0.53	5.09	0.477
PAL TE	17 ± 13	15 ± 11	2 ± 11	0.40	58.43	0.537

¹*n* = 23. Values are means ± SDs. DMS, delayed matching to sample; FAMS, first attempt memory score; IC, incongruency cost; ML, median latency; MSE, mean square error; MT, movement time; MTC, multitasking cost; MTT, multitasking test; PAL, paired associates leaning; RT, reaction time; RTI, reaction time task; SL, span length; SSP, spatial span; TC, total correct; TE, total number of errors.

²Repeated-measures ANOVA with period and gender as between-subject factors and participant number and treatment as fixed factors.

extended to the left temporal lobe. The fourth brain cluster was located in the left frontal lobe. Further, cognitive performance within the domain of psychomotor speed improved, but no changes were observed in executive function or memory. Finally, fasting and postload glucose and insulin concentrations did not change, and glucose concentrations—measured during daily life with a CGM—were also not affected.

Effects of soy products on CBF have not been studied before in humans, to our knowledge. However, consumption of specific substances that are also present in soy, such as phytoestrogens (isoflavones), *cis*-PUFAs, and plant proteins, may improve CBF (13–15). Specifically, isoflavones in soy are distinct from those of other plant products, and mainly consist of genistein and daidzein. Effects of these isoflavones on CBF are not known. Yet, supplementation for 12 wk with a blueberry concentrate rich in the flavonoid anthocyanin increased in older adults CBF in the occipital and parietal lobes, which agrees with our findings (30). Further, flavanol-rich cocoa acutely increased CBF in 2 clusters located in the frontal lobe and parietal lobe in older adults (31). Although the different classes of flavonoids may have different effects, such as antioxidant and anti-inflammatory activities (32), they may all increase NO bioavailability, thereby improving CBF (33).

Other soy components that may account for the observed effects on CBF include *cis*-PUFAs and high-quality plant proteins (11). Soy mainly contains linoleic acid (18:2n–6), but effects of linoleic acid on CBF have not been studied. However, it has been suggested that the majority of linoleic acid entering the brain is converted into relatively polar compounds (34), including linoleic acid–derived oxylipins that may increase CBF (35). The soy nuts also provided daily ~0.8 g of α -linolenic acid (ALA, 18:3n–3) that can be converted into the long-chain n–3 PUFAs EPA (20:5n–3) and DHA (22:6n–3), although in limited amounts (36). Daily supplementation for 12 wk with fish (37), krill, or sardine oil (38), which are rich in EPA and DHA, increased CBF in the prefrontal cortex. These studies measured CBF using near-infrared spectroscopy (NIRS) during a cognitive task in healthy young (37) or older adults (38). Circulating DHA gets incorporated into human brain lipids (39), thereby possibly affecting CBF responses. Finally, isolated soy proteins (8 g) acutely increased CBF in the prefrontal cortex in young, healthy

adults as measured with NIRS in superficial cortical regions, which may be due to their beneficial effects on neurotransmission (40) and NO metabolism (41).

Soy nut consumption significantly improved cognitive performance within the domain of psychomotor speed. Effects of soy foods on cognitive performance have hardly been studied. In contrast to our findings, consumption of a soy drink did not affect psychomotor speed in postmenopausal women (42). This may relate to the shorter study duration (12 wk compared with 16 wk) and the lower daily intake of isoflavones (10–60 mg compared with 174 mg in our study). Interestingly, several reviews have reported beneficial effects of soy isoflavones on cognitive performance (5, 43–45). A recent meta-analysis of 16 randomized controlled trials (RCTs) in mainly postmenopausal women indeed concluded that isolated soy isoflavones with intakes ranging from 60 to 160 mg/d improved overall cognitive performance (5). However, we only observed effects on psychomotor speed. Of note, the only study in the meta-analysis involving a similar study population of healthy older men and women also observed an improved psychomotor speed (46). Whether effects on cognitive performance depend on the study population warrants further study. Effects on cognitive performance may also relate to the increased intake of *cis*-PUFAs and plant proteins. Although positive associations have been observed (7), no RCTs have addressed the effects of linoleic acid on cognitive performance. However, some evidence exists that in healthy older adults EPA and DHA have beneficial effects on psychomotor speed (47). Finally, daily consumption of 50 g isolated soy protein for 8 wk improved results of a multichoice reaction time task, which agrees with our findings, whereas memory was also not affected (48).

A relation was found between regional CBF increase and the favorable effects observed on psychomotor speed. The brain clusters were located in cortical regions that are known to be affected by aging (16) and may thus be more sensitive to the effects of diet. Specifically, clusters 1 and 3 were partly located in the lateral occipital cortex that is involved in object recognition (49), whereas the occipital pole (clusters 1–3) and temporal fusiform cortex (cluster 1) have been linked to visual information processing (50, 51). Furthermore, cluster 4 was located in the frontal gyrus, which is part of the ventral attention network

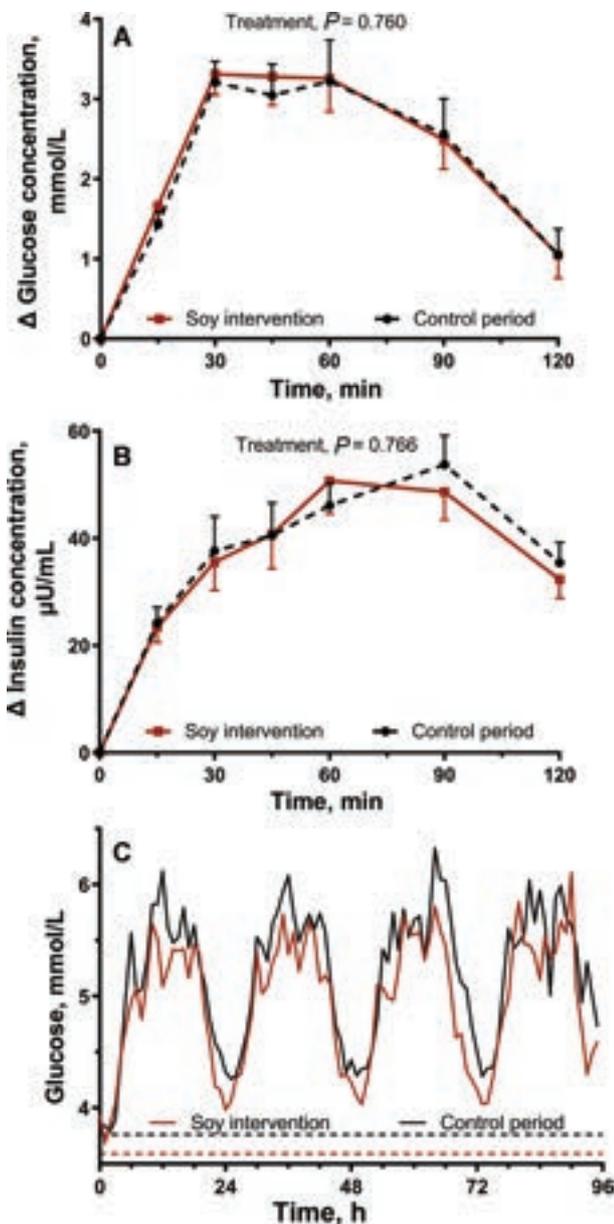


FIGURE 4 Glucose and insulin measurements. Mean \pm SEM differences in (A) glucose and (B) insulin concentrations during a 7-point oral-glucose-tolerance test ($n = 23$). Data were analyzed using linear mixed models to test the difference between each time point and baseline after the soy nut intervention and control periods. No treatment effect was observed for glucose ($P = 0.760$) and insulin concentrations ($P = 0.766$). (C) Mean 96-h continuous glucose measurements. The horizontal dashed lines (red = soy nut intervention period, black = control period) represent the mean minimal fasting glucose concentration of each day.

that is involved when performing the 5-choice reaction time psychomotor speed tasks (49). The faster movement time during the psychomotor speed test may thus be due to faster recognition and processing of the target in combination with improved reorientation to the stimuli. Interestingly, concomitant changes in regional CBF and performance during a psychomotor speed test have already been reported in older adults after 12 wk of supplementation with an anthocyanin-rich blueberry concentrate (30).

Effects on CBF or cognitive performance in our study were not related to changes in glucose metabolism, as suggested by other studies (12–15). In fact, markers of glucose metabolism did not change at all. This is in line with results of a meta-analysis including 24 RCTs (52). In 9 studies soy foods were used, in 10 studies soy isoflavone extracts, and in 5 studies soy proteins. No effects on fasting or postload glucose and insulin concentrations were observed (52). A more recent meta-analysis reported that plant-derived *cis*-PUFAs also did not affect fasting glucose concentrations. However, a dose-dependent decrease in fasting insulin concentrations was observed (53). Based on the results of that meta-analysis, however, the additional intake of *cis*-PUFAs in our study was probably too low to affect fasting insulin concentrations (53). Intervention studies with dietary ALA (54) in healthy individuals also did not show beneficial effects on glucose metabolism or plasma insulin concentrations.

We used the MRI perfusion method ASL, which is considered the noninvasive gold standard (11), to quantify changes in CBF, and CANTAB as a standardized, validated, and sensitive method to detect changes in cognitive performance after dietary interventions (55). Our focus was on both older men and women who had to adhere to food-based dietary guidelines (56), meaning that soy nut effects were evaluated as part of a recommended diet. Compliance based on serum isoflavone concentrations was excellent. An inherent limitation of our study was that participants could not be blinded. Except for the research assistant, however, researchers were blinded. Even though body weight remained stable, it should be considered that participants only partly compensated for the extra energy from the intake of the nuts, because energy intake tended to increase during the soy nut intervention. In addition, soy nut effects cannot be disentangled from those due to the replacement of food products by the intake of the soy nuts. Some studies have suggested that people who can convert daidzein into equol benefit more from the potential health benefits of soy. However, only 6 participants (24%) were equol-producers, which is in line with other studies (57). Unfortunately, this number is too low to compare effects between equol producers and nonproducers with sufficient statistical power.

In conclusion, a longer-term soy nut intervention increased regional CBF. These effects may underlie the observed beneficial effects on cognitive performance in the psychomotor speed domain, suggesting a potential mechanism by which an increased intake of soy-rich foods beneficially affects cognitive performance in older men and women.

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The authors' responsibilities were as follows—JPDK: analyzed the data and performed the statistical analysis; LT: conducted the research; LT and TCA: reviewed the manuscript; RPM and PJJ: designed the research and had primary responsibility for the final content; JPDK, RPM, and PJJ: wrote the paper; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

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

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