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Original Research Article

Glycemic control contributes to the neuroprotective effects of Mediterranean and green-Mediterranean diets on brain age: the DIRECT PLUS brain-magnetic resonance imaging randomized controlled trial

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ABSTRACT

Background: We recently reported that Mediterranean (MED) and green-MED diets significantly attenuated age-related brain atrophy by ~50% within 18 mo.

Objective: The objective of this study was to explore the contribution of specific diet-induced parameters to brain-volume deviation from chronologic age.

Methods: A post hoc analysis of the 18-mo DIRECT PLUS trial, where participants were randomly assigned to the following groups: *1*) healthy dietary guidelines, *2*) MED diet, or *3*) green-MED diet, high in polyphenols, and low in red meat. Both MED groups consumed 28 g walnuts/d (+440 mg/d polyphenols). The green-MED group further consumed green tea (3–4 cups/d) and Mankai green shake (Wolffia globosa aquatic plant) (+800 mg/d polyphenols). We collected blood samples through the intervention and followed brain structure volumes by magnetic resonance imaging (MRI). We used hippocampal occupancy (HOC) score (hippocampal and inferior lateral-ventricle volumes ratio) as a neurodegeneration marker and brain-age proxy. We applied multivariate linear regression models.

Results: Of 284 participants [88% male; age = 51.1 y; body mass index = 31.2 kg/m^2; hemoglobin A1c (HbA1c) = 5.48%; APOE-ε4 genotype = 15.7%], 224 completed the trial with eligible whole-brain MRIs. Individuals with higher HOC deviations (i.e., younger brain age) presented lower body weight [r = –0.204; 95% confidence interval (CI): –0.298, –0.101], waist circumference (r = –0.207; 95% CI: –0.310, –0.103), diastolic (r = –0.186; 95% CI: –0.304, –0.072), systolic blood pressure (r = –0.189; 95% CI: –0.308, –0.061), insulin (r = –0.099; 95% CI: –0.194, –0.004), and HbA1c (r = –0.164; 95% CI: –0.337, –0.006) levels. After 18 mo, greater changes in HOC deviations (i.e., brain-age decline attenuation) were independently associated with improved HbA1c (β = –0.254; 95% CI: –0.392, –0.117), HOMA-IR (β = –0.200; 95% CI: –0.346, –0.055), fasting glucose (β = –0.155; 95% CI: –0.293, –0.016), and c-reactive protein (β = –0.153; 95% CI: –0.296, –0.010). Improvement in diabetes status was associated with greater HOC

Abbreviations: AD, Alzheimer's disease; BBB, blood–brain barrier; BP, blood pressure; CI, confidence interval; green-MED diet, Mediterranean diet higher in polyphenols and lower in red/processed meat; HbA1c, hemoglobin A1c; HDG, healthy dietary guidelines; HOC, hippocampal occupancy score; MCI, mild cognitive impairment; MED, Mediterranean diet; PA, physical activity; TG, triglyceride; WC, waist circumference.

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deviation changes than either no change in diabetes status (0.010; 95% CI: 0.002, 0.019) or with an unfavorable change (0.012; 95% CI: 0.002, 0.023). A decline in HbA1c was further associated with greater deviation changes in the thalamus, caudate nucleus, and cerebellum ($P < 0.05$). Greater consumption of Mankai and green tea (green-MED diet components) were associated with greater HOC deviation changes beyond weight loss.
Conclusions: Glycemic control contributes to the neuroprotective effects of the MED and green-MED diets on brain age. Polyphenols-rich diet components as Mankai and green tea may contribute to a more youthful brain age.
This trial was registered at clinicaltrials.gov at clinicaltrials.gov as NCT03020186.

Keywords: aging, brain age, dietary intervention, glycemic control, green-Mediterranean, hippocampal occupancy score, polyphenols

Introduction

Age-related brain atrophy, a natural aging process, is characterized by a reduction in brain volume and has been identified as an early biomarker for cognitive decline and brain aging [1,2]. Although age-related brain atrophy is an unavoidable process, type 2 diabetes, inflammation, hypertension, high cholesterol, and accumulation of β amyloid and tau markers have all been found to be associated with accelerated brain atrophy and cognitive impairment [3–8]. People with type 2 diabetes are characterized by greater structural brain abnormalities [9], such as atrophy [10–13], particularly in the hippocampus [14,15]. A longer duration of type 2 diabetes is also a significant risk factor for brain atrophy regions [16], such as ventricular enlargement [17]. Hippocampal atrophy is a morphologic feature of a mild cognitive impairment (MCI) and Alzheimer's disease (AD) [1]. The hippocampal occupancy (HOC) score, which measures the degree of hippocampal atrophy in relation to inferior lateral-ventricle expansion, is considered an effective tool for assessing the undesirable progression from MCI to AD [18]. Recently, as part of the 18-mo DIRECT PLUS trial (a dietary intervention randomized controlled trial study), we explored the effects of a caloric-restricted Mediterranean (MED) diet, and of a further enriched high-polyphenol green-MED diet, on age-related brain atrophy [19], using MRI. In participants aged ≥50 y, both the MED and the green-MED diet groups experienced a 50% attenuation of HOC decline compared with the healthy dietary guidelines (HDG) control group. We also found that successful weight loss following the lifestyle intervention might benefit the trajectory of brain aging, based on MRI-assessed resting-state functional connectivity [20].

Based on these findings, this study aims to explore specific diet-induced parameters that may contribute to brain-volume deviation compared with chronologic age – using the HOC score (i.e., the ratio of the hippocampal volume and the inferior lateral-ventricle volume) as a neurodegeneration marker and a proxy for brain age. We hypothesized that improved glycemic control contributes to the neuroprotective effects of diet on brain age and may play a key role in promoting a younger brain age.

Methods

Study design

The 18-mo DIRECT PLUS trial included 294 participants and was conducted in an isolated workplace (Nuclear Research Center Negev).

The inclusion criteria were age ≥30 y with abdominal obesity [waist circumference (WC): male >102 cm; female >88 cm) or dyslipidemia (triglycerides>150 mg/dL; HDL cholesterol ≤40 mg/dL for male; ≤50 mg/dL for female] (exclusion criteria are detailed in Supplemental Methods 1). The study was approved by the Institutional Review Board at the Soroka University Medical Center. The participants provided their written informed consent and did not receive any compensation for participating in the study.

Randomization and interventions

At a 1:1:1 ratio, the participants were randomly assigned to 1 of the following 3 intervention groups: *1*) HDG, an active control group, *2*) a traditional calorie-restricted MED diet, low in simple carbohydrates, or *3*) the green-MED diet. We conducted the randomization in a single phase, with a parallel assignment intervention model, and participants were aware of their assigned intervention (open-label protocol). The first participant was enrolled on 28 January, 2017, and the last participant was enrolled on 30 April 2017. The trial was initiated and conducted in a single phase between May 2017 and November 2018 (for randomization rules, refer to Supplemental Methods 2).

Each intervention group received distinctive nutritional guidance in addition to the physical activity (PA) instruction. For the HDG, the participants received basic health-promoting guidelines for maintaining a healthy diet. For the MED diet, the participants received guidelines for maintaining a calorie-restricted traditional MED diet, low in simple carbohydrates, as described in our previous articles [21,22]. The MED diet was rich in vegetables, with beef and lamb being replaced by poultry and fish.

Both MED diets included 28 g of walnuts per day. In addition to 28 g walnuts/d provided, the green-MED was lower in processed and red meat than the MED diet, and richer in plants and polyphenols – consumed via 3–4 cups/d of green tea and 500 mL of a Mankai-based (cultivated duckweed product) [23–25], green shake at dinner. Both MED diets were equally calorie-restricted (1500–1800 kcal/d for males and 1200–1400 kcal/d for females).

All participants received a free gym membership and PA guidelines; additional lifestyle interventions included periodical 90-min nutritional and PA sessions in the workplace, provided by a multidisciplinary team of physicians, clinical dietitians, and fitness instructors (lifestyle sessions are detailed in Supplemental Methods 3).

Clinical measurement outcomes

Clinical and anthropometric biomarkers were measured at the baseline and 18 mo later. Height was measured to the nearest millimeter using a standard wall-mounted stadiometer. Body weight was measured without shoes and rounded to the nearest 0.1 kg. WC was measured halfway between the lowest rib and the iliac crest, to the nearest millimeter, using standard procedures and an anthropometric measuring tape. Two blood pressure (BP) measurements and heart rate measurements were recorded after resting using an automatic BP monitor. BP was calculated as the mean of the 2 measurements. Blood samples were taken at 8 am, following a 12-h fast (further laboratory methods are detailed in Supplemental Methods 4).

MRI and image analysis outcomes

Brain MRI was assessed at the baseline ($n = 284$ participants) and then again, 18 mo later ($n = 224$ participants) using a 3.0 T magnetic resonance scanner (Philips Ingenia). Retention rates during the DIRECT PLUS trial were 98% at 6 mo into the intervention and 90% at

the end of the 18-mo intervention; eligible brain MRIs at the 18-mo timepoint were achieved for 224 participants (79%). Reasons for dropout were limited to a lack of motivation and medical issues not related to the study. The attrition rate was similar across the intervention groups (HDG: 16%, MED: 25%, green-MED: 23%; $P = 0.24$ between groups).

Brain MRI-derived data were quantified and segmented in a fully automated manner, using the NeuroQuant (FDA-approved software), to yield hippocampal and lateral-ventricle volume measurements. Our a priori primary assessment addressed differences in changes to brain volumes: the HOC score, calculated as the average between hippocampal volume to [hippocampal volume + inferior lateral-ventricle volume] for each hemisphere separately [18].

Statistical analysis

Our primary endpoint was a change in the HOC (refer to Supplemental Methods 5 for the sample size calculations). Continuous variables are presented as mean ± SD and *n* (%) for categorical variables. To determine normal distribution, the dependent variables were analyzed using the Shapiro–Wilk test and histogram interrogation. Baseline characteristics of the study population were analyzed across sex-specific tertiles of the HOC deviation, measured by the residuals (deviation of HOC volume) from the predicted chronologic age values [19] (for more details, refer to Supplemental Methods 6). The Kendall τ correlation was used to examine the *P* trend in variable changes across groups. Associations between HOC deviation and baseline characteristics were explored via partial linear correlations, adjusted for age. Multivariate linear regression models were used to identify changes to metabolic markers associated with the 18-mo HOC-deviation changes. Three stepwise models were also performed: model 1, crude; model 2, model 1 + age (years) and sex (male/female); model 3, model 2 + weight change (kg) and intervention group (HDG/MED/green-MED). A χ^2 test is used in statistics to determine if there is a significant association between 2 categorical variables. Changes in outcomes were assessed using ANOVA tests, adjusted to the values of the parameter of interest. HOC change and HOC deviation change across 3 diabetes status change groups are adjusted for age, sex, weight change, and lifestyle intervention. The 18-mo HOC deviation changes across 2 levels "green" dietary components consumption comparison is adjusted for age, sex, and weight change. Finally, mediation analyses

TABLE 1
Baseline characteristics of the DIRECT PLUS trial participants across sex-specific tertiles of brain aging: MRI-assessed HOC deviation from expected for age.

	Lower tertile: HOC lower than expected for age (older)	Median tertile: HOC expected for age	Higher tertile: HOC higher than expected for age (younger)	Entire (*n* = 284)	Correlation with HOC deviation[1] *r*; 95% CI
Mean HOC deviation male (*n* = 251)	–0.04 ± 0.03	0.00 ± 00	0.04 ± 0.04	–0.002 ± 0.39	
Mean HOC deviation female (*n* = 33)	–0.01 ± 0.01	0.00 ± 00	0.04 ± 0.001	0.0134 ± 0.22	
Age (y)	50.48 ± 10.85	50.25 ± 9.77	52.41 ± 11.31	51.1 ± 10.5	
Weight (kg)	96.48 ± 14.86	93.73 ± 14.00	90.65 ± 13.96	93.63 ± 14.4	–0.204; –0.298, –0.101
BMI (kg/m^2)	32.37 ± 6.34	31.03 ± 3.82	30.31 ± 3.39	31.2 ± 3.9	–0.229; –0.337, –0.118
WC (cm)	111.61 ± 10.43	109.44 ± 8.59	108.01 ± 9.27	109.7 ± 9.5	–0.207; –0.310, –0.103
DBP (mm Hg)	82.63 ± 11.47	81.43 ± 9.46	79.34 ± 9.83	81.14 ± 10.34	–0.186; –0.304, –0.072
SBP (mm Hg)	131.90 ± 14.74	130.03 ± 13.86	129.33 ± 13.61	130.4 ± 14.07	–0.189; –0.308, –0.061
Glucose (mg/dL)	101.82 ± 17.69	101.77 ± 18.59	102.61 ± 15.69	102.0 ± 17.32	–0.028; –0.136, 0.102
Insulin (μU/mL)	15.24 ± 7.65	14.82 ± 8.86	13.68 ± 6.46	14.58 ± 7.7	–0.099; –0.194, –0.004
HOMA-IR	3.85 ± 2.11	3.81 ± 2.72	3.57 ± 1.97	3.74 ± 2.29	–0.064; –0.166, 0.040
HbA1c, %	5.55 ± 0.82	5.48 ± 0.57	5.44 ± 0.53	5.48 ± 0.64	–0.164;
HbA1c (mmol/mol)	37.2	36.4	36	36.4	–0.337, –0.006
HDL-c (mg/dL)	45.37 ± 10.44	46.16 ± 11.94	46.59 ± 12.67	46.04 ± 11.69	0.070; –0.072, 0.193
LDL-c (mg/dL)	122.27 ± 31.79	129.00 ± 29.79	125.89 ± 31.76	125.7 ± 31.1	0.040; –0.074, 0.144
CRP	3.22 ± 2.17	3.07 ± 1.85	2.88 ± 2.15	3.05 ± 2.06	–0.021; –0.142, 0.096
TG[2]	4.86 ± 0.36	4.9 ± 0.42	4.92 ± 0.5	4.90 ± 0.43	0.060; –0.055, 0.167
APOE-ε4 allele, %	15.1	12.6	19.6	15.71	0.039[3]: –0.039, 0.116

Mean ± SD for continuous variables and *n* (%) for categorical variables. ApoE-ε4 was considered positive if there was 1 APOE-ε4 allele.
Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HOC, hippocampal occupancy score; SBP, systolic blood pressure; TG, triglycerides; WC, waist circumference.
[1] Partial correlation between baseline HOC deviation and other parameters adjusted for age and 95% CIs are presented.
[2] Ln transformed values (normally distributed) were used.
[3] Kendall's tau-b test between APOE-ε4 allele groups and HOC deviation. Sex-specific tertiles: 1: male: ≤–0.136 cm^3; female: ≤0.007 cm^3; 2: male: –0.136 cm^3 to 0.018 cm^3; female: 0.007 cm^3 to 0.024 cm^3; 3: male: >0.018 cm^3; female: >0.024 cm^3.

were performed to assess whether glycemic biomarkers mediated the relationship between the levels of "green" dietary components and the 18-mo changes in HOC deviations. Significance was set at $P < 0.05$. Statistical analyses were performed using SPSS software, version 28.0 (IBM), and R, Version 4.2.0 (R Foundation for Statistical Computing).

Results

Table 1 presents the baseline characteristics of the DIRECT PLUS trial participants across sex-specific tertiles of brain aging ($n = 284$). At the baseline, the participants' mean measures were as follows: weight = 94 kg, BMI = 31.2 kg/m^2, WC = 110 cm, BP = 130/81 mmHg, insulin concentrations = 15 mg/dL, and hemoglobin A1c (HbA1c) = 5.5%. Higher than expected HOC deviations by chronologic age (i.e., younger brain than anticipated for the given age) were significantly associated with lower body weight [$r = -0.204$; 95% confidence interval (CI): −0.298, −0.101], BMI ($r = -0.229$; 95% CI: −0.337, −0.118), WC ($r = -0.207$; 95% CI: −0.310, −0.103), systolic BP ($r = -0.189$; 95% CI: −0.308, −0.061), diastolic BP ($r = -0.186$; 95% CI: −0.304, −0.072), insulin ($r = -0.099$; 95% CI: −0.194, −0.004, and HbA1c ($r = -0.164$; 95% CI: –0.337, –0.006) – age-adjusted for all. The means of significant baseline characteristics for each tertile with the absolute HOC score scale distribution between HOC deviation tertiles and the correlation between the HOC deviation and the baseline characteristics is presented in Supplemental Figure 1 (for more details on obesity status and HOC deviation, refer to Supplemental Figure 2).

HOC deviation: dynamics during the intervention

Table 2 presents the 18-mo changes in HOC deviations from the expected scores by chronologic age, based on changes in anthropometric measures and biomarkers. In the multiple linear regression models (adjusted for age, sex, weight change, and intervention group), greater HOC 18-mo change deviations (i.e., less aging than expected for the given intervention time period) were significantly associated with reduced fasting glucose ($\beta = -0.155$; 95% CI: −0.293, −0.016), HbA1c ($\beta = -0.254$; 95% CI: −0.392, −0.117), HOMA-IR ($\beta = -0.200$; 95% CI: −0.346, −0.055), and C-reactive protein ($\beta = -0.153$; 95% CI: −0.296, −0.010). In addition, we also found that glycemic control improvement, adjusted for age, sex, intervention groups, and weight change, was associated with greater changes in other brain region deviations following the interventions. A reduction in HbA1c was significantly associated with greater changes in the thalamus, caudate nucleus, and cerebellum with $\beta = -0.145$; 95% CI: −0.285, –0.006; $\beta = -0.176$; 95% CI: –0.314, –0.039; and $\beta = -0.159$; 95% CI: –0.3, –0.018, respectively. For more details on the improvement in glycemic control parameters and other brain regions, refer to Supplemental Table 1. Favorable changes to diabetes status [26,27] during the intervention were directly associated with HOC deviation changes (adjusted for sex, weight change, and intervention group, $P = 0.009$ between groups; Figure 1A). Such favorable diabetes status changes were defined as a decrease from HbA1c (mmol/mol) ≥38.8 (5.7%) prediabetes/diabetes to HbA1c (mmol/mol) <38.8 (5.7%) normal levels status.

Participants who exhibited a favorable change in their diabetes status also presented greater mean HOC deviation changes compared with their counterparts with either no change to their diabetes status (0.010; 95% CI: 0.002, 0.019) or with an unfavorable change (from normal to prediabetes/diabetes: 0.012; 95% CI: 0.002, 0.023). Significance values were corrected for multiple comparisons using Bonferroni's method. Similarly, this favorable diabetes status change was also associated with greater HOC changes (between groups, $P = 0.005$; Figure 1B). A favorable change in diabetes status was associated with greater mean HOC changes compared with their counterparts with either no change in diabetes status (1.248; 95% CI: 0.272, 2.224) or with an unfavorable change (from normal to prediabetes/diabetes, 1.541; 95% CI: 0.334, 2.748). Significance values were corrected for multiple comparisons using Bonferroni's method. Participants' glycemic parameter responses in the lower tertile (insulin mean ± SD = –7.85 ± 4.78, glucose = –11.51 ± 12.82, and HOMA-IR = –2.26 ± 1.78) were directly associated with greater HOC deviation changes (means of 0.003, 0.002, and 0.002 compared with –0.002, –0.002, and

TABLE 2
Multivariate models for changes in HOC deviation with changes in anthropometric parameters and biomarkers.

18 mo changes	Entire group, $n = 224$					
	Model 1 – crude		Model 2 – adjusted for age, sex		Model 3 – adjusted for age, sex, weight change, and intervention groups	
	β	95% CI	β	95% CI	β	95% CI
ΔWeight	–0.021	–0.153, 0.112	–0.028	–0.164, 0.109	— [1]	— [1]
ΔWC	0.006	–0.126, 0.139	0.000	–0.136, 0.136		
ΔDBP	–0.096	–0.229, 0.037	–0.095	–0.229, 0.039	–0.097	–0.237, 0.043
ΔSBP	–0.093	–0.226, 0.04	–0.089	–0.223, 0.044	–0.085	–0.221, 0.052
ΔGlucose	–0.126	–0.259, 0.007	–0.146	–0.282, –0.010	–0.155	–0.293, –0.016
ΔHbA1c	–0.225	–0.355, –0.095	–0.235	–0.366, –0.105	–0.254	–0.392, –0.117
ΔInsulin	–0.101	–0.234, 0.031	–0.109	–0.244, 0.025	–0.127	–0.274, 0.021
ΔHOMA–IR	–0.158	–0.290, –0.026	–0.173	–0.308, –0.038	–0.200	–0.346, –0.055
ΔCRP	–0.147	–0.287, –0.007	–0.151	–0.291, –0.010	–0.153	–0.296, –0.010
ΔHDL-c	–0.066	–0.199, 0.067	–0.073	–0.211, 0.066	–0.091	–0.247, 0.065
ΔLDL-c	–0.085	–0.217, 0.048	–0.083	–0.215, 0.050	–0.076	–0.210, 0.059
ΔTG	–0.014	–0.147, 0.119	–0.024	–0.16, 0.112	–0.018	–0.173, 0.137

Multivariable linear regressions were conducted to assess the association between HOC deviation change and 18-mo parameters change.
Abbreviations: CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HOC, hippocampal occupancy score; TG, triglyceride; SBP, systolic blood pressure; WC, waist circumference.
[1] Cannot be tested in a multivariate model due to collinearity of weight with WC. Model 1: crude analysis; Model 2: adjusted for age and sex; and Model 3: adjusted for age and sex, weight change and intervention group. Coefficients (β) and 95% CIs are reported for each model.

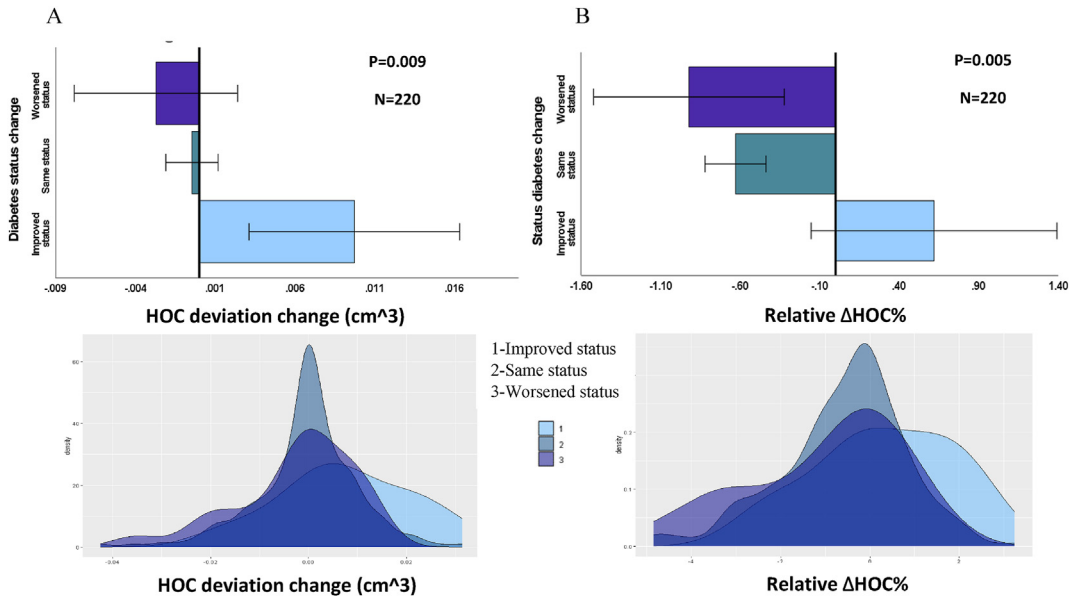

FIGURE 1. (A) HOC deviation change across diabetes status change following 18 mo of intervention. (B) HOC change across diabetes status change following 18 mo of intervention. MRI-assessed hippocampal occupancy score (HOC) 18-mo change deviation from expected for chronologic age (A), and MRI-assessed HOC 18-mo related change (B) with 18-mo diabetes status change during the intervention. Three groups based on diabetes status change: 1. Improved status; 2. Same status; 3. Worsened status (healthy participant-HbA1c (mmol/mol) <38.8 (5.7%), prediabetes and diabetes participants-HbA1c (mmol/mol) ≥38.8 (5.7%). (A) Mean differences between groups: Group 1 compared with group 2: 0.010 (95% CI: 0.002, 0.019), group 1 compared with group 3: 0.012 (95% CI: 0.002, 0.023), group 2 compared with group 3: 0.002 (95% CI: –0.004, 0.009). (B) Mean differences between groups: Group 1 compared with group 2: 1.248 (95% CI: 0.272, 2.224), group 1 compared with group 3: 1.541 (95% CI: 0.334, 2.748), group 2 compared with group 3: 0.293 (95% CI: –0.480, 1.066). Adjusted for age, sex, weight change, and lifestyle intervention, after Bonferroni correction. Density plots represent the HOC/HOC deviation distribution according to the 3 diabetes status groups.

–0.002 for insulin, glucose, and HOMA-IR, respectively) – adjusted for age, sex, weight change, and intervention group ($P < 0.05$ for all) (for further details, refer to Supplemental Figure 3).

We also examined the improvement in glycemic status following the study, which refers to a transition from HbA1c (mmol/mol) ≥ 38.8 (5.7%) – indicating prediabetes or diabetes, to HbA1c (mmol/mol) < 38.8 (5.7%) – indicating normal glycemic concentrations. The greatest glycemic improvement was seen among the MED diet groups, especially the green-MED diet one, where 58.33% of the participants showed such an improvement, compared with 31.62% and 28.57% of the participants on the MED diet and HDG, respectively ($P = 0.04$; 95% CI: 0.037, 0.044 between the green-MED and the HDG group; $P = 0.03$; 95% CI: 0.029, 0.036 between the 2 MED diet groups and the HDG group).

Finally, participants from the green-MED diet group tended to exhibit greater HOC deviation changes (that is, attenuation of brain-age decline) compared with those in the MED and HDG groups (mean = 0.002 compared with mean = –0.001; $P = 0.082$, 0.003, 95% CI: −0.0004, 0.006). Higher consumption of Mankai and green tea, the specific green-MED dietary components (Figure 2), was directly linked

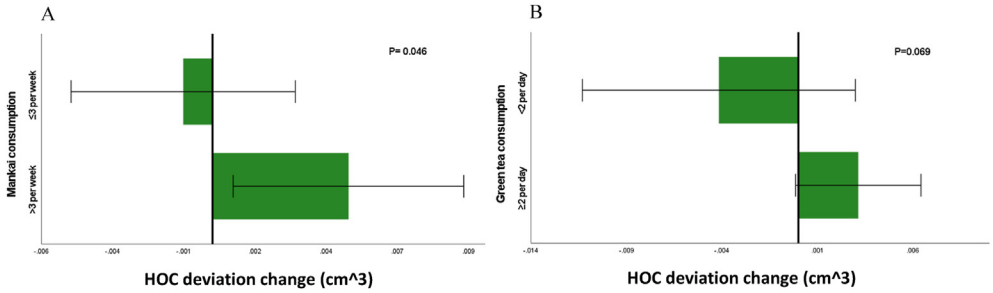

FIGURE 2. 18-mo HOC deviation changes based on the specific *Mankai* plant and *green tea*; "green" dietary components. (A,B) HOC deviation change according to specific "green" dietary components. (A) Weekly Mankai consumption: low was defined as ≤3/wk ($n = 37$), and high as >3/wk ($n = 35$). (B) Daily green tea consumption: low was defined as >2/d ($n = 12$), and high as ≥2/d ($n = 56$). Mean differences between groups: (A) low compared with high Mankai consumption: –0.006 (95% CI: –0.0115, –0.0001). (B) Low compared with high daily green tea consumption: –0.007 (95% CI: –0.0152, 0.0006). Adjusted for age, sex, and weight change. HOC, hippocampal occupancy score.

to greater HOC deviation changes adjusted for age, sex, and weight change. Specifically, consumption of the Mankai shake >3 times/wk was found to be associated with greater mean HOC deviation changes compared with lower consumption (0.006; $P = 0.046$; 95% CI: 0.0001, 0.0115). Participants who consumed ≥2 cups/d green tea tended to exhibit greater HOC deviation changes (0.007; $P = 0.069$; 95% CI: −0.001, 0.015).

In another set of mediation analyses, when the treatment is the intake levels of Mankai, adjusted for age, sex, and weight change, we found significant differences in the average direct effect for HOMA-IR and insulin ($P = 0.026$ and 0.032, respectively), with a tendency for HbA1c and glucose ($P = 0.08$ and 0.056, respectively). There was also a tendency in the total effect for glucose, insulin, and HOMA-IR ($P =$ 0.06, 0.056, and 0.076, respectively). No significant effects were found for the green tea treatment in this model (for further details of the mediation analyses, refer to Supplemental Result 1).

Discussion

In this ancillary study of the 18-mo DIRECT PLUS brain-MRI trial, which included 284 participants with abdominal obesity or dyslipidemia, we found that improved glycemic control following weight loss interventions may have an independent beneficial effect on MRI-assessed brain age. Following the 18-mo lifestyle intervention, we observed a significant greater change in HOC residuals (that is, attenuation of brain-age decline), mainly in participants with improved glycemic control markers. Younger brain age was driven by greater consumption of high polyphenols: green tea and Mankai. Our findings suggest a potential mechanistic pathway for driving the favorable impact of high-polyphenol diets. Moreover, the consumption of polyphenol-rich foods, such as green tea and Mankai, may enrich the blood–brain barrier (BBB), reduce BP, and attenuate age-related brain atrophy [28] (for further details, refer to Supplemental Discussion 1).

We also found that lower weight, BP, WC, insulin, and HbA1c at the baseline were linked to younger HOC than expected for the given chronologic age. The hippocampus plays a major role in learning and memory [29], and accumulating evidence suggests that age-related hippocampal atrophy may serve as an early biomarker for cognitive decline. At the same time, we previously showed that ventricular volume increases with age [19]. Hence, the HOC score, as the ratio of the hippocampal volume to the sum of the hippocampal volume and the ventricular volume, serves as a sensitive predictive measurement of cognitive decline and the progression from MCI to AD [30]. Disturbed metabolic parameters (such as BMI, cholesterol, and glycemic parameters) are known to be correlated with accelerated brain atrophy and cognitive decline [31,32]. This atrophy acceleration of brain aging can be assessed by the brain-age gap [33], which is calculated as the difference between the anatomical brain age and the chronologic age.

In this study, we observed a correlation between baseline HOC, age, and BMI, which is in line with a previous study in which high BMI was found to serve as a biomarker of older brain age [34]. However, it should be noted that in contrast to the observed associations between the BMI and HOC residuals at the baseline, the participants' weight changes did not significantly contribute to the prediction of HOC residual changes. This suggests that such changes may take longer to manifest, compared with changes in glycemic concentrations. Supporting this hypothesis, we recently reported that successful weight loss following a lifestyle intervention might benefit the trajectory of brain aging, as assessed by MRI-assessed resting-state functional connectivity [20]. However, further studies are needed to fully explore this effect.

The fact that greater changes to HOC residuals were observed in participants with improved glycemic control and inflammation markers beyond weight loss supports our hypothesis that reduction in glycemic biomarkers has an independent effect on the neuroprotective benefits of diet and may play a major role in attenuating neurodegeneration. Few studies have examined the relationship between biomarker changes and brain atrophy following a lifestyle intervention [35]. Our results indicate that improvements in simple-to-measure biomarkers, through lifestyle interventions, improve brain aging. Similarly, we demonstrated that lifestyle-induced improvements in diabetes status (prediabetes to normal status, assessed by routine clinical measurements) were directly associated with greater HOC deviation changes. Moreover, improved (that is, reduced) concentrations of the C-reactive protein inflammatory marker, associated with diabetes [36], were also found to be linked to HOC improvements.

In a previous study, we reported that Mankai (as a study-case green plant) could ameliorate the occurrence of postprandial glucose spikes [37], possibly explaining the apparent beneficial effect of Mankai on HOC. Several processes have been suggested regarding the effect of glucose metabolism disruption in the brain, such as tau protein degradation, neuroinflammatory responses, and amyloidogenesis [4]. Impaired glucose metabolism is known to increase oxidative stress, resulting in an accumulation of amyloid β-protein and brain neurodegeneration. Furthermore, high brain insulin concentrations may increase amyloid β-protein secretion and inhibit its degradation, by competing for insulin-degrading enzymes. Given the selective distribution of insulin receptors in the hippocampus, insulin resistance, and hyperinsulinemia may particularly contribute to atrophy in these areas [13].

In addition, there is evidence that subjects with a midlife glycemic dysfunction exhibit higher hippocampal atrophy than those with late-life glycemic dysfunction [13,16]. It also has been shown regarding the cognitive aspect that patients with AD or MCI with type 2 diabetes had similar amyloid β deposition but were associated with greater cerebrospinal fluid total tau and phosphorylated tau concentrations than those without type 2 diabetes [38]. In addition, in human postmortem brain tissue, insulin mRNA transcripts have been identified, especially in the hippocampus and hypothalamus, with low concentrations in AD individuals. Insulin receptors are expressed on all cell types in the brain, with the highest density in several regions, such as the hippocampus [39]. The variance of insulin receptor distribution in the brain can indicate that insulin signaling has essential and diverse roles in the brain.

In this analysis, beyond weight change, higher consumption of green-MED components such as Mankai and green tea was directly associated with greater HOC deviation changes; participants from the green-MED diet group also tended to exhibit greater HOC deviation changes compared with the MED and HDG diet groups. A previous clinical trial provides evidence that a MED diet rich in polyphenols may attenuate age-related cognitive decline [40]. The potential underlying mechanism of such a favorable association between MED diets and age-related neurodegeneration could be partially attributed to the high content of polyphenols that are present in plant-based food sources [41]. The Mankai plant includes >200 polyphenols and phenolic metabolites [24], and has high phenolic and antioxidant content, with a high concentration of the flavonoid-class polyphenols luteolin and apigenin derivatives. Green tea contains catechins, specifically epigallocatechin, epicatechin gallate, and epigallocatechin gallate polyphenols [42]. Consuming green tea, as either a beverage or an extract, has numerous reported

health benefits, including improvements in cardiometabolic health [43], weight management [42], and cognitive function [44]. Polyphenols are known to be able to cross the BBB, reduce neuroinflammation, and induce cell proliferation and adult-onset neurogenesis in the hippocampus [45]. However, limited intervention-trial data exists regarding the potential for reducing age-related atrophy in response to polyphenol consumption [46].

Limitations and future research

Despite the important contribution of this study to the literature, a number of research limitations should be addressed. First, although HOC and other structural changes are predictors of cognitive impairments [47], this study lacks data about the participants' educational or cognitive status. Moreover, the high proportion of male participants in this trial (88%) may limit the generalizability of our findings to females. In addition, the change in diabetes status was assessed based on HbA1c values following the intervention, although it could also be determined using other biomarkers, such as glucose concentrations. At the same time, the HbA1c test is the primary tool for assessing glycemic status in both clinical practice and clinical trials, and it is strongly linked to diabetes complications [48]. Also, we cannot attribute the effect solely to polyphenols; it is possible that the lower consumption of red meat also had an impact. However, under the same reduced consumption of red meat, a high intake of green components was associated with changes in HOC deviation throughout the intervention. Finally, HOC is only our primary outcome and is used as a proxy for brain age, but additional brain regions were evaluated in this analysis. We found that changes in glycemic biomarkers following the intervention play a major role in other brain regions as well (for further details regarding the improvement in glycemic control parameters and other brain regions, refer to Supplemental Table 1). Future studies could benefit from assessing additional brain regions and expanding the population groups in the study. The study's strengths include MRI brain scans, considered the state of the art in imaging techniques, large sample size, and the use of accurate imaging techniques with validated brain-volumetric methods. In addition, this trial is characterized by its long duration and high adherence. Another strength of the study is the recruitment of participants from a closed workplace environment, thereby providing a unique opportunity for closely monitoring the participants' dietary intake. The workplace also offered on-site access to a medical clinic, ongoing dietary guidance, and regular group meetings with a multidisciplinary team. Finally, the participants in the green-MED diet group were provided with polyphenol-rich food sources, free of charge.

In conclusion, the secondary analysis of the DIRECT PLUS trial presented in this study suggests that improved glycemic control contributes to the neuroprotective benefit of MED and green-MED diets on brain age. The study also indicates that polyphenols-rich diet components such as Mankai and green tea may contribute to a more youthful brain age. If confirmed by additional studies, this finding may indicate an accessible, low-risk, and practical approach to attenuating age-related neurodegeneration, which could hold potential clinical significance for future applications in cognitive health.

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Author contributions

The authors' responsibilities were as follows – DP, AK, GT, I Shai: designed the research, wrote the paper, and had primary responsibility for the final content; AK, GT, HZ, AYM, ER, GL: conducted the research; DP, AK, SH, FB, PK, MvB, UC: analyzed the data; MS, YY, VW, MB, MS, FBH, MJS, AF, I Shelef, GA: reviewed and edited the manuscript; and all authors: read and approved the final manuscript.

Conflict of interest

MB received honoraria for lectures and consultancy from Amgen, Astra Zeneca, Bayer, Boehringer-Ingelheim, Lilly, Novartis, Novo Nordisk, and Sanofi. All other authors report no conflicts of interest.

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Data availability

All data described in the article, code book, and analytic code will be made available upon request, pending the approval of the principal investigator (irish@bgu.ac.il).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.09.013.

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