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Glycemic Control Contributes to the Neuroprotective Effects of Mediterranean and Green-Mediterranean Diets on Brain Age; The DIRECT PLUS Brain-MRI 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 months.

Objective: To explore the contribution of specific diet-induced parameters to brain volume deviation from chronological age.

Methods: A post-hoc analysis of the 18-month DIRECT-PLUS trial, where participants were randomly assigned to: (1)-healthy-dietary-guidelines (HDG); (2)-MED diet; or (3)-green-MED diet, high in polyphenols and low in red meat. Both MED groups consumed 28g walnuts/day (+440mg/day polyphenols). The green-MED group further consumed green-tea (3–4 cups/day) and Mankai green shake (Wolffia-globosa aquatic plant) (+800mg/day 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=51.1years; BMI=31.2kg/m^2; 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%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]), and 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 months, 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 s-CRP (β=-0.153;95%[CI -0.296,-0.010]). Improvement in diabetes status was associated with greater HOC-deviation changes compared to 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 is 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.

Abbreviations: AD, Alzheimer disease; ADE, Average direct effect; BBB, Blood-brain barrier; BP, Blood pressure; CRP, C-reactive protein; HOMA-IR, Homeostatic model assessment of insulin resistance; ECG, Epicatechin gallate; EGC, Epigallocatechin; EGCG, Epigallocatechin gallate; green-MED diet, Mediterranean diet higher in polyphenols and lower in red/processed meat; HbA1c, Hemoglobin A1c; HDG, Healthy dietary guidelines; HDL-c, High-density lipoprotein cholesterol; HOC, Hippocampal occupancy score; LDL-c, Low-density lipoprotein cholesterol; MCI, Mild cognitive impairment; MED, Mediterranean diet; PA, Physical activity; RSFC, Resting-state functional connectivity; TG, Triglyceride; WC, Waist circumference.

48 **Keywords:** aging, brain age, dietary intervention, glycemic control, green-

49 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). While 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 found to be associated with accelerated brain atrophy and cognitive impairment (3-8). People with type 2 diabetes have been found to be 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 morphological feature of a mild cognitive impairment (MCI) and of 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 as an effective tool for assessing the undesirable progression from MCI to AD (18). Recently, as part of the 18-month 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 magnetic resonance imaging (MRI). In participants aged ≥50 years, both the MED and the green-MED diet groups experienced a 50% attenuation of HOC decline compared to the healthy dietary guidelines (HDG) control group. We also found that successful weight loss following the lifestyle intervention might have a beneficial effect on the trajectory of brain aging, based on MRI-assessed resting-state functional connectivity (RSFC) (20).

Based on these findings, the current study aims to explore specific diet-induced parameters that may contribute to brain-volume deviation compared to chronological age – using the hippocampal occupancy (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-month DIRECT PLUS trial included 294 participants and was conducted in an isolated workplace (Nuclear Research Center Negev, Dimona, Israel).

The inclusion criteria were age ≥30 years with abdominal obesity (waist circumference (WC): men>102 cm, women >88 cm) or dyslipidemia (triglycerides>150 mg/dL; HDL cholesterol≤40mg/dL for men, ≤50mg/dL for women). (Exclusion criteria are detailed in Supplementary 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 one of the following three 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 January 28, 2017, and the last participant was enrolled on April 30, 2017. The trial was initiated and conducted in a single phase between May 2017 and November 2018. (For randomization rules, please see Supplementary 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 papers (21, 22). The MED diet was rich in vegetables, with beef and lamb being replaced by poultry and fish.

Both MED diets included 28gr walnuts per day. In addition to 28 gr walnuts/day 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/day of green tea and 500ml of a Mankai-based (cultivated duckweed product) (23-25), green shake at dinner. Both MED diets were equally calorie-restricted (1500–1800 kcal/day for males and 1200–1400 kcal/day 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 Supplementary Methods 3.)

Clinical measurement outcomes

Clinical and anthropometric biomarkers were measured at the baseline and 18 months 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 0.1kg. WC was measured halfway between the lowest rib and the iliac crest, to the nearest millimeter, using standard procedures and an anthropo-metric 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 two measurements. Blood samples were taken at 8 am, following a 12-hour fast. (Further laboratory methods are detailed in Supplementary Methods 4.)

MRI and image analysis outcomes

Brain MRI was assessed at the baseline (n=284 participants) and then again, 18 months later (n=224 participants) using a 3.0T magnetic resonance scanner (Philips Ingenia, Best, The Netherlands). Retention rates during the DIRECT PLUS trial were 98% at six months into the intervention and 90% at the end of the 18-month intervention; eligible brain MRIs at the 18-month 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 hippocampal occupancy score (HOC), 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. (Supplementary Methods 5 details 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 chronological age values (19) (For more details, please see Supplementary 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-month 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 chi-square test is used in statistics to determine if there is a significant association between two 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 three diabetes status change groups are adjusted for age, sex, weight change, and lifestyle intervention. The 18-month HOC deviation changes across two levels 'green' dietary components consumption comparison is adjusted for age, sex, and weight change. Finally, mediation analyses were performed to assess whether glycemic biomarkers mediated the relationship between the levels of 'green' dietary components and the 18-month changes in HOC deviations. Significance was set at $p<0.05$. Statistical analyses were performed using

SPSS software, version 28.0 (IBM, Armonk, NY, USA), and using R, Version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

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=94kg, BMI=31.2kg/m^2, WC=110cm, BP=130/81 mmHg, insulin levels=15mg/dL, and hemoglobin A1c (HbA1c)=5.5%. Higher than expected HOC deviations by chronological age (i.e., younger brain than anticipated for the given age) were significantly associated with lower body weight (r=-0.204, 95% 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 Supplementary Figure 1. For more details on obesity status and HOC deviation, please refer to Supplementary Figure 2.

HOC deviation: dynamics during the intervention

Table 2 presents the 18-month changes in HOC deviations from the expected scores by chronological age, based on changes in anthropometric measures and in biomarkers. In the multiple linear regression models (adjusted for age, sex, weight change, and intervention group), greater HOC 18-months change deviations (i.e., less aging than expected for the given intervention time period) were significantly

associated with reduced fasting glucose (β=-0.155, 95% CI [-0.293, -0.016]), HbA1c (β=-0.254, 95% CI [-0.392, -0.117]), HOMA-IR (β=-0.200, 95% CI [-0.346, -0.055]), and C-reactive protein (CRP) (β=-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 β = -0.145; 95% CI [-0.285, -0.006], β = -0.176; 95% CI [-0.314, -0.039], and β = -0.159; 95% CI [-0.3, -0.018], respectively. For more details on the improvement in glycemic control parameters and other brain regions, please see Supplementary 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%) pre-diabetes/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 to 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 pre-diabetes/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 to 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 pre-diabetes/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 to -0.002, -0.002, and -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, please see Supplementary 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 pre-diabetes or diabetes, to HbA1c (mmol/mol)<38.8 (5.7%) – indicating normal glycemic levels. The greatest glycemic improvement was seen among the MED-diet groups, especially the green-MED diet one, where 58.33% of the participants show such an improvement, compared to 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 two MED-diet groups and the HDG group).

Finally, participants from the green-MED diet group tended to exhibit greater HOC deviation changes (i.e., attenuation of brain-age decline) compared to those in the MED and HDG groups (mean=0.002 compared to 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 to greater HOC deviation changes adjusted for age, sex, and weight change. Specifically, consumption of the Mankai shake more than three times/week was found to be associated with greater mean HOC deviation changes compared to lower consumption (0.006, p=0.046, 95% CI [0.0001, 0.0115]). Participants who consumed

≥two cups/day 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 (ADE) 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, please refer to Supplementary Result 1.)

Discussion

In this ancillary study of the 18-month DIRECT PLUS brain-MRI trial, that 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-month lifestyle intervention, we observed a significant greater change in HOC residuals (i.e., 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, please see Supplementary Discussion 1.)

We also found that lower weight, blood pressure, WC, insulin, and HbA1c at the baseline were linked to younger HOC than expected for the given chronological 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 increase 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, is serves as a sensitive predictive measurement of cognitive decline and of 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 a 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 chronological age.

In this study, we observed a correlation between the baseline HOC, age, and BMI – 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 to changes in glycemic levels. Supporting this hypothesis, we recently reported that successful weight loss following a lifestyle intervention might have a beneficial effect on the trajectory of brain aging, as assessed by MRI-assessed RSFC (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 have 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 (pre-diabetes to normal status, assessed by routine clinical measurements) were directly associated with a greater HOC deviation changes. Moreover, improved (i.e., reduced) levels of the CRP inflammatory marker, which is associated with diabetes (36), was 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 levels 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 AD or MCI patients with type 2 diabetes had similar amyloid β deposition but were associated

with greater CSF total tau and phosphorylated tau concentrations than those without type 2 diabetes (38). In addition, in human post-mortem brain tissue, insulin mRNA transcripts have been identified, especially in the hippocampus and hypothalamus, with low levels 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 the current 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 to the MED and HDG diet groups. A previous clinical trial provides evidence that a MED diet, that is 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 over 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 (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) 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 levels. 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 please see Supplementary Table 1.) Future studies could benefit from assessing additional brain regions and from 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.

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 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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The authors' responsibilities were as follows: DP, AK, GT, and I Shai designed the research, wrote the paper, and had primary responsibility for the final content; AK, GT, HZ, AYM, ER, and GL conducted the research; DP, AK, SH, FB, PK, MvB, and

UC analyzed the data; MS, YY, VW, MB, MS, FBH, MJS, AF, I Shelef, and GA reviewed and edited the manuscript; and finally, all authors read and approved the submitted manuscript.

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).

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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[3]
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, years	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 CI [-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 CI [-0.337, -0.118]
WC, cm	111.61±10.43	109.44 ±8.59	108.01± 9.27	109.7±9.5	-0.207 CI [-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 CI [-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 CI [-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 CI [-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 CI [-0.194, -0.004]
HOMA-IR	3.85 ±2.11	3.81 ±2.72	3.57 ±1.97	3.74±2.29	-0.064 CI [-0.166, 0.040]
HbA1c, % **HbA1c, mmol/mol**	5.55 ±0.82 37.2	5.48 ±0.57 36.4	5.44 ±0.53 36	5.48±0.64 36.4	-0.164 CI [-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 CI [-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 CI [-0.074, 0.144]
CRP	3.22±2.17	3.07±1.85	2.88±2.15	3.05±2.06	-0.021 CI [-0.142, 0.096]
TG[1]	4.86 ±0.36	4.9 ±0.42	4.92 ±0.5	4.90 ±0.43	0.060 CI [-0.055, 0.167]
APOE-ε4 allele, %	15.1	12.6	19.6	15.71	0.039[2] CI [-0.039, 0.116]

Mean ± SD for continuous variables and n (%) for categorical variables. [2]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. BMI = body mass index, WC = Waist circumference, DBP = Diastolic blood pressure, SBP = Systolic blood pressure, HOMA-IR = homeostatic model assessment of insulin

resistance, HbA1c = hemoglobin A1c, HDL-c = high-density lipoprotein cholesterol, LDL-c = low-density lipoprotein cholesterol, TG = Triglycerides. ApoE-ε4 was considered positive if there was 1 APOE-ε4 allele. [1]Ln transformed values (normally distributed) were used. [3]Partial correlation between baseline HOC deviation and other parameters adjusted for age and 95% confidence intervals are presented.

Table 2: Multivariate models for changes in HOC deviation with changes in anthropometric parameters and biomarkers

18 months 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]	-	-
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-month parameters change. * 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% confidence intervals are reported for each model. Abbreviations: WC, Waist circumference; DBP, Diastolic blood pressure; SBP, Systolic blood pressure; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance;

HbA1c, Hemoglobin A1c; CRP, C-Reactive Protein; HDL-c, High-Density Lipoprotein Cholesterol; LDL-c, Low-Density Lipoprotein Cholesterol; TG, Triglyceride.

Figure Captions:

Figure 1:

A. HOC ***Deviation*** Change Across Diabetes Status Change Following 18 Months of Intervention

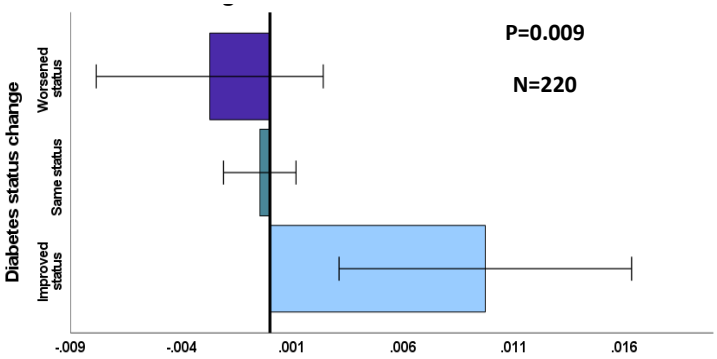
B. HOC Change Across Diabetes Status Change Following 18 Months of Intervention

A+B: MRI-assessed hippocampal occupancy score (HOC) 18-month change deviation from expected for chronological age (A), and MRI-assessed HOC 18-month related change (B) with 18-month 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%), Pre-diabetes and diabetes participants- HbA1c (mmol/mol) ≥ 38.8 (5.7%)). A. Mean differences between groups: Group 1 vs. Group 2: 0.010 [CI: 0.002, 0.019], Group 1 vs. Group 3: 0.012 [CI: 0.002, 0.023], Group 2 vs. Group 3: 0.002 [CI: -0.004, 0.009]. B. Mean differences between groups: Group 1 vs. Group 2: 1.248 [CI: 0.272, 2.224], Group 1 vs. Group 3: 1.541 [CI: 0.334, 2.748], Group 2 vs. Group 3: 0.293 [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 three diabetes status groups.

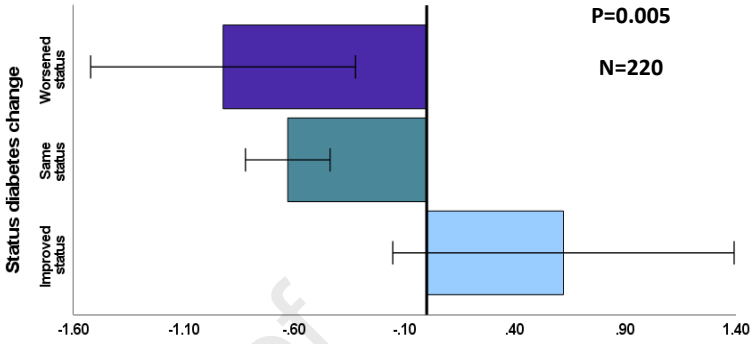
Figure 2: 18-month HOC deviation changes based on the specific *Mankai* Plant and *Green Tea;* 'green' dietary components

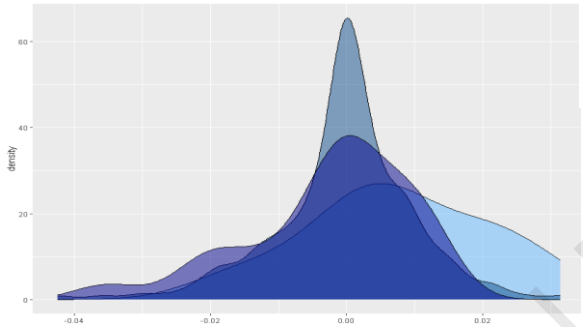
A+B: HOC deviation change according to specific "green" dietary components. (A) Weekly Mankai consumption: low was defined as ≤3/week (n=37), and high as >3/week (n=35). (B) Daily green tea consumption: low was defined as <2/day (n=12), and high as ≥2/day (n=56). Mean differences between groups: A. Low compared to high Mankai consumption: -0.006 [CI: -0.0115, -0.0001]. B. Low compared to high daily Green-Tea consumption: -0.007 [CI: -0.0152, 0.0006]. Adjusted for age, sex, and weight change.

A

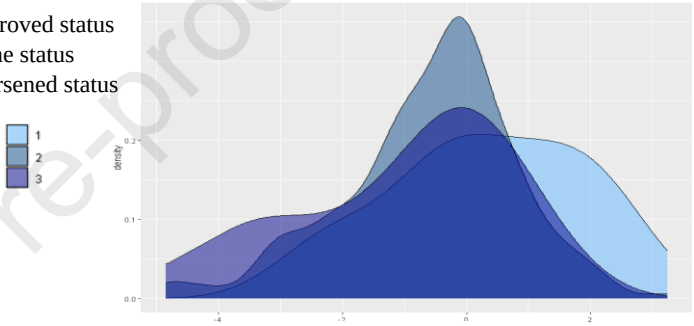


B

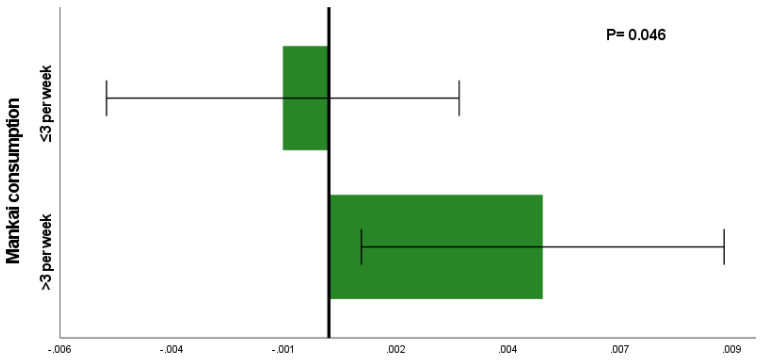




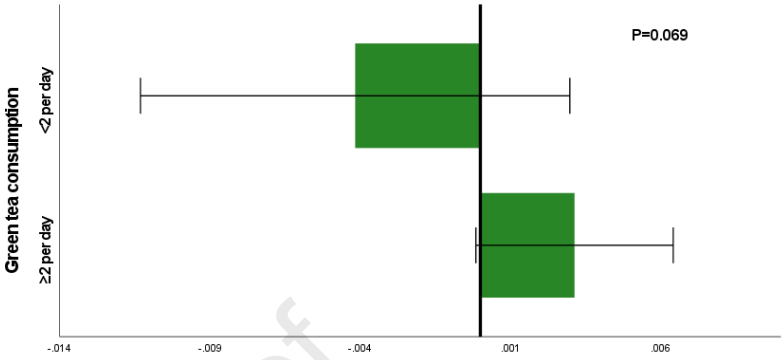
1-Improved status
2-Same status
3-Worsened status



A



B



Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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