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ORIGINAL COMMUNICATION

MemAID: Memory advancement with intranasal insulin vs. placebo in type 2 diabetes and control participants: a randomized clinical trial

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Abstract

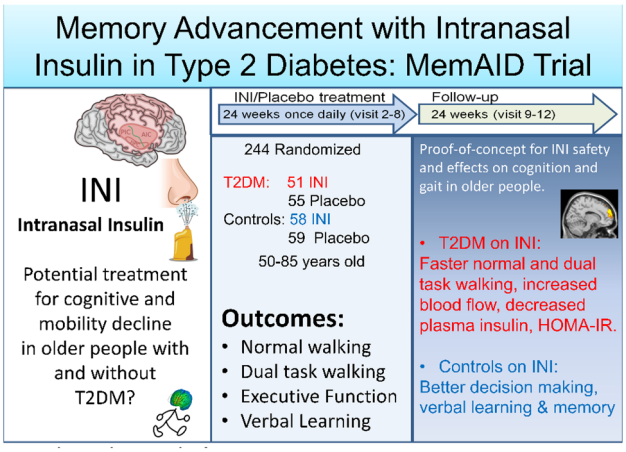
Background This study aimed at assessing the long-term effects of intranasal insulin (INI) on cognition and gait in older people with and without type 2 diabetes mellitus (T2DM).

Methods Phase 2 randomized, double-blinded trial consisted of 24 week treatment with 40 IU of INI (Novolin® R, off-label use) or placebo (sterile saline) once daily and 24 week follow-up. Primary outcomes were cognition, normal (NW), and dual-task (DTW) walking speeds. Of 244 randomized, 223 completed baseline (51 DM-INI, 55 DM-Placebo, 58 Control-INI, 59 Control-Placebo; 109 female, 65.8 ± 9.1; 50–85 years old); 174 completed treatment (84 DM, 90 Controls); 156 completed follow-up (69 DM).

Results DM-INI had faster NW (~7 cm/s; $p = 0.025$) and DTW on-treatment ($p = 0.007$; $p = 0.812$ adjusted for baseline difference) than DM-Placebo. Control-INI had better executive functioning on-treatment ($p = 0.008$) and post-treatment ($p = 0.007$) and verbal memory post-treatment ($p = 0.004$) than Control-Placebo. DM-INI increased cerebral blood flow in medio-prefrontal cortex ($p < 0.001$) on MRI. Better vasoreactivity was associated with faster DTW ($p < 0.008$). In DM-INI, plasma insulin ($p = 0.006$) and HOMA-IR ($p < 0.013$) decreased post-treatment. Overall INI effect demonstrated faster walking ($p = 0.002$) and better executive function ($p = 0.002$) and verbal memory ($p = 0.02$) (combined DM-INI and Control-INI cohort, hemoglobin A1c-adjusted). INI was not associated with serious adverse events, hypoglycemic episodes, or weight gain.

Conclusion There is evidence for positive INI effects on cognition and gait. INI-treated T2DM participants walked faster, showed increased cerebral blood flow and decreased plasma insulin, while controls improved executive functioning and verbal memory. The MemAID trial provides proof-of-concept for preliminary safety and efficacy and supports future evaluation of INI role to treat T2DM and age-related functional decline.

Graphical abstract



Extended author information available on the last page of the article

Keywords Type 2 diabetes · Intranasal insulin · Cognition · Gait · Brain blood flow · Aging

Abbreviations

AE	Adverse events
BRAVO	T1-weighted 3D brain volume sequence
CBF	Cerebral blood flow
CGM	Continuous glucose monitoring
DTW	Dual-task walking
HbA1c	Hemoglobin A1c
HOMA-IR	Homeostatic assessment model of insulin resistance
INI	Intranasal insulin
mPFC	Medial prefrontal cortex
MMSE	Mini mental state Exam (scale 0–30)
NW	Normal walk
PAL	Paired associated learning, total errors adjusted (scale 0–120)
PCASL	Pseudo continuous arterial spin labeling
RARE	Rapid acquisition with relaxation enhancement
SMBG	Self-monitored plasma glucose
SWM	Spatial working memory: between errors (scale 0–42); strategy (scale 8–56)
PAL	Paired associated learning total errors adjusted (scale 0–120)
Executive function	Composite *z* score (SWM, PAL: lower score = better performance)
VRM	Verbal recognition memory: free recall (scale 0–12); recognition immediate, delayed (scale 0–24)
Verbal learning	Composite *z* score (VRM: higher score = better performance)
WHODAS 2.0	World health organization disability assessment schedule 2 complex score (scale 0–100)
WTAR-IQ	Wechsler adult reading test (adjusted scale 50–128)
On-treatment	24 Weeks of INI/Placebo treatment (visit 2–8)
Post-treatment	24 Weeks of follow-up (visit 9–12)
ITT-Model	Intention-to-treat model (subjects with at least baseline)
PP-Model	Per-protocol model (participants treatment compliant)

Introduction

Type 2 diabetes mellitus (T2DM) accelerates brain aging [1, 2] and increases risk for dementia [3]. Insulin plays a key role in energy metabolism, neurovascular coupling [4] and signaling among functional networks [5–7]. Brain insulin resistance, microvascular disease and impaired insulin signaling may be common pathways for cognitive decline in aging [5], diabetes and Alzheimer disease [3, 4, 8]. Intranasal insulin (INI) enters the brain along the olfactory, trigeminal pathways and perivascular channels, bypassing the blood–brain barrier, and binds to receptors in multiple cortical regions, insula, hippocampus, hypothalamus, cerebellum and substantia nigra, thus stimulating dopaminergic and hypothalamic pathways [9–12]. INI has been shown to improve verbal memory [13] and has emerged as a potential treatment for cognitive impairment in elderly [8, 14, 15]. Underlying mechanisms supporting INI effects include enhancement of brain metabolism [16], regional perfusion [17, 18] and functional connectivity [10, 19–22]. INI improvements in verbal and visuospatial memory in older T2DM and healthy adults are likely mediated by regional vasodilatation [21, 23] and functional connectivity between hippocampus and default mode networks [23, 24]. INI effects on gait have not been studied. Gait is a complex task and walking speed is an important indicator of overall health [25]. Slower walking speed correlates with brain hypoperfusion in T2DM [26] and can predict cognitive impairment and disability [23, 25].

We conducted a prospective randomized placebo-controlled Phase 2 trial—Memory Advancement with Intranasal Insulin in Type 2 Diabetes (MemAID)—to determine long-term INI effects on cognition and walking speed in participants with and without diabetes and safety.

Research design and methods

Trial design

MemAID was a prospective double-blinded, placebo-controlled Phase 2 trial that assessed effects of INI compared to placebo treatment over 24 weeks with 24 weeks of follow-up in participants with and without diabetes randomized into four treatment arms. Primary hypotheses were that INI-treated T2DM participants would have better outcomes in memory and other specific cognitive domains, faster normal (NW) and dual-task walking (DTW) speeds, and better daily functionality, as compared

to placebo-treated participants with diabetes. We also hypothesized that INI-treated controls would perform better on these outcomes, compared to placebo-treated controls [28].

A Magnetic Resonance Imaging (MRI) Substudy evaluated INI effects on cerebral blood flow (CBF) and vasoreactivity to hypercapnia and hypocapnia. We evaluated long-term INI safety including adverse events, metabolic profile, weight and hypoglycemic episodes. A Safety Substudy was conducted in subcutaneous insulin-dependent T2DM participants (T2DM-IDDM).

Standard protocol approvals, registrations, and patient consents

The trial was conducted at the Syncope and falls in the Elderly Laboratory (SAFE, V.N. Principal Investigator) at the Clinical Research Center at Beth Israel Deaconess Medical Center (BIDMC, Boston, MA, USA) and the Center for Clinical Investigation at Brigham and Women's Hospital (BWH, P.N. Site Principal Investigator, Boston, MA, USA). Cognitive training was done at Harvard Medical School (R.M.G., Site Principal Investigator). The trial was advertised at Joslin Diabetes Center. The trial was approved by the US Food and Drug Administration (FDA; IND#107,690) and registered on www.clinicaltrials.gov (NCT02415556 on 3/23/2015). The study was conducted in accordance with the Guideline for Good Clinical Practice and followed Consolidated Standards for Reporting in Clinical Trials (CONSORT).

This study was carried out in accordance with the recommendations of ethical standards of the BIDMC, BWH and Harvard Medical School. The BIDMC Committee on Clinical Investigation, BWH and Harvard Catalyst CEDE reviewed and approved the study. All participants signed the informed consent in accordance with the Declaration of Helsinki. A Data and Safety Monitoring Board monitored progress and adverse events (AEs). Study screening began October 6, 2015 at BIDMC and June 22, 2017 at BWH. The first patient was randomized at BIDMC on November 5, 2015. In October 2017, the trial protocol and sample size were modified following National Institute of Diabetes and Kidney Diseases (NIDDK) guidance. The Safety substudy in T2DM-IDDM and enrollment of T2DM-IDDM participants were stopped due to high rate of dropouts. Retention strategies were implemented to reduce drop-out rate (participation incentives, transportation, flexible scheduling and skipping visits; Supplementary Appendix). On March 25, 2020, due to COVID-19 pandemic, on-site visits and MRI scans were stopped. The last patient was seen via virtual visit on May 21, 2020, and the study was concluded on May 31, 2020.

Participants and study protocol

The total of 668 subjects were contacted, 289 signed informed consent, 244 were eligible, enrolled and randomized; 223 completed baseline, 221 initiated treatment, 174 (84 DM; 90 controls) completed the 24 week (168 days) treatment and 156 (69 DM; 87 controls) completed the 24 week follow-up. The trial remained under-enrolled for T2DM groups (84 of 120 planned enrollment; 70%), but reached the target for controls (90 of 90 planned enrollment; 100%) (Fig. 1A, Consort diagram). Retention strategies and stopping enrollment of T2DM-IDDM group reduced overall drop-out rate from 28 to 17%. Due to COVID-19, 13 (11 T2DM) participants missed 23 on-site assessments and seven end-of treatment MRI scans, and ten participants were censored due to study ending. Participants were recruited from the community, BIDMC, BWH, and Joslin Diabetes Center.

Inclusion criteria and study procedures

Eligible participants were 50–85 years old and able to walk for six minutes. T2DM participants were treated with diet, non-insulin oral or injectable agents. Controls had fasting plasma glucose.

(<126 mg/dL) and hemoglobin A1c (HbA_{1c}, $<6.5\%$). Excluded were persons with T1DM, T2DM-IDDM (enrollment stopped in 2017), intolerance to insulin, history of severe hypoglycemia, more than one hypoglycemic episode during the entire study [29], dementia or mini mental state exam (MMSE) scores ≤ 20, serious systemic diseases, recent hospitalizations or drug abuse. INI or placebo was added to participants' routine medication regimen.

Study procedures consisted of a phone screen, on-site screening (visit 1), baseline (visit 2), and four assessments at 8 week intervals, three medication refill visits during 24 week treatment (visits 2–8), and four assessments during 24 week post-treatment (visits 9–12) (Fig. 1B). Baseline and the first intervention assessment were done on the same day (visit 2). Visit nine was at 1 week after visit eight, and it was replaced by a phone call in 2017. Assessment visits included fasting metabolic panels, anthropometrics, cognitive, functional and mood tests, NW and DTW [28], duration about 3 h. Baseline Charlson co-morbidity index used ICD-10 codes [30]. Participants kept a diary of daily medication usage and weekly self-monitored blood glucose (SMBG). Safety outcomes, AEs and medication adherence were assessed at each visit. The study physicians evaluated participants at baseline and any AE occurrence.

Participants were randomized into four groups. The study statistician (L.N.) designed code that used randomly selected blocks with sizes four, eight and twelve

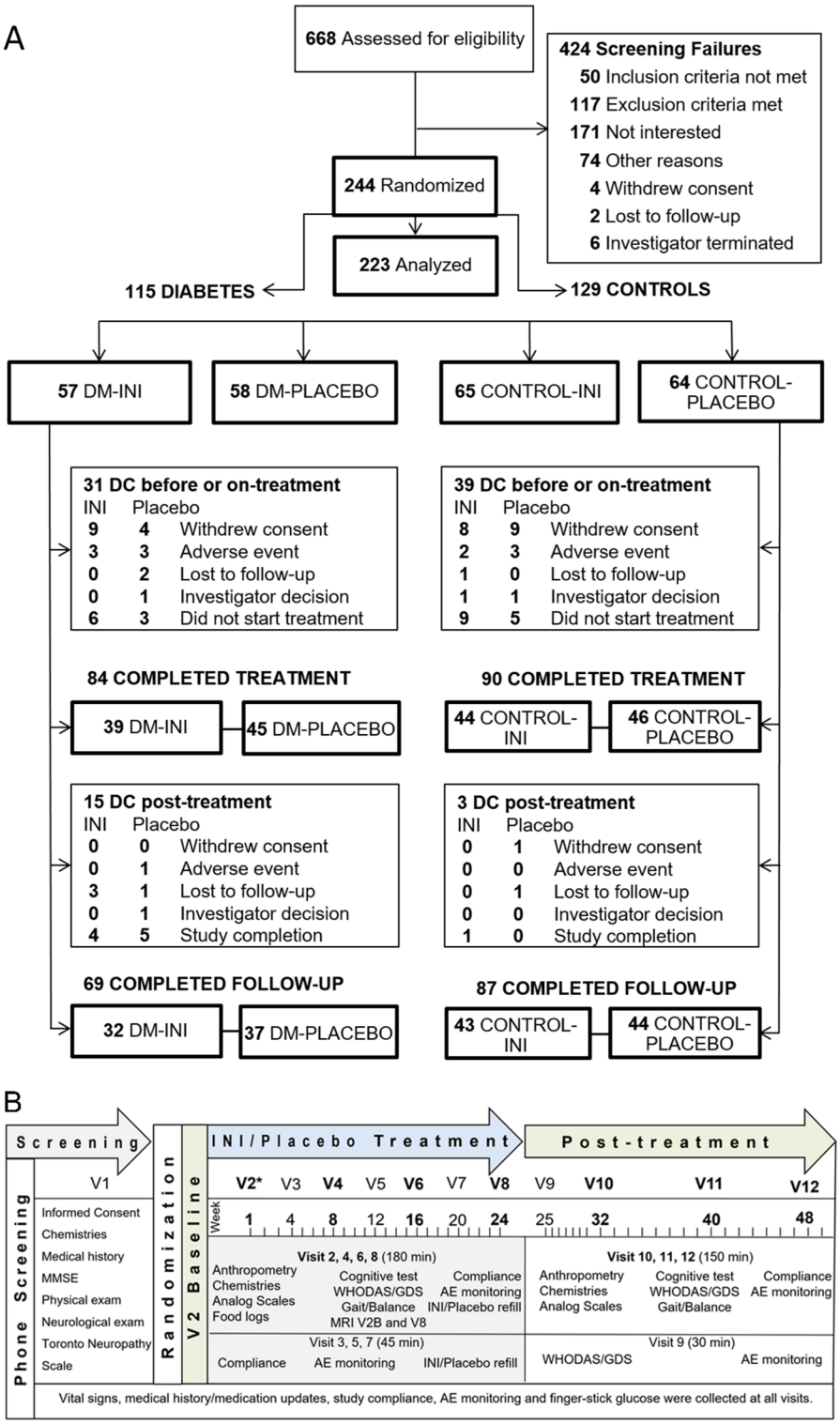


Fig. 1 Consort diagram for MemAID trial and study flow chart. **A** Consort diagram for Memory Advancement with Intranasal insulin trial. **B** Study Flow chart. DM: type 2 diabetes mellitus; INI-intranasal insulin. V: Visit number, V2* consisted of two assessments: baseline and intervention 1 that were done on the same day. V8: end-of-treatment. MRI was done at V2 baseline (V2B) and after V8 at the end-of-treatment. V9*:* Phone call. V12: end of the follow-up. A subset of patients also completed food logs for three days before V2 baseline, V4, V6, V8. *MMSE* mini mental state examination, *WHODAS 2.0* world health organization disability assessment schedule, GDS geriatric depression scale, AE adverse event

to ensure uniform distribution of baseline characteristics between DM-INI vs. DM-placebo and Control-INI vs. Control-Placebo.

The PI, co-investigators, staff, participants and participants' providers were blinded to assignments.

Interventions and device

Insulin/placebo was delivered using the ViaNase™ electronic atomizers (Kurve Technology, Inc. Lynnwood, WA, USA). Participants administered 40 IU (0.4 mL) of human

insulin (rDNA origin; Novolin® R, Novo Nordisk Inc., Bagsværd, Denmark) or placebo (0.4 mL bacteriostatic sodium chloride 0.9% solution) intranasally once daily before breakfast. Devices were calibrated to dispense a single 0.1 mL dose over 20 s. Participants sprayed a single 0.1 mL dose four times (twice into each nostril) over a 2 min period to administer the daily 0.4 mL dose of INI (40 IU) INI or placebo. Novolin® R was used off-label [31]. BIDMC and BWH research pharmacy performed sterility procedures, reconstituted study drug and dispensed according to the randomization code.

Cognition and gait outcomes

Primary outcomes were cognitive measures, NW and DTW speeds. The Cambridge Cognition computerized system (CANTAB) was used to assess attention, memory and executive function using a battery of validated tests with parallel versions to reduce practice effects [32]. Cognitive outcomes were converted to scaled z scores and summed to create composite measures [33].

The executive function composite score included the following: paired associates learning (PAL, total errors adjusted) and spatial working memory (SWM, total errors and strategy to complete tasks). Lower score indicates better performance. Verbal memory composite score included verbal immediate free recall and immediate and delayed verbal recognition memory (VRM). Higher score indicates better performance.

Gait was measured during a 6 min walk at usual speed and 6 min dual-task walking (counting backwards subtracting seven) using the Mobility Lab System (APDM, Inc., Portland, OR). Gait speed was calculated from the total distance walked over six minutes in 45 m hallway, excluding turns.

Mood was assessed with the geriatric depression scale, a validated self-report measure of mood (scale 0–30). Disability was assessed with the World Health Organization Disability Assessment Schedule (WHODAS) 2.0, a validated self-reported measure of cognition, mobility, self-care, getting along and life activities domains. The summary score was converted into a percentage score (0 no disability—100 full disability). The Wechsler Adult Reading and Comprehension test was used as a proxy for intelligence and was adjusted for age, sex and education (scale 50–128). Laboratory chemistries included blood, metabolic, lipid and renal panels collected at baseline. Fasting serum glucose, finger stick glucose, HbA_{1c}, fructosamine, serum insulin and C-reactive peptide were collected at assessment visits (Quest Diagnostics™, Secaucus, NJ, USA).

Magnetic resonance imaging

MRIs were acquired using a GE Discovery MR750 3 Tesla scanner (GE Medical Systems, Milwaukee, WI) with a receive-only 32-channel head-array coil, and a body transmit coil.

T1-weighted anatomical images were acquired with a 3D brain volume (BRAVO) sequence. Perfusion images were acquired at normocapnia (supine rest for 6 min), hypercapnia (rebreathing of 5% CO_2 and 95% air for 2 min) and hypocapnia (hyperventilation for 2 min) with vital signs and CO_2 monitoring using a pseudo-continuous arterial spin labeling (PCASL) sequence and 3D stack of spirals rapid acquisition with relaxation enhancement (RARE) sequence. Resting cerebral blood flow (CBF) maps [34, 35] were divided by their global mean to normalize differences among subjects. CBF responses to CO_2 challenge were calculated as the difference in CBF maps between hypercapnia and normocapnia (vasodilatation), and between hypocapnia and normocapnia (vasoconstriction), respectively. Vasodilation reactivity and vasoconstriction reactivity maps were normalized by dividing the vasodilatation and vasoconstriction maps by the corresponding CO_2 changes. Vasomotor range was calculated as the difference between vasodilatation and vasoconstriction maps, divided by CO_2 changes [26, 36]. CBF and vasoreactivity maps were analyzed on a voxel-by-voxel basis using statistical non-parametric mapping (SnPM) software, voxel-level threshold $p < 0.005$. The non-parametric approach used was more robust with the nominal false positive rate of 5% [37] compared to the parametric approach.

Data management

MemAID database (Study TRAX© Macon, GA, USA) is FDA- and Health Insurance Portability and Accountability Act-compliant web-based data management software. It was used to enter and manage data, determine eligibility, track enrollment, study progress and AEs. Data quality audits were conducted regularly, to review data and correct errors. A geographically redundant storage, 256-bit encryption and a dedicated firewall protected the data.

Statistical analyses

Primary outcomes were cognition and gait (executive function, verbal learning composite scores, NW and DTW). We hypothesized that INI-treated participants with diabetes would have faster walking speed than placebo-treated T2DM participants. We also tested this hypothesis for DTW, executive function, and verbal memory. The same four hypotheses were tested in controls. Variables were collected at baseline, on-treatment visits 2–8 (0, 1, 55, 113, 165 average days from baseline) and post-treatment

visits 9–12 (173, 227, 282, 333 average days from baseline). Linear mixed-effects models were used to estimate the effects of INI in the DM and Control groups. Intention-to-treat analyses (ITT-Model) included data from 223 randomized subjects who completed the baseline visit. Per-protocol analyses (PP-Model) included data from 175 participants who were compliant with treatment and used medication daily for more than 109 days (65% of 168 days treatment period); 48 non-compliant participants (22%) were excluded.

Primary outcomes were compared between DM-INI and DM-Placebo groups and between Control-INI and Control placebo groups at baseline, on-treatment and post-treatment. A spatial power variance–covariance structure was used to model within-subject correlated measurements where the number of days from the baseline visit was used as the power of the autoregressive correlation coefficient. Each efficacy and safety outcome variable was modeled separately.

The independent variables in the model included a four-level indicator variable for the following four treatment groups: a three-level time indicator variable (TIMEG) representing baseline, on-treatment and post-treatment period and an interaction term between TIMEG and treatment group.

An average number of treatment days at each assessment visit was used as a continuous repeated variable and subjects were included as random effects. Restricted maximum likelihood estimation method and linear contrasts were used to obtain the estimated mean difference and 95% confidence interval between INI and Placebo for each outcome variable. Safety analyses used the same linear mixed-effects models. Nominal *p* values without adjustment for multiple comparisons were specified to show the effects of each primary outcome variable modeled separately within DM and Control groups. Multiple comparison adjustment of the type-I error of 0.05 was not implemented because the null hypothesis of our study was not based on a composite hypothesis of the "or" conditions [15, 38, 39]. We did not test the "composite" null hypothesis that INI had an effect on NW or DT walking speed or executive function or verbal memory or differences between the DM and Control groups. However, we specifically and a priori set four separate null hypotheses testing each of these outcomes of interest separately. For each of the four outcomes, we used a type-I error of 0.05. No stratification of data was used to obtain the comparisons between INI and placebo for each group (DM, Controls) or time point (baseline, on-treatment, post-treatment) because that would cause a loss in statistical power. Rather, we used a single linear mixed effects model with all observations for each outcome. We used linear contrasts (linear combinations of the model betas of main effects and interaction with time period), which yielded the estimated mean difference of the outcome (e.g. gait speed) between INI vs. placebo for each group within each time period (two-tailed p-values).

Potential confounding effects of baseline differences were accounted for by randomization and by including baseline in the model. We carried out additional analyses by examining the INI effect in the combined cohort of DM and Control participants. We also examined models that would yield the overall INI effect from both DM and Control cohort by first testing for the significance of the interaction between cohort (DM, Control) and treatment group (INI, Placebo). Due to non-significance, we estimated the overall INI effect by removing the interaction and adjusting the model using HbA_{1c}, a good proxy for DM versus Control classification. For each outcome, we computed ITT and PP models (as described above), adjusted for HbA_{1c} as a continuous variable (Supplementary Appendix). We carried out additional subgroup analyses in the Control group by examining the INI effect in controls with pre-diabetes vs. normoglycemic controls using linear mixed-effects models as described above.

Adverse events analyses used Fisher's exact test and chi-square test. Data were converted from Study TRAX© (Macon, GA, USA). The code and data analyses were generated using Statistical Analyses Software (SAS), Version 9.4 TS level; SAS System for Windows (X64_8PRO platform, Copyright© 2002–2012 SAS Institute Inc. (Cary, NC, USA) and JMP® Pro, Version 15 (SAS Institute Inc. Cary, NC, USA). Data-sharing of de-identified datasets will be done through collaborations and publications.

Sample size

We computed the required sample size based on the expected estimated mean difference at the end of treatment between the DM-Placebo and DM-INI group for cognitive (SWM errors, SWM strategy, VRM total recall) and gait variables. We set type-I error rate at 0.05, power of 0.80 or above, effect size of 15% improvement due to INI, and obtained $n = 120$ for the DM group (60 DM-INI; 60 DM-Placebo) and $n = 90$ for the Control group (45 Control-INI; 45 Control-Placebo) yielding 210 patients with data at the end of the treatment period.

Results

Primary outcomes

Baseline characteristics of 223 randomized participants were similar between DM-INI and DM Placebo and between Control-INI and Control-Placebo (Table 1). DM participants had more co-morbidities and worse cognition and gait; 48 controls (41%) had pre-diabetes. Figure 2 shows intention-to-treat analyses for NW, DTW, executive function and verbal memory and comparisons between DM-INI

Table 1 Baseline characteristics of the study cohort in four randomized groups

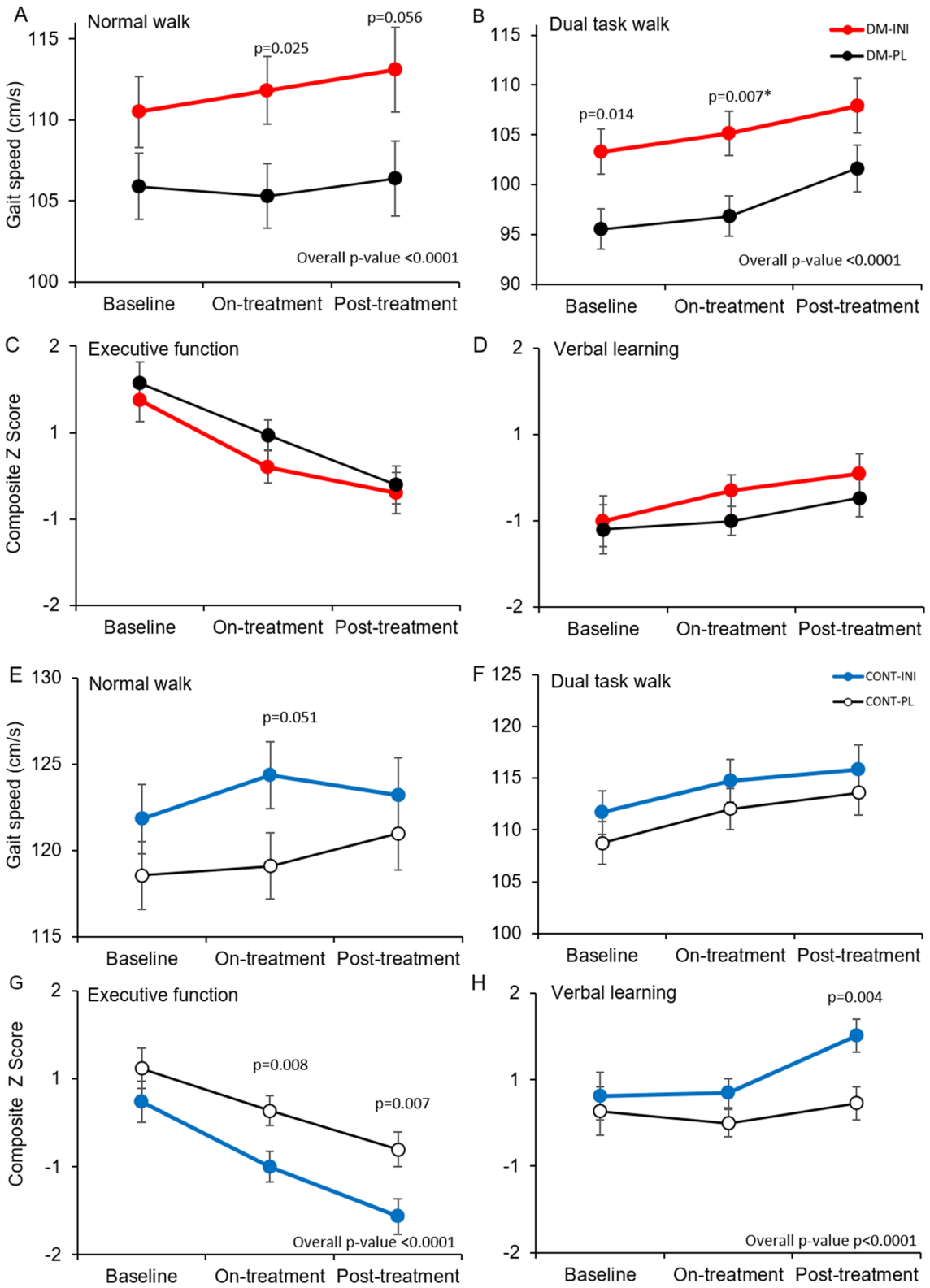
	Whole cohort	DM-INI	DM-Placebo	Control-INI	Control-Placebo
Number of participants-baseline	223	51	55	58	59
Age, years	65.4 ± 8.9	63.6 ± 8.5	65.7 ± 8.7	66.1 ± 9.2	66.1 ± 9.2
Range	50–84	50–83	51–84	50–84	50–84
Female, *n* (%)	109 (48.9)	24 (47.1)	25 (45.5)	29 (50.0)	31 (52.5)
Diabetes duration, years	10.9 ± 7.5	10.6 ± 8.4	11.2 ± 6.6	N/A	N/A
Range	1–47	1–47	1–26	N/A	N/A
Race					
White, *n* (%)	173 (77.6)	36 (70.6)	34 (61.8)	52 (89.7)	51 (86.4)
Black, *n* (%)	34 (15.2)	8 (15.7)	16 (29.1)	4 (6.9)	6 (10.2)
Asian, *n* (%)	8 (3.6)	4 (7.8)	1 (1.8)	2 (3.4)	1 (1.7)
Other, *n* (%)	8 (3.6)	3 (5.9)	4 (7.3)	0 (0)	1 (1.7)
Ethnicity-Hispanic, *n* (%)	13 (5.8)	3 (5.9)	6 (10.9)	1 (1.7)	3 (5.1)
Functional scales					
Education, years	16.3 ± 3.4	15.4 ± 3.9	15.8 ± 3.3	17.1 ± 3.3	16.6 ± 3.0
WTAR-IQ adjusted (scale 50–128)	112.7 ± 13.7	109.0 ± 14.9	107.9 ± 15.8	115.6 ± 10.8	117.6 ± 10.6
Mini-mental state exam (scale 0–30)	28.3 ± 1.8	28.4 ± 1.6	27.9 ± 2.1	28.4 ± 1.6	28.5 ± 1.7
Charlson co-morbidity Index (scale 0–24)	3.4 ± 1.7	3.9 ± 1.6	4.1 ± 1.5	2.7 ± 1.4	2.9 ± 1.8
WHODAS 2.0 complex (scale 0–100)	12.0 ± 12.2	16.8 ± 12.5	16.3 ± 14.2	8.3 ± 10.9	7.3 ± 7.3
Geriatric depression Scale (0–30)	5.7 ± 5.3	6.5 ± 5.6	7.3 ± 5.0	5.0 ± 5.7	4.1 ± 4.5
Toronto Neuropathy Scale (0–19)	3.1 ± 3.6	4.3 ± 4.2	4.7 ± 3.9	1.8 ± 2.5	1.9 ± 2.7
Metabolic and cardiovascular parameters					
BMI, kg/m^2	29.5 ± 6.2	32.1 ± 6.7	32.0 ± 6.1	27.1 ± 4.6	27.2 ± 5.5
Waist circumference, cm	103.9 ± 16.1	110.8 ± 14.6	111.0 ± 14.8	97.0 ± 12.2	98.1 ± 17.2
HbA_{1c},% $HbA_{1c,}$ mmol/mol	6.3 ± 1.3	7.4 ± 1.6	7.1 ± 1.1	5.6 ± 0.3	5.6 ± 0.3
	45.8 ± 13.8	54.5 ± 15.6	53.3 ± 12.6	37.4 ± 3.8	37.4 ± 3.0
Fasting serum glucose, mg/dL	114.4 ± 41.4	138.6 ± 44.6	141.3 ± 51.2	92.1 ± 7.7	90.5 ± 7.7
C-reactive protein, mg/L	3.2 ± 4.2	4.5 ± 7.2	4.0 ± 3.0	1.8 ± 1.9	2.2 ± 3.0
Total cholesterol, mg/dL	178.0 ± 41.8	170.1 ± 37.9	168.7 ± 43.3	190.7 ± 42.1	180.8 ± 40.8
Microalbumin urine, ug/mL	19.3 ± 47.8	17.9 ± 30.5	30.6 ± 78.7	15.1 ± 38.6	14.4 ± 24.7
Microalbumin-to-creatinine ratio, mg/gcreat	26.9 ± 68.0	20.1 ± 27.5	50.0 ± 117.8	19.0 ± 43.1	18.3 ± 38.1
Hypertension diagnosis, *n* (%)	107 (48)	33 (64.7)	38 (69.1)	18 (31.0)	18 (30.5)
Systolic blood pressure, mmHg	131.0 ± 16.5	133.3 ± 17.1	134.9 ± 17.1	128.3 ± 14.1	127.9 ± 16.9
Diastolic blood pressure, mmHg	73.7 ± 11.8	73.7 ± 12.9	75.8 ± 12.3	71.8 ± 11.3	73.8 ± 11.0
Medications					
Use of s.c. insulin, n (%)	10 (4.5)	6 (11.8)	4 (7.3)	N/A	N/A
Use of oral antidiabetic drugs, n (%)	87 (39.0)	41 (80.4)	46 (83.6)	N/A	N/A
Use of injectable antidiabetic drugs, n (%)	7 (3.1)	3 (5.8)	4 (7.3)	N/A	N/A
Use of antihypertensive drugs, n (%)	112 (50.2)	37 (72.5)	40 (72.7)	17 (29.3)	18 (30.5)
Use of lipid lowering drugs, n (%)	105 (47.1)	31 (60.8)	38 (69.1)	18 (31.0)	18 (30.5)
Use of antidepressants, n (%)	36 (16.1)	6 (11.8)	12 (21.8)	9 (15.5)	9 (15.3)

Diabetes-Intranasal Insulin (DM-INI), Diabetes-Placebo (DM-Placebo), Control-Intranasal Insulin (Control-INI), Control-Placebo. DM: diabetes mellitus; Intranasal Insulin: INI WTAR-IQ: Wechsler Test of Adult Reading Intelligence Quotient (age, sex, education adjusted score). WHODAS 2.0. Complex score: World Health Organization Disability Assessment schedule 2.0

Data are mean ± SD unless otherwise indicated. Comparisons between DM-INI vs. DM-Placebo and between Control-INI vs. Control-Placebo using Wilcoxon and Fisher exact test unadjusted, did not show statistical differences

BMI body mass index, *HbA_{1c}*: hemoglobin A1c

and DM-Placebo, and between Control-INI and Control-Placebo groups at baseline, during treatment and post-treatment. Table 2 shows differences and confidence intervals for DM-INI vs. DM-Placebo and for Control-INI vs. Control-Placebo at baseline, on-treatment and post-treatment using intention-to-treat (ITT-Model, 223 randomized participants,



overall $p < 0.0001$) and per-protocol (PP-Model, treatment compliant participants, overall $p < 0.0001$) analyses. Baseline walking speeds were within the normal range (T2DM participants: NW 107.6 ± 22.3; DTW 98.4 ± 23.5 cm/s; controls: NW 120.5 ± 18.9; DTW 109.8 ± 20.7 cm/s). INI-treated participants with diabetes walked faster compared to placebo-treated: DM-INI had faster NW on-treatment ($p = 0.025$) and post-treatment (ITT-Model $p = 0.057$,

◂**Fig. 2** Cognitive and gait outcome variables for Diabetes-Intranasal Insulin and Diabetes-Placebo groups, and for Control-Intranasal Insulin and Control-Placebo groups. Primary outcome variables: normal and dual-task walking speeds, executive function and verbal memory composite scores for diabetes (**A**–**D**) and control (**E**–**H**) groups at baseline, during 24 weeks of treatment (on-treatment, visit 2–8) and 24 weeks of follow-up (post-treatment, visit 9–12). Graphs show the estimates of the intention-to-treat models (mean ± SE) for each variable. *P* values reflect the interaction between group and time period e.g. diabetes-intranasal insulin (DM-INI) vs. diabetes-placebo (DM-Placebo) and control-intranasal insulin (Control-INI) vs. control-placebo (Control-Placebo) at baseline, during treatment and post-treatment. Overall p value < 0.0001 denotes significance of the whole intention-to-treat model calculated separately for each variable. **A** Normal walking speed was faster during treatment in diabetes-intranasal insulin group (DM-INI; red line, full circles; $p = 0.025$) and borderline post-treatment ($p = 0.056$), compared to diabetes-placebo group (DM-Placebo; black line, full circles). Per-protocol model has shown that DM participants, compliant with intranasal insulin treatment, walked faster post-treatment ($p = 0.041$), compared to DM-Placebo. **B** Dual-task walking speed was faster in DM-INI at baseline ($p = 0.014$) and during intranasal insulin-treatment ($p = 0.0007$) compared to DM-Placebo group. *On-treatment DTW was not significant after adjusting for baseline difference ($p = 0.812$). **C** Executive function performance (lower composite score indicates better performance) was not different between DM-INI and DM-Placebo groups. **D** Verbal memory performance (higher composite score indicates better performance) was not different between DM-INI and DM-Placebo groups. **E** Normal walking speed was borderline faster during INI treatment in control-intranasal insulin group (Control-INI; blue line, full circles; $p = 0.051$), compared to control-placebo group (Control-Placebo; black line, empty circles). **F** Dual task walking speed was not different between Control-INI and Control-Placebo. **G** Executive function performance was better in Control-INI during intranasal insulin treatment ($p = 0.008$) and post-treatment ($p = 0.007$) compared to Control-Placebo (lower composite score indicates better performance). **H** Verbal memory performance was better in Control-INI group after intranasal insulin treatment compared to Control-Placebo ($p = 0.004$; (higher composite score indicates better performance)

PP-Model $p = 0.041$) compared to DM-Placebo. NW difference DM-INI vs. DM-Placebo was 6.5 cm/s on-treatment, compared to 4.3 cm/s at baseline (ITT-Model, Table 2). DM-INI also had faster DTW at baseline ($p = 0.013$), during treatment ($p = 0.007$) and borderline post-treatment ($p = 0.087$; Fig. 2A, B). On-treatment DTW was not significant after adjusting for baseline difference ($p = 0.812$). Executive function and verbal memory composite scores did not differ between DM-INI and DM-Placebo (Fig. 2C, D).

Control-INI improved cognition and had borderline faster NW on-treatment ($p = 0.051$; difference 5.28 cm/s) (ITT-Model, Table 2) compared to Control-Placebo, but DTW did not change (Fig. 2E, F). Control-INI achieved better executive function composite scores on treatment (lower score indicates better performance, $p = 0.008$; model estimate difference -0.64) and post-treatment ($p = 0.007$; difference -0.76) (Fig. 2G, Table 2), and better verbal memory composite scores post-treatment (higher score indicates better performance, $p = 0.004$; difference 0.79) compared to Control-Placebo (Fig. 2H). On-treatment, Control-INI made fewer errors during learning (PAL $p = 0.026$, SWM $p = 0.03$), demonstrated better strategy during visuospatial memory task (SWM, $p = 0.034$) and delayed verbal recall (VRM $p = 0.002$) as compared to Control-Placebo. Post-treatment, Control-INI also made fewer errors (PAL, $p = 0.003$) and showed better decision making strategy (SWM, $p = 0.023$), immediate ($p = 0.013$) and delayed recall ($p = 0.002$), and recognition memory (VRM, $p = 0.008$).

We also evaluated an overall INI treatment effect in a combined cohort of DM and control participants. INI-treated participants (DM-INI and Control-INI) had similar baseline characteristics to Placebo-treated participants (DM-Placebo and Control-Placebo) and there were no differences in main outcomes (Supplementary Table A.1.) We modeled the overall INI treatment effect from both DM and Control participants, adjusting for HbA_{1c} (Table 3). INI-treated participants walked faster on-treatment (NW ~ 6.42 cm/s; ITT-Model = 0.002, PP-Model = 0.003; DTW ~ 5.8 cm/s; ITT-Model = 0.006, PP-Model = 0.01). INI-treated participants had better executive function during treatment (ITT-Model = 0.002, PP-Model = 0.004) and post-treatment (ITT-Model = 0.026, PP-Model = 0.038) as compared to placebo-treated participants. INI-treated participants had better verbal memory during treatment (ITT-Model = 0.018) and post-treatment (ITT-Model = 0.004, PP-Model = 0.009). HbA_{1c} effect was significant in all models. No effects were found on mood or WHODAS 2.0. Sex, age, race, ethnicity and insulin sensitizers had no significant effects on cognitive or gait outcomes because of randomization.

We have identified that of 117 controls, 48 participants met criteria for pre-diabetes (baseline HbA_{1c} 5.7–6.4%) and 69 participants were normoglycemic (baseline $HbA_{1c} < 5.7\%$). INI treated participants with pre-diabetes ($n = 21$) have shown trend toward the better performance on NW and DTW as compared to placebo-treated participants with pre-diabetes ($n = 27$) (Table 4). INI-treated participants with pre-diabetes performed better on executive function tests on-treatment ($p = 0.003$) and post-treatment ($p = 0.009$) and on verbal memory tests post-treatment ($p = 0.005$), compared to placebo-treated participants with pre-diabetes. A similar trend was observed for normoglycemic controls (INI: $n = 37$, Placebo $n = 32$).

MRI substudy

We aimed to enroll 40 T2DM participants; 18 completed baseline scans; 11 completed end-of-treatment scans; seven scans were cancelled due to COVID-19. All 11 participants were treatment compliant. INI treatment increased resting CBF in right medial pre-frontal cortex (mPFC) as compared to baseline (corrected cluster-level p = 0.03; similar trend on the left; Fig. 3A, i). Regional analysis of the mPFC cluster showed that CBF increased in all eight T2DM-INI

Table 2 Primary cognitive and gait outcomes

Variable	Baseline		On-treatment		Post-treatment	
	Estimate (CI)	*p*	Estimate (CI)	*p*	Estimate (CI)	*p*
DM-INI vs. DM-Placebo						
Normal walking speed (cm/s)						
ITT-Model	4.26 (− 1.62–10.13)	0.155	6.52 (0.81–12.23)	**0.025**	6.71 (− 0.17–13.58)	0.057
PP-Model	3.05 (− 3.30–9.41)	0.345	7.00 (0.78–13.21)	**0.028**	7.25 (0.31–14.19)	**0.041**
Dual-Task walking speed (cm/s)						
ITT-Model	7.75 (1.61–13.89)	**0.013**[a]	8.31 (2.33–14.29)	**0.007**	6.31 (−0.91–13.52)	0.087
PP-Model	8.08 (1.55–14.61)	**0.015**[a]	8.66 (2.30–15.02)	**0.008**	6.46 (− 0.76–13.67)	0.079
Executive function composite z score (lower score = better performance)						
ITT-Model	− 0.20 (− 0.88–0.49)	0.574	− 0.37 (− 0.87–0.13)	0.144	− 0.10 (− 0.73–0.53)	0.759
PP-Model	− 0.36 (− 1.09–0.37)	0.335	− 0.32 (−0.84–0.20)	0.221	− 0.10 (− 0.73–0.53)	0.750
Verbal memory composite z score (higher score = better performance)						
ITT-Model	0.09 (− 0.72–0.91)	0.819	0.35 (− 0.13–0.83)	0.149	0.29 (− 0.32–0.89)	0.357
PP-Model	0.24 (− 0.62–1.09)	0.586	0.30 (− 0.18–0.79)	0.219	0.29 (− 0.30–0.87)	0.341
Control-INI vs. Control-Placebo						
Normal walking speed (cm/s)						
ITT-Model	3.28 (− 2.20–8.76)	0.240	5.28 (− 0.03–10.60)	0.051	2.17 (− 3.77–8.10)	0.473
PP-Model	2.32 (− 3.62–8.26)	0.443	5.14 (− 0.66–10.93)	0.082	1.19 (− 4.91–7.28)	0.702
Dual-Task walking speed (cm/s)						
ITT-Model	2.96 (− 2.81–8.72)	0.314	2.71 (− 2.90–8.32)	0.342	2.21 (− 4.06–8.47)	0.489
PP-Model	4.54 (− 1.61–10.68)	0.148	2.49 (− 3.49–8.46)	0.413	1.26 (− 5.08–7.60)	0.697
Executive function composite *z* score (lower score = better performance)						
ITT-Model	− 0.38 (− 1.03–0.27)	0.253	− 0.64 (− 1.11–0.16)	**0.008**	− 0.76 (− 1.32– − 0.21)	**0.007**
PP-Model	− 0.35 (− 1.05–0.36)	0.336	− 0.65 (− 1.14–0.16)	**0.010**	− 0.70 (− 1.25 – − 0.14)	**0.014**
Verbal memory composite z score (higher score = better performance)						
ITT-Model	0.17 (− 0.60–0.95)	0.658	0.35 (− 0.11–0.80)	0.134	0.79 (0.25–1.32)	**0.004**
PP-Model	− 0.07 (− 0.90–0.75)	0.860	0.23 (− 0.23–0.69)	0.334	0.64 (0.13–1.16)	**0.015**

Baseline; On-treatment: 24 weeks of treatment, visits 2–8; Post-treatment: 24-weeks of follow-up, visits 9–12. Comparisons of gait and cognitive outcomes between DM-INI and DM Placebo, and between Control-INI and Control-Placebo at baseline, on-treatment and post-treatment using mixed models: ITT-Model: Intention-to-Treat analysis ($n = 223$ subjects with at least baseline) and PP-Model: Per-Protocol analysis ($n = 175$ treatment compliant subjects). Estimates, confidence intervals (CI) and *p* values are the differences between DM-INI and DM-Placebo and between Control-INI and Control Placebo at each period (group and treatment period interaction), $p < 0.05$ in bold. All models had overall *p* value < 0.0001.

INI intranasal insulin, DM diabetes mellitus

[a]On-treatment DTW was not significant after adjusting for baseline difference (ITT $p = 0.812$; PP $p = 0.726$)

participants and decreased in three T2DM-Placebo subjects (Fig. 3B, i). Hypercapnia-induced CBF increase was attenuated in the anterior/middle cingulate ($p = 0.023$), inferior parietal cortex ($p = 0.024$) and posterior cingulate/precuneus cortex ($p = 0.003$) (Fig. 3A, ii and Fig. 3B, ii). Hypocapnia-induced CBF reduction was smaller in the occipital and parietal junction ($p = 0.024$; Fig. 3A iii, Fig. 3B, iii). A faster DTW was associated with increased post-treatment vasodilatation reactivity in cerebellum ($p = 0.008$; $n = 11$; Fig. 3C, i), decreased post-treatment vasoconstriction reactivity in the entire brain except the right temporal regions ($p = 0.006$; Fig. 3C, ii) and decreased post-treatment vasomotor range in the entire brain, due to decreased vasoconstriction ($p = 0.007$; Fig. 3C, iii). Statistically different changes were observed within each cluster for relative CBF ($t = 7.35$, $p < 10^{-4}$), vasodilation ($t = 4.36$, $p = 0.0018$), and vasoconstriction ($t = 5.82$, $p < 10^{-4}$). Better performance on SWM (less errors) was associated with absolute vasodilatation in occipital and parietal regions ($p = 0.041$).

Study adherence and safety

Study compliance was 78%; 175 participants self-reported medication device usage > 65% (> 109 days). DM-INI group had lower plasma insulin ($p = 0.006$) and HOMA-IR (Homeostatic Model Assessment of Insulin Resistance; $p = 0.013$) (Fig. 4 A, B). INI treatment did not change HbA_{1c}, weight, waist circumference or BMI (Fig. 4C, D), but fasting serum

Table 3 Primary cognitive and gait outcomes in the whole cohort

Variable	Baseline		On-treatment		Post-treatment	
	Estimate (CI)	*p*	Estimate (CI)	*p*	Estimate (CI)	*p*
Whole cohort: INI vs. Placebo						
Normal walking speed (cm/s)						
ITT-Model	4.28 (0.22–8.35)	**0.039**	6.42 (2.47–10.37)	**0.002**	4.33 (− 0.23–8.90)	0.062
PP-Model	3.24 (-1.19–7.67)	0.151	6.6 (2.27–10.95)	**0.003**	4.14 (− 0.55–8.83)	**0.083**
Dual-task walking speed (cm/s)						
ITT-Model	5.64 (1.38–9.89)	0.096	5.80 (1.64–9.95)	**0.006**	4.19 (− 0.61–8.99)	0.087
PP-Model	6.60 (2.03–11.2)	**0.005**	5.87 (1.42–10.3)	**0.010**	3.83 (− 1.04–8.69)	0.122
Executive function composite z score (lower score = better performance)						
ITT-Model	− 0.34 (− 0.80–0.13)	0.157	− 0.55 (− 0.88– −0.21)	**0.002**	− 0.47 (− 0.88– −0.06)	**0.026**
PP-Model	− 0.38 (− 0.88–0.12)	0.134	− 0.51 (− 0.87– −0.17)	**0.004**	− 0.43 (− 0.84–− 0.02)	**0.038**
Verbal memory composite z score (higher score = better performance)						
ITT-Model	0.18 (− 0.38–0.74)	0.521	0.40 (0.07–0.73)	**0.018**	0.60 (0.20–1.00)	**0.004**
PP-Model	0.11 (− 0.48–0.71)	0.706	0.32 (− 0.02–0.65)	0.061	0.52 (0.13–0.91)	**0.009**

INI: Intranasal Insulin; INI- treated participants; Placebo-treated participants. Baseline; On-treatment: 24 weeks of INI/Placebo treatment, visits 2–8; Post-treatment: 24 weeks of follow-up, visits 9–12. Comparisons of gait and cognitive outcomes between INI-treated and Placebo-treated participants at baseline, on-treatment and post-treatment using mixed models: ITT-Model: Intention-to-Treat analysis ($n = 223$ subjects with at least baseline) and PP-Model: Per-Protocol analysis ($n = 175$ treatment compliant subjects), adjusted for hemoglobin A1c. Estimates, confidence intervals (CI) and p-values are the differences between INI-treated participants and Placebo-treated participants at each period, p value < 0.05 in bold. Hemoglobin A1c effect p value = 0.02–0.001. All models had overall p value < 0.0001

Table 4 Subgroup analyses of cognitive and gait outcomes in pre-diabetes and normoglycemic controls

Variable	Baseline		On-treatment		Post-treatment	
	Estimate (CI)	*p*	Estimate (CI)	*p*	Estimate (CI)	*p*
Pre-diabetes: INI vs. Placebo						
Normal walking speed (cm/s)						
LMM Model	3.97 (− 5.46–13.4)	0.405	5.38 (− 3.96–14.74)	0.255	1.30 (− 8.45–11.05)	0.792
Dual-Task walking speed (cm/s)						
LMM Model	4.75 (− 5.04–14.54)	0.337	4.07 (− 5.53–13.68)	0.401	2.92 (− 7.43–13.26)	0.577
Executive function composite z score (lower score = better performance)						
LMM Model	− 0.65 (− 1.67–0.38)	0.212	− 1.09 (− 1.81–0.38)	**0.0032**	− 1.08 (− 1.89– −0.27)	**0.009**
Verbal memory composite z score (higher score = better performance)						
LMM Model	0.25 (− 0.94–1.44)	0.674	0.41 (− 0.31–1.13)	0.259	1.17 (0.36–1.98)	**0.005**
Normoglycemic Controls: INI vs. Placebo						
Normal walking speed (cm/s)						
LMM Model	1.12 (− 5.6–7.8)	0.740	3.15 (− 2.5–8.8)	0.268	3.39 (− 6.10–7.34)	0.854
Dual-Task walking speed (cm/s)						
LMM Model	0.59 (− 6.51–7.69)	0.869	0.08 (− 6.68–6.84)	0.981	− 0.51 (− 8.38–7.36)	0.898
Executive function composite z score (lower score = better performance)						
LMM Model	− 0.004 (− 0.91–0.90)	0.993	− 0.14 (− 0.79–0.50)	0.659	− 0.31 (− 1.09–0.46)	0.424
Verbal memory composite z score (higher score = better performance)						
LMM Model	0.10 (− 0.93–1.12)	0.853	0.20 (− 0.40–0.79)	0.513	0.35 (− 0.36–1.06)	0.332

INI: Intranasal Insulin. Of 117 Controls, 48 participants had pre-diabetes (baseline hemoglobin A1c (HbA1c) 5.7–6.4%) and 69 were normoglycemic (baseline HbA1c < 5.7%). Participants with pre-diabetes: 21 INI, 27 Placebo; Normoglycemic Controls: 37 INI, 32 Placebo. Baseline; On-treatment: 24 weeks of INI/Placebo treatment, visits 2–8; Post-treatment: 24 weeks of follow-up, visits 9–12. Comparisons of gait and cognitive outcomes between INI and Placebo in pre-diabetes and normoglycemic participants at baseline, on-treatment and post-treatment using linear mixed-effects model (LMM). Estimates, confidence intervals (CI) and p-values are the differences between INI-treated participants and Placebo-treated participants at each period; p value < 0.05 in bold. All models had overall p value < 0.0001

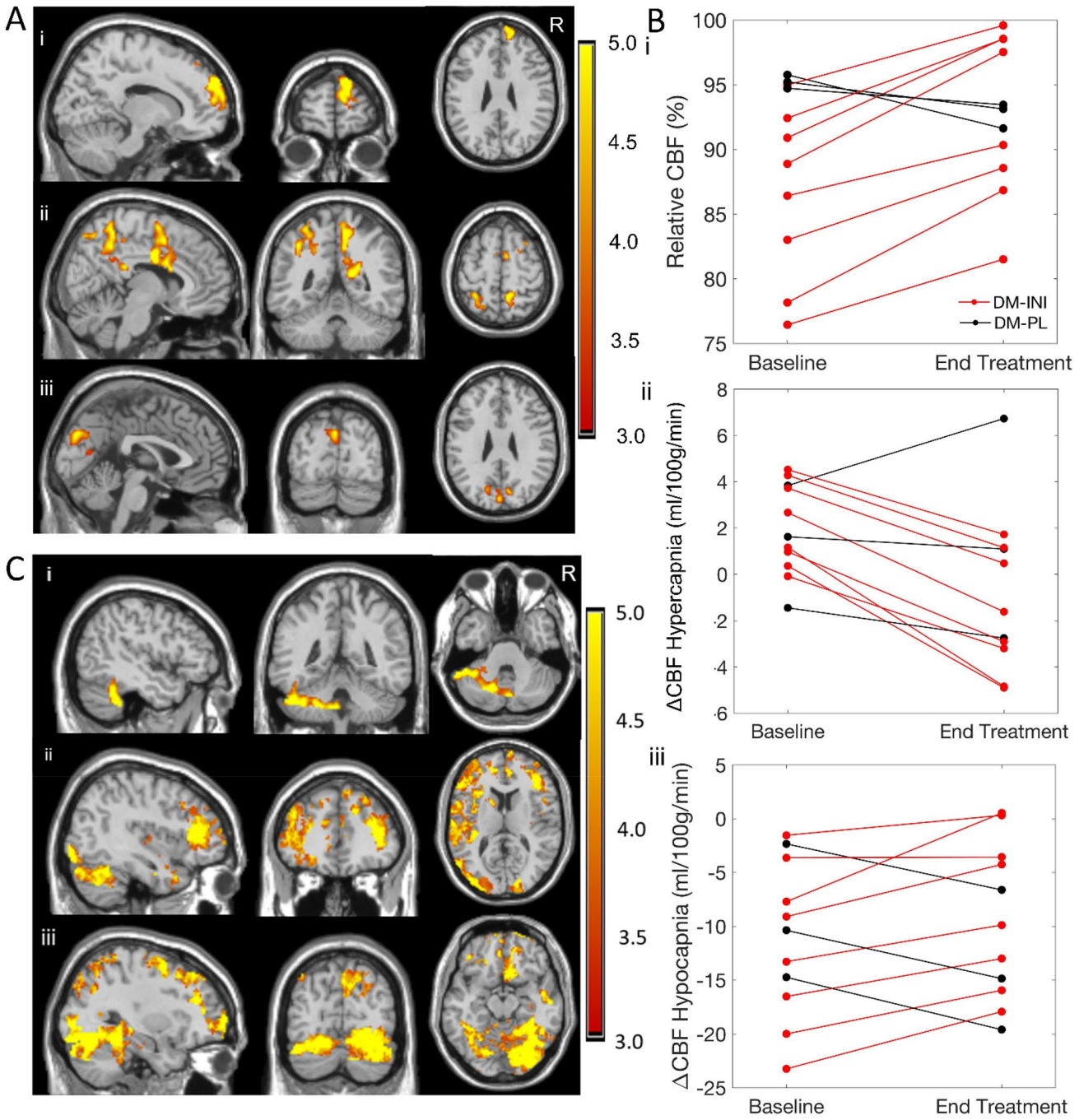


Fig. 3 Cerebral blood flow responses to intranasal insulin or placebo in participants with diabetes. **A** Cerebral blood flow (CBF) changes after intranasal insulin treatment in participants with diabetes: (i) Resting state CBF increased in the right medio-prefrontal cortex (mPFC) ($p=0.03$). (ii) Hypercapnia-induced CBF increase in the anterior/middle cingulate ($p=0.02$), inferior parietal cortex ($p=0.02$), posterior cingulate/precuneus cortex ($p=0.003$); (iii) Hypocapnia-induced CBF decrease in the occipito-parietal junction ($p=0.02$), compared to baseline. R denotes R hemisphere. **B** (i) Regional changes of relative cerebral blood flow (CBF %) in the right medio-prefrontal cortex (mPFC) in eight insulin-treated (DM-INI; red line, full circles) and three placebo-treated (DM-Placebo; black line, full circles) participants between baseline and the end-of treatment. (ii) Hypercapnia-induced CBF increase was attenuated in the posterior cingulate cortex/precuneus, and (iii) hypocapnia-induced CBF decrease was lower in the occipito-parietal junction between baseline and end-of treatment. Statistically different changes within each cluster were observed for relative CBF ($t=7.35$, $p<10^{-4}$), ΔCBF to hypercapnia ($t=4.36$, $p=0.0018$), and ΔCBF to hypocapnia ($t=5.82$, $p<10^{-4}$). **C** A faster dual-task walking speed was associated with (i) increased post-treatment vasodilatation reactivity in cerebellum ($p=0.008$), (ii) decreased post-treatment vasoconstriction reactivity the entire brain except the right temporal regions ($p=0.006$), and (iii) decreased post-treatment vasomotor range in the entire brain ($p=0.007$). R denotes the right hemisphere

glucose decreased in DM-Placebo group ($p = 0.03$) (Supplementary Table A.2.). These outcomes did not differ between Control-INI and Control-Placebo (Figure E–H). INI did not have effects on appetite, body composition and energy consumption.

Adverse events

INI treatment was not associated with any serious or moderate AEs. AE distribution was similar among the INI and placebo groups. There were 288 AEs in 145 of 244 randomized participants (Supplementary Table A.3); 134 participants had 262 AEs during treatment and follow-up. Thirteen participants were discontinued for AEs (one pre-treatment, five during INI, six during placebo treatment, one post-placebo treatment). Eight serious AEs were unrelated to treatment. Eleven mild AEs were likely related to INI (epistaxis, dizziness, headache, hypoglycemia, skin irritation, cold sore, insomnia), and two to placebo (epistaxis). Two occurred before treatment (dizziness and skin ulceration). AEs affecting > 5% of participants were flu-like symptoms (22%), falls (8%), hypoglycemia (8%), dizziness (5%) or diarrhea (5%) (Supplementary Table A.4).

Hypoglycemia rate was 7% during treatment, while the expected rate for Novolin® R subcutaneous administration is 36%. Nineteen participants experienced 23 asymptomatic hypoglycemic AEs after randomization (Supplementary Table A.5). During treatment period, 16 participants (five DM-INI, three DM-Placebo, four Control-INI, four Control-Placebo) had 19 asymptomatic hypoglycemic AEs (16 level-1 hypoglycemia, three level-2 hypoglycemia) that were recorded on CGM, SMBG or plasma. Six participants discontinued treatment for hypoglycemic AEs (two DM-INI, three DM-Placebo, one Control-INI).

Safety substudy in T2DM-IDDM

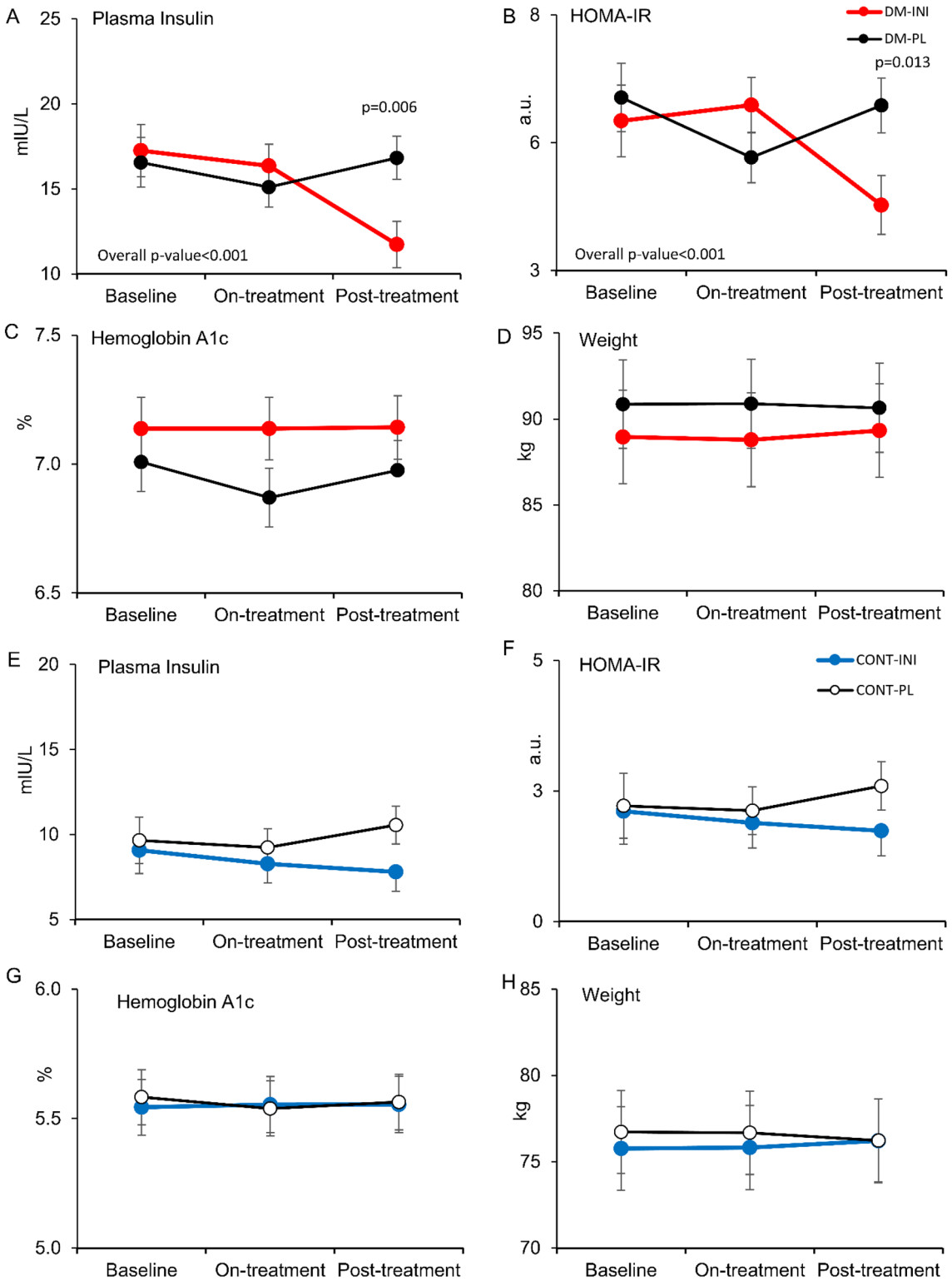
We aimed to enroll 20 T2DM-IDDM participants. We screened 86 T2DM-IDDM participants, 14 (nine INI, five Placebo) were randomized and five (two INI, three Placebo) completed treatment and follow-up (Fig A.1). Participants completed one week of continuous glucose monitoring (CGM) (Medtronic iPro2; Medtronic, Northridge CA, USA) and measured SMBG five times daily (Accu-Check®, Aviva PluF. Hoffman-La Roche Ltd, Basel, CH) [40] before and after INI/Placebo treatment initiation. Capillary glucose on CGM did not decline for 2 h after INI or placebo administration (Fig. 5 A, B). No interactions were observed between INI and subcutaneous insulins. Five T2DM–IDDM subjects had seven hypoglycemic AEs (Supplementary Table A.6). Two T2DM-IDDM subjects treated with INI had two asymptomatic level-2 hypoglycemic AEs (five episodes on CGM) and five subjects (two INI and three Placebo) had five level-1 hypoglycemic AEs. CGM-recorded hypoglycemic episodes occurred at nighttime, before INI administration (done in the morning). Distribution of hypoglycemic episodes on CGM was similar between INI and placebo, and between baseline and the first week on treatment. In two INI-treated participants, HbA_{1c}, fasting plasma and capillary glucose declined from baseline, but the average values during treatment and follow-up were similar to the three placebo-treated participants (Supplementary Table A.6).

Discussion

MemAID Phase 2 trial provided preliminary evidence for positive INI effects on cognition and gait. INI-treated T2DM participants demonstrated faster walking speed, increased cerebral blood flow and lower plasma insulin and insulin resistance (HOMA-IR) as compared to placebo, while INI-treated controls showed improved executive functioning, verbal memory and borderline faster NW. In the combined cohort of INI-treated participants, overall INI effect demonstrated faster walking and better executive functioning and memory. In the Control group, INI-treated participants with pre-diabetes demonstrated better executive functioning and memory. The four main outcomes and the individual outcomes all showed trends toward improvement on INI treatment with clinically relevant effect sizes.

At baseline, participants with diabetes walked slower, had worse cognition, microvascular disease, neuropathy and moderate disability. Controls served as a clinical reference for normal aging population with preserved cognition, mobility and functionality (overweight, 41% pre-diabetic, 30% hypertensive, none-to-mild disability). DM-INI group had faster normal and dual-task walking during treatment and post-treatment, as compared to DM-Placebo, likely due to an improvement of brain perfusion [12, 22, 24]. For NW, the DM-INI effect was ~ 6.5 cm/s for NW and ~ 5.28 cm/s for Control-INI on-treatment. We also analyzed the INI effects in the combined cohort of T2DM and control participants, adjusting for HbA_{1c} (range 4.8–11.8% from normal, pre-diabetes, controlled and uncontrolled diabetes). INI-treated participants walked faster on-treatment (NW ~ 6.42 cm/s; DTW ~ 5.8 cm/s). Walking speed is an important vital sign in the elderly and slows down ~ 1 cm/s per year (from 112 cm/s at age 50 to 84 cm/s for 80 + years old) [41]. Therefore, in our cohort of 50–85 year olds with DM and control participants, we would expect an average decline of walking by ~ 0.5 cm/s or more per 6 month period. Slower walking is a clinical predictor of well-being that correlates with cognitive decline [27], hospitalizations, disability and death [25, 42].

The Control-INI group performed better on executive functioning during treatment and post-treatment compared



to Control-Placebo. They made fewer errors and exhibited a better decision-making strategy on visuospatial tasks. For executive function, INI effect on-treatment (-0.64) was larger by 68% (− 0.64 + 0.38)/(− 0.38) × 100) than the baseline INI-placebo difference (− 0.38) and was larger by 106% for verbal memory (0.35 vs. 0.17)(Table 2). For the overall combined effect of INI (Table 3, ITT-Model), INI effect on-treatment was larger than INI-placebo difference at baseline (− 0.55 vs. − 0.34) by 62% and for verbal memory (0.40 vs. 0.18) by 122%. INI-treated participants with pre-diabetes

◂**Fig. 4** Metabolic outcome variables for Diabetes-Intranasal Insulin and Diabetes-Placebo groups, and for Control-Intranasal Insulin and Control-Placebo groups. Safety outcome variables: plasma insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), hemoglobin A1c and weight for diabetes (**A**–**D**) and control (**E**–**H**) groups at baseline, during 24 weeks of treatment (on-treatment, visit 2–8) treatment and during 24 weeks of follow-up (post-treatment, visit 9–12). Graphs show the estimates of the intention-to-treat models (mean ± SE) for each variable. *P* values reflect the interaction between group and time period e.g. diabetes-intranasal insulin (DM-INI) vs. diabetes-placebo (DM-Placebo) and control-intranasal insulin (Control-INI) vs. control-placebo (Control-Placebo) at baseline, during treatment and post-treatment. Overall p-value < 0.0001 denotes significance of the whole intention-to-treat model calculated separately for each variable. **A** Plasma insulin declined after intranasal insulin treatment in diabetes-intranasal insulin group (DM-INI; red line, full circles), compared to diabetes-placebo group (DM-Placebo; black line, full circles) ($p = 0.006$). **B** HOMA-IR declined after intranasal insulin treatment in DM-INI group, compared to DM-Placebo group ($p = 0.013$). **C** Hemoglobin A1c did not differ between DM-INI and DM-Placebo at baseline, during treatment and post-treatment. **D** Weight was not different between DM-INI and DM-Placebo group. **E** Plasma insulin was not different between control-intranasal insulin group (Control-INI; blue line, full circles) and control-placebo group (Control-Placebo; black line, empty circles) at baseline, during treatment and post-treatment. **F** HOMA-IR was not different between Control-INI and Control-Placebo. **G** Hemoglobin A1c was not different between Control-INI and Control-Placebo group. **H** Weight was not different between Control-INI and Control-Placebo

performed better on executing functioning and verbal memory than placebo-treated participants.

In T2DM participants, resting CBF increased after INI-treatment in the right mPFC cortex, while CBF declined after Placebo-treatment. On-INI improvement of regional perfusion was consistent with other studies in non-diabetic participants [17, 18]. A faster DTW on INI treatment was associated with increased vasodilatation reactivity and decreased vasoconstriction reactivity in cerebellum, motor and visuospatial processing regions. DTW is a complex daily task that involves interactions among visuospatial, motor, memory and executive function networks. T2DM was associated with decreased CBF in the resting state default mode, visual and cerebellum networks. T2DM-related resting state perfusion pattern involved basal ganglia, insular, frontal and temporal cortex and limbic system and was associated walking speed and cognitive performance [34]. Our results suggest that INI may improve perfusion deficit in the prefrontal cortex in T2DM.

Attenuation of vasodilatation responses in the cingulate may be related to CBF increases in mPFC, due to connections between limbic system and pre-frontal cortex and to their role in modulating emotions and cognitive functions. T2DM participants had exaggerated vasoconstriction reactivity (about twice as much than non-diabetic controls), which correlated with intracellular adhesion molecules in the frontal, temporal parietal and occipital regions, and they also had reduced vasodilatation reactivity which correlated with vascular adhesion molecules in the parietal and occipital regions [36]. Lower baseline vasoreactivity was associated with slower NW and DTW over the 2 year period [26]. Therefore, in persons with diabetes a lower resting state perfusion [43], worse vasoreactivity and functional connectivity [23, 34] were associated with vascular inflammation and microvascular disease [36], which correlated with worse cognitive performance [2] and slower walking [26]. In addition, diabetic peripheral neuropathy was associated with gray matter and cerebellar atrophy, reduced functional connectivity between frontal lobe and motor areas and worse executive function [44, 45]. Possible beneficial effects of INI treatment on vasoreactivity in T2DM need confirmation in larger studies.

Insulin resistance negatively affects learning and executive function in normal aging, pre-diabetes and diabetes [8, 22]. In pre-diabetes, HbA_{1c} correlates with reduction of insular gray matter and in diabetes with cerebellar gray matter atrophy and decreased integrity of frontal lobe white matter pathways [46]. Therefore, the age and diabetes-related structural changes in the gray and white matter, insulin resistance and microvascular disease may limit responses to INI treatment. In T2DM participants, serum insulin and HOMA-IR declined after INI treatment, suggesting a decrease in insulin resistance. A dose of 160 IU of human insulin improved whole-body insulin sensitivity, as assessed by the hyperinsulinemic-euglycemic clamp. Insulin-sensitizing effect correlated with increased CBF in hypothalamus, larger blood flow fluctuations in insular cortex, and increased heart rate variability (an estimate of parasympathetic autonomic nervous system activity) suggests modulation of central metabolic and homeostatic activity by INI [19, 47]. INI-induced decline in insulin resistance or increased whole-body insulin sensitivity and/or less endogenous insulin production [19, 47] reflects pleiotrophic effects of INI on hypothalamic energy metabolism regulation, perfusion and neuroprotection [5–8]. Therefore, improvement of insulin sensitivity by INI may potentially have protective effects for prevention of dementia in T2DM [48].

Response to INI may depend upon the dose, treatment duration and method to effectively deliver INI to the olfactory epithelium in the upper nasal cavity, which warrants further investigation. INI dose of 40 IU increased regional blood flow and brain energy [17, 18] and modulated activity in the hypothalamus and orbitofrontal cortex [10]. INI modulates signaling in insular, cortex, mesolimbic system and hypothalamus [22]. High 160 IU dose increased functional connectivity between mPFC and midbrain in people with high insulin resistance, but the effect declined after an hour and was similar to 40 IU dose response [20], suggesting interactions among the dose, insulin resistance and time course of the response. A 160 IU dose decreased blood

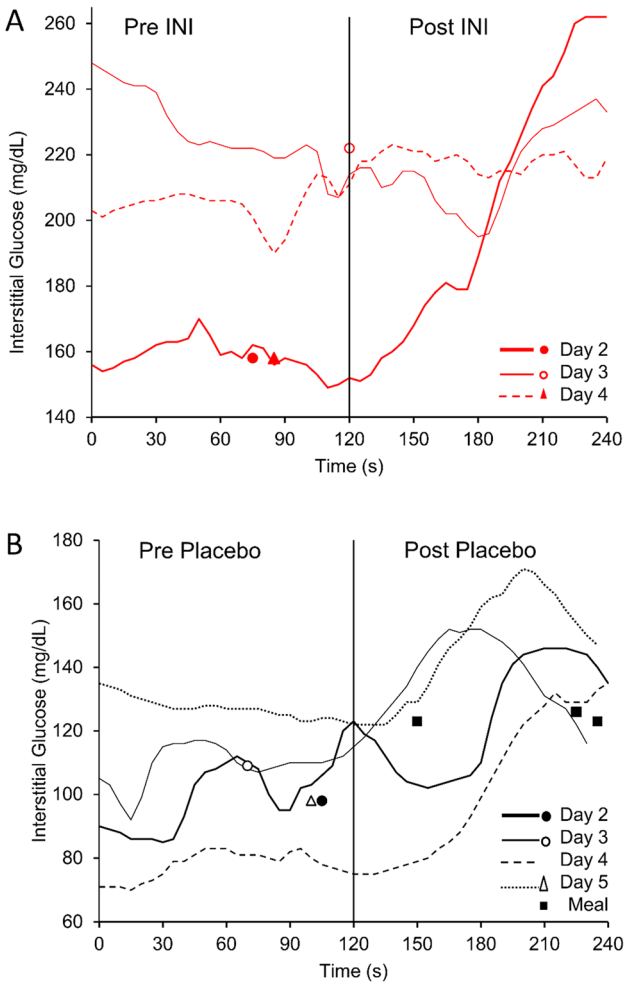


Fig. 5 Interstitial glucose recordings before and after intranasal insulin or placebo administration. Interstitial glucose recordings during continuous glucose monitoring (CGM) for 2 h before and after intranasal insulin (INI) administration in INI-treated T2DM-IDDM participant (**A**) and in placebo-treated T2DM-IDDM participant (**B**). **A** Intranasal insulin-treated T2DM-IDDM treated participant (INI-1) completed CGM monitoring for four days with self-monitored plasma glucose (SMBG). Day 2: CGM red solid thick line, SMBG red solid circle; Day 3: red solid thin line, red empty circle; Day 4: red dashed line, red triangle. **B** Placebo-treated T2DM-IDDM treated participant (PL-4) completed CGM monitoring for five days with SMBG. Day 2: CGM black solid thick line, SMBG black solid circle; Day 3: black solid thin line, black empty circle; Day 4: black dashed line, no SMBG; Day 5: black dotted line; black empty triangle. Black squares indicate SMBG before meals on Days 3–5

flow to limbic system regions [49]. However, these studies varied in the INI dose, insulin type (short-intermediate-long acting), method of delivery, MRI analyses and functional assessments [50]. Future longitudinal studies using dose–response design and effective nose-to-brain delivery methods are needed to detect thresholds for INI efficacy which may be different in older people with and without DM.

Safety and limitations

MemAID trial findings, although of potential clinical significance, demonstrated potential for improvement of cognition and gait but not for disability and daily living activities in INI-treated subjects. Due to COVID-19 pandemic, 23 on-site assessments and seven end-of treatment MRI scans were stopped and diabetes groups remained under-enrolled. Analyses of combined cohort (INI-treated vs. Placebo-treated participants) have overcome this limitation and have shown positive INI effects on the main outcomes. INI treatment was safe and was not associated with severe AEs or hypoglycemia, consistently with previous studies [51]. T2DM-IDDM subgroup had a highest rate of screen failures due to exclusion criteria and lack of interest in participating, and a high drop-out rate. INI treatment in T2DM-IDDM did not show risk of hypoglycemia or interactions with subcutaneous insulins, despite of intensive glucose lowering therapy. The study population was well-educated, had normal walking speeds and minimal cognitive impairment. Gait speed could have been influenced by participants acclimating to the testing environment. These factors may have limited the observed INI differences and may affect the generalizability of results.

Conclusions

The MemAID trial provided evidence for positive INI effects on cognition and gait in older people with and without T2DM and proof-of-concept for preliminary safety and efficacy.

INI-treated diabetic participants had faster walking speed, increased cerebral blood flow and less insulin resistance, while INI-treated controls performed better on executive function and verbal memory tasks. Overall, INI effect demonstrated improvements of walking speed, executive function and verbal memory. These findings are clinically relevant and warrant further investigation in a larger clinical trial.

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Author contributions VN: designed the study and obtained funding, overseen all aspects of the study conduct, experiments, and writing the manuscript. CSM, LN, PN and WD: contributed to study design, conduct and manuscript preparation. LN: designed and conducted analyses, overseen data processing and integrity. WD: designed and analyzed MRI data. PN: was BWH-site PI. RMG: was Harvard Study Site PI. RMG, CBF: overseen neuropsychological testing. FK and LAB: contributed to study and conduct analysis. SB, VL, PN: were study physicians. All authors contributed to the data interpretation and manuscript preparation. VN and LN: are the guarantors of this work, had full access to all of the data from the study, and take responsibility of the data and accuracy of the data analyses.

Declarations

Conflicts of interest V. Novak, R. McGlinchey, C. B. Fortier, W. Dai, F. Khan and Laura Aponte Becerra report no disclosures relevant to the manuscript. C.S. Mantzoros provided consultations for Coherus, Redwood City CA, AltrixBio Cambridge, MA, California Walnut Commission, Folsom, CA, Genfit, Cambridge MA, Regeneron, Westchester NY, Ansh, Webster TX, Amgen, Thousand Oaks CA, Intercept, New York, NY, Aegerion, Cambridge, MA, AltrixBio, Cambridge MA, and has provided educational services through Elsevier, New York, NY, CMHC, Baton Rouge, FL, TMIOA, Tarzana, CA (all unrelated to this project since 2015-2020). C.S. Mantzoros provided consultations for Novo Nordisk, Inc. Bagsværd, Denmark on Obesity Advisory Board, and has received grant support through BIDMC, which could be considered as related to this project given that Novo Nordisk, Inc. provided medication. L. Ngo provided consultation to the Radiological Society; to the Journal of Cardiovascular Magnetic Resonance; to Five Island Consulting LLC, Georgetown ME; and to Vinmec Inc. Hanoi, Vietnam between 2015 and 2020. P. Novak is advisor—independent contractor for Dysimmune Diseases Foundation, Boston MA, USA. P. Novak received speaker's honoraria from KabaFusion, Cerritos, CA, USA and Lundbeck, Copenhagen, Denmark. He is a member of the Scientific Advisory Board of Endonovo Therapeutics, Woodland Hills, CA, USA. He obtained royalties from Oxford Press, Oxford, UK. V. Lioutas provided consultation for Qmetis, New York, NY, USA since 2020, unrelated to this project. S. Buss provided consultation for Kinto Care, Cambridge, MA, USA between 2019 and 2020, unrelated to this project.

Ethical standard statement Ethocal state was added in section Standard protocol approvals, registrations, and patient consents. This study was carried out in accordance with the recommendations of ethical standards of the BIDMC, BWH and Harvard Medical School. The BIDMC Committee on Clinical Investigation, BWH and Harvard Catalyst CEDE reviewed and approved the study. All participants signed the informed consent in accordance with the Declaration of Helsinki.

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