

Supplementary Material

Supplementary Methods 1: Exclusion Criteria

DIRECT¹ (NCT00160108) excluded participants who were pregnant or lactating, had a serum creatinine level of ≥ 2 mg/dL (177 μ mol/L) or higher, or had liver dysfunction (defined as an alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level at least twofold above the upper limit of normal). Additional exclusions included active cancer, gastrointestinal disorders preventing adherence to the dietary protocols, or concurrent participation in another diet trial.

CASCADE² (NCT00784433) excluded individuals who consumed more than one alcoholic drink per week, had a personal or family history of addiction, smoking, stroke, or myocardial infarction, underwent major surgery within the prior three months, were using more than two insulin injections per day or an insulin pump, or had a triglyceride level >4.52 mmol/L (400 mg/dL). Additional exclusion criteria were a hemoglobin A1c (HbA1c) level $<6.4\%$ or $\geq 10\%$, women with first-degree relatives diagnosed with breast cancer, and pregnancy.

CENTRAL³ (NCT01530724) excluded participants with a serum creatinine level ≥ 2 mg/dL, impaired liver function (ALT or AST ≥ 3 times the upper limit of normal), active cancer, pregnancy, or lactation. Additional exclusions included being physically active for more than three hours per week or being unable to participate in the physical activity component, and participation in another clinical trial.

DIRECT-PLUS⁴ (NCT03020186) excluded participants unable to engage in physical activity, with a serum creatinine level ≥ 2 mg/dL, or with liver dysfunction. Additional exclusions were a major illness requiring hospitalization, pregnancy or lactation, active cancer or chemotherapy within the prior three years, participation in another trial, chronic warfarin treatment (due to potential interactions with vitamin K), and having an implanted pacemaker or platinum implant (due to MRI incompatibility).

Sample size calculation

A multiple linear regression model was constructed to predict Total Score using the following predictors: Total brain percent of ICV, its interaction with VAT, Age, Sex, BMI, and MED score. The model yielded an R^2 of 0.248, indicating that approximately 24.8% of the variance in the dependent variable is explained by the model. To evaluate the required sample size for adequate statistical power, the Cohen's f^2 effect size was computed using the formula:

$$f^2 = R^2 / (1 - R^2) = 0.248 / (1 - 0.248) = 0.33,$$

which corresponds to a medium-to-large effect size as per Cohen's convention.

A post-hoc power analysis was then conducted using the `pwr.f2.test` function from the `pwr` package in R, with the following parameters: Effect size (f^2): 0.33, Significance level (α): 0.05, Power ($1-\beta$): 0.90.

The analysis indicated that a minimum sample size of 63 participants is required to detect an effect of this magnitude with 90% power at a 5% significance level.

Supplementary Methods 2: Interventions

All trials promoted long-term weight loss and cardiometabolic health through dietary modifications, emphasizing reduced consumption of trans fats and refined carbohydrates and increased intake of vegetables. In DIRECT, participants were randomized to a low-fat diet, a Mediterranean (MED) diet, or a low-carbohydrate non-restricted calorie diet.¹ The CASCADE trial randomized participants to consume 150 mL of mineral water, red wine, or white wine daily with dinner, as part of a MED diet.^{2, 5} In the CENTRAL trial, participants were randomized to low-fat or MED/low-carbohydrate diets, with a secondary randomization at six months into either physical activity (PA) intervention or no additional activity.³ The DIRECT-PLUS trial randomized participants to healthy dietary guidelines, MED diet, or green-MED diet (enriched in polyphenols and green plant-based proteins), with all groups incorporating PA.^{4, 6, 7}

Supplementary Methods 3: Magnetic Resonance Imaging

Brain Imaging

Brain structure volumes were assessed at three time points: baseline and 18 months during the DIRECT-PLUS trial, and at the FIT follow-up. Imaging was performed on the 3.0-Tesla Philips Ingenia scanner using a 3D T1-weighted turbo field echo (T1-TFE) sequence with 1.0 mm isotropic voxel resolution. Acquisition parameters were: TR = 2500 ms, TE = 30 ms, and a matrix field of view of $240 \times 220 \times 150$. An MR technologist reviewed all scans for quality assurance immediately after acquisition. Brain volumes were quantified using NeuroQuant® Software Version 4.1.2, an FDA-approved, fully automated platform for volumetric brain analysis (Cortechs.ai; <https://www.cortechs.ai/neuroquant>). The NeuroQuant pipeline includes skull stripping, atlas-based brain mapping, image filtering, artifact correction, segmentation, error measurement, and report generation. Quantitative volumetric data

were generated for brain regions per hemisphere. Key metrics used in the analysis included total brain volume (TB, %ICV), cortical gray matter (GM) volume (%ICV), white matter (WM) volume (%ICV), total ventricular volume (%ICV), and the hippocampal occupancy score (HOC). All T1-weighted MRI scans were de-identified prior to analysis with NeuroQuant.

Abdominal Fat Imaging

Body fat depots were assessed using whole-body magnetic resonance imaging (MRI) scans performed on a 3.0-Tesla Philips Ingenia scanner (Philips Healthcare). Scans were acquired at baseline and 18 months during the CENTRAL and DIRECT-PLUS trials, and at the FIT follow-up. Abdominal adipose tissue compartments were quantified using a 3D modified DIXON (mDIXON) sequence without interslice gaps, with a slice thickness and spacing of 2 mm. This sequence employed a fast low-angle shot technique and a multiecho two-excitation pulse for phase-sensitive fat and water signal encoding. Acquisition parameters included a repetition time (TR) of 3.6 ms, echo times (TE) of 1.19 ms and 2.3 ms, a field of view (FOV) of $520 \times 440 \times 80$ mm, and a voxel size of $2 \times 1.4 \times 1$ mm. Each scan generated four image types: in-phase, out-of-phase, fat-phase, and water-phase.⁸ Breath-hold techniques were used during abdominal scans to minimize motion artifacts.

Image analyses were conducted by trained raters who were blinded to group allocation and time point. Quantification of abdominal fat depots was performed using a MATLAB-based semi-automated software. The fascia superficialis was manually traced to delineate deep subcutaneous adipose tissue (SAT) from superficial SAT. Fat measurements were derived from two axial slices at the L4–L5 and L5–S1 vertebral levels. For each fat depot, both the absolute area and the proportion relative to total abdominal fat were quantified. Inter- and intrarater reliability were high, with intraclass and interclass correlation coefficients exceeding 0.96 ($p < 0.001$).^{3, 6}

Supplementary Methods 4: Clinical Parameters and Laboratory Methodology

Clinical and laboratory assessments were conducted at baseline, at the end of the intervention, and at 5 to 16 years post-intervention. Height was measured to the nearest millimeter using a wall-mounted stadiometer, and body weight was recorded to the nearest 0.1 kg without shoes. Waist circumference (WC) was measured at the midpoint between the last rib and the iliac crest using a standard anthropometric tape, with readings recorded to the nearest millimeter. Blood pressure (BP) was measured

twice after participants had rested, using an automated monitor (Accutorr-4, Datascope). The mean of the two readings was used for analyses.

Laboratory Methods

Fasting blood samples were collected at 8:00 AM following an overnight fast (12 hours for DIRECT, CENTRAL, DIRECT-PLUS, and FIT follow-up; 8 hours for CASCADE). Samples were obtained at baseline, at the end of intervention (18 or 24 months, depending on trial), and at 5-16 years post-intervention. Blood samples were centrifuged and stored at -80°C until analysis.

Serum total cholesterol (coefficient of variation [CV]: 1.3%), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (CV: 2.1%) were measured enzymatically. For DIRECT, analyses were performed using a Wako R-30 analyzer; for CASCADE, a Cobas 6000 system (Roche); and for CENTRAL, DIRECT-PLUS and FIT follow-up, a Cobas 8000 analyzer (Roche).

Fasting plasma glucose was measured using the Roche GLUC3 hexokinase method (for CASCADE, the Roche Glucose Hexokinase, generation 3 assay was used).

Glycated hemoglobin (HbA1c) was determined using Cobas Integra reagents and equipment (Roche) in DIRECT, Tina-quant hemoglobin A1c, generation 3 (Roche) in CASCADE, and high-performance liquid chromatography (HPLC) using the Tosoh system (Tosoh Bioscience) in the FIT follow-up.

Fasting insulin was measured by enzyme immunoassay using the Immulite automated analyzer (Diagnostic Products, CV: 2.5%) for DIRECT, CASCADE, and CENTRAL; and by the Roche Elecsys platform for DIRECT-PLUS and FIT follow-up. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: $\text{HOMA-IR} = (\text{Fasting Insulin} \times \text{Fasting Glucose}) / 405$.⁹ High-sensitivity C-reactive protein (CV: 1.9%) was measured by ELISA (DiaMed) in DIRECT and Tina-quant® hsCRP assay from Roche in CENTRAL, DIRECT-PLUS, and FIT follow-up. All biochemical assays were performed in laboratories at the University of Leipzig, Germany.

Supplementary Table 1: Participant characteristics stratified by three brain slope categories

Mean slopes based on repeated measurements (x-axis based on months)			
Variable	Mean	3rd_Quartile	1st_Quartile
slope_Whole_Brain_Total_Percent_Of_Icv (TB)	-0.01789	-0.00966	-0.02475
slope_Cortical_Gray_Matter_Total_Percent_Of_Icv (GM)	-0.01296	-0.007053	-0.017134
slope_Ventricle_Total_Percent_Of_Icv (TV)	0.002994	0.004099	0.000989
slope_Cerebral_White_Matter_Total_Percent_Of_Icv (WM)	-0.004069	0.001485	-0.009648
slope_Hippocampal_Occupancy_Score (HOC)	-0.000265	-1.00E-05	-0.000455

Annualized slopes of change were calculated for five brain MRI measures (TB, GM, TV, WM, and HOC) based on repeated measurements across three time points. For each region, the mean slope and the 1st and 3rd quartiles of the slope distribution are presented. These quartile thresholds were subsequently used to define categories of brain decline in Supplementary Table 2.

Supplementary Table 2: VAT AUC values across fast, moderate, and slow brain decline groups

	VAT AUC		
	Fast Decline	Moderate Decline	Slow Decline
Brain regions	n=56	n=111	n=56
TB	9530	8730	7993
GM	9416	8743	8131
WM	9959	8448	8011
HOC	10436	8405	7713
	Fast Increase	Moderate Increase	Slow Increase
TV	10046	8366	8290
(slope per year TBV = slope Whole Brain Total Percent Of Icv * 12)			
mean	3rd Quartile	1st Quartile	
-0.21	-0.12	-0.3	

Participants were categorized based on slope distribution into: Moderate decline (middle 50%), fast decline (lower quartile), and slow decline (upper quartile). Visceral adipose tissue (VAT) area under the curve (AUC) values are shown for each group. This categorization was used for descriptive visualization purposes in density plots.

The individual slopes (based on brain volume change across three MRI time points) were first computed per month and then converted to annualized values (percentage brain volume loss per year). The interquartile range in our cohort corresponded to 0.12%–0.3% loss per year, a range that aligns closely with values reported for healthy individuals in previous literature (Takao et al., 2012; de Jong et al., 2017).

Supplementary References:

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