Enhancement of Vasoreactivity and Cognition by Intranasal Insulin in Type 2 Diabetes

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Short title: Intranasal insulin effects on cognition

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Total words: Abstract: 242; MS Characters in title: 73; Figures: 2 Tables 2
Current word count: 3925
Abstract

**Objective:** To determine acute effects of intranasal insulin on regional cerebral perfusion and cognition in older adults with type 2 diabetes.

**Research design and methods:** This was a proof-of-concept, randomized, double-blind, placebo-controlled intervention evaluating the effects of a single 40IU dose of insulin or saline on vasoreactivity and cognition in 15 diabetic and 14 control subjects. Measurements included regional perfusion, vasodilatation to hypercapnia at 3 Tesla MRI and neuropsychological evaluation.

**Results:** Intranasal insulin administration was well tolerated and did not affect systemic glucose levels. No serious adverse events were reported. Across all subjects, intranasal insulin improved visuospatial memory ($p<0.05$). In the diabetes group, an increase of perfusion after insulin administration was greater in the insular cortex as compared to the control group ($p=0.0003$). Cognitive performance following insulin administration was related to regional vasoreactivity. Improvements of visuospatial memory after insulin administration in the diabetes group ($R^2_{adj}=0.44$, $p=0.0098$) and of verbal fluency test in the control group ($R^2_{adj}=0.64$, $p=0.0087$) were correlated with vasodilatation in the middle cerebral artery territory.

**Conclusions:** Intranasal insulin administration appears safe and does not affect systemic glucose control, and may provide acute improvements of cognitive function in patients with type 2 diabetes, potentially through vasoreactivity mechanisms. Intranasal insulin-induced changes in cognitive function may be related to vasodilatation in the anterior brain regions, such as insular cortex that regulates attention-related task performance. Larger studies are warranted to
identify long-term effects and predictors of positive cognitive response to intranasal insulin therapy.
Introduction

Type 2 diabetes mellitus (DM) is a major risk factor for Alzheimer's and vascular dementia. Associated brain atrophy is widespread and generalized, advancing brain age (1) and accelerating cognitive decline in older diabetic populations (2),(3),(4). Although the underlying pathophysiology of gray matter atrophy is complicated, hyperglycemia-induced small vessel disease is a potential pathway for altered neurovascular coupling, impaired vasoreactivity and regional hypoperfusion (5),(6),(7), and neurotoxicity (8). Typically, vasodilatatory responses to hypercapnia or cognitive task performance are diminished in multiple brain regions (1),(6).

Insulin plays an important role in the brain as a neuromodulator. Central insulin receptors are abundant, yet are mostly dependent upon insulin transport through the blood-brain barrier. Therefore, inadequate insulin delivery may affect perfusion and cortical activity in associative regions with high energy demands, such as cognitive networks (9). Clinical studies suggest that augmenting cerebral insulin may enhance cognitive function and memory in healthy young and older adults and in cognitively impaired non-diabetic people with both acute and chronic intranasal administration (10),(11),(12). Intranasal administration of insulin delivers the compound to the brain, thus bypassing the blood-brain barrier and avoiding systemic effects (13). Intranasal insulin increases rapidly in cerebrospinal fluid and binds to receptors along trigeminal and autonomic pathways in the frontal lobe, limbic system, hypothalamus and other areas (14),(15).

We aimed to determine the acute effects of intranasal insulin on regional perfusion, vasoreactivity and cognition in older adults with and without type 2 diabetes in a proof-of-concept, double-blind, placebo-controlled, cross-over study. We hypothesized that intranasal insulin improves acutely regional perfusion, and that improvement of cognition may be
dependent upon regional vasoreactivity in older diabetic adults, as compared to non-diabetic adults and to placebo treatment.

**Research Design and Methods**

This was a single-center, randomized, double-blind, placebo-controlled safety and efficacy pilot intervention with cross-over assignment (FDA-IND 107690; www.clinicaltrials.gov NCT01206322) to evaluate acute effects of intranasal insulin on regional vasoreactivity and cognition in older diabetic and non-diabetic adults. Primary end-points were insulin-related changes in regional perfusion, vasoreactivity to CO₂ challenges and cognitive exam scores in the diabetes group as compared with placebo and with the control group. As no preliminary data on the effects of intranasal insulin on these endpoints in diabetic subjects were available at the time of study design, we based our vasoreactivity estimates on perfusion response to hypoglycemia (16) and our cognitive outcome estimates on intranasal insulin studies in non-diabetic subjects (10),(11),(17). We estimated that a total of 60 subjects would be needed to detect a 10% improvement in cognitive performance with 81% power, alpha 0.05.

**Subjects**

Studies were conducted at the Syncope and Falls in the Elderly (SAFE) Laboratory, the Center for Advanced MR imaging and the Clinical Research Center (CRC) at Beth Israel Deaconess Medical Center (BIDMC). This study was approved by the BIDMC Review Board. Participants were recruited prospectively via advertisements in the local community. Of 262 participants screened over the phone, 94 were eligible and 64 completed a screening visit and provided
written informed consent. Of these, 29 (15 diabetic and 14 control subjects) completed the protocol (Table 1), 28 were excluded and seven withdrew consent.

Diabetic participants were included if they were diagnosed with type 2 DM for > 5 years and treated with oral antidiabetic agents). Controls were required to be normotensive, have fasting blood glucose <100 mg/dL and not be treated for any systemic disease, including hypertension.

Exclusion criteria were: type 1 DM, insulin treatment or allergy, hypoglycemia, intranasal medications, clinically significant heart disease, arrhythmias, nephropathy, malignancies, strokes, major surgery within 6 months, uncontrolled hypertension, sub-threshold Mini Mental Status Exam (MMSE) scores (≥3 points below the comparative normal value for the subject’s age group and education level, or ≤ 24), current recreational drug or alcohol abuse, morbid obesity (BMI >40), claustrophobia, or 3T MRI-incompatible metal implants, pacemakers or arterial stents.

On-site screening included: fasting laboratory chemistries, electrocardiogram (ECG), vital signs, detailed medical history and medication review, anthropometric measurements and transcranial Doppler (TCD) insonation assessment. Of 64 subjects who completed screening visit, seven participants withdrew consent and 27 participants were found ineligible, and one control subject presented with elevated blood pressure upon CRC admission and after insulin administration and was therefore excluded from the study for untreated hypertension (data not included in the analyses). All exclusions of study participants occurred before randomization during the screening phase, except for one participant who was excluded after randomization.

Participants were excluded for the following reasons: diagnosis of DM <5 years (n=3), insulin treatment (n=1), intranasal medication usage (n=1), abnormal laboratory results (n=3), controls with HbA1C ≥6% (n=4), uncontrolled hypertension (n=4), sub-threshold MMSE scores (n=2),
psychological disorder (n=1), brain biopsy surgery (n=1), substance abuse (n=1), MRI-incompatible stents (n=1), hypoglycemic episodes during home monitoring (n=2), health care provider disapproval (n=1) and lost to follow-up (n=3).

Protocol

Studies were conducted at the CRC at BIDMC. Diabetic subjects monitored their BP and glucose via finger-stick four times daily for three days prior to admission while following their usual medication regimen. On CRC admission Day 1, participants completed a baseline neuropsychological assessment. They adhered to a diabetic diet and were fasting from midnight until the protocol completion on Day 2. Protocols for Day 2 and Day 3 included fasting blood draws; glucose, vital signs and cerebrovascular monitoring; insulin/placebo administration; anatomical and perfusion MR imaging and cognitive assessment (Table 2). Glycemic control and other medications were allowed during the study, but were held in the morning before the intervention, MRI and cognitive testing. Medications were administered at a usual dose after the completion of these procedures on Day 2 and Day 3. The medication classes included: glycemic control agents ((biguanides (metformin), sulfonylureas (glyburide, glipizide, glimepiride) and thiazolidinediones (pioglitazone)), antihypertensive and other prescribed medications.

Glucose, cardiovascular and cerebrovascular monitoring

Interstitial (via finger stick) and intravenous glucose were measured after an overnight fast and at 10, 40 and 60 minute intervals during the protocol with insulin or placebo administration and before each meal afterwards. ECG, BP using both sphygmomanometer and beat-to-beat
(Portapres®, Finapres Medical Systems, NL) instrumentation, end tidal CO₂ (Capnomac Ultima, Datex-Ohmeda, Madison WI) and blood flow velocities in the anterior (ACA) and middle cerebral (MCA) arteries (TCD System Spencer Technologies, Seattle, WA) were continuously monitored during a 10-minute baseline period, throughout insulin/placebo administration and for five minutes post-administration. Vitals signs were also monitored during MRI using a Medrad® Veris® MR Vital Signs Monitor (Warrendale, PA).

**Insulin/placebo administration**

Intranasal insulin (Novolin® Novo Nordisk) or sterile saline were administered in random order as determined by a random numbers generator on Day 2 or Day 3 with cross-over assignment. Insulin administration contained 40 IU of insulin mixed with 0.4 ml of saline and an additional residual volume of 0.66ml (30IU of insulin mixed with 0.33 ml of saline) required for ViaNase electronic atomizers (Kurve Technologies, Seattle, WA). The placebo contained an equivalent volume of sterile saline.

**Magnetic Resonance Imaging**

Anatomical and perfusion studies were performed on a 3-Tesla GE HDx MRI scanner (GE Medical Systems, Milwaukee, WI) using the 3-D magnetization-prepared rapid gradient echo (MP-RAGE) and 3-D continuous arterial spin labeling (CASL). After a localizer scan, perfusion scans were taken during normocapnia (6 min and 2 min), hypercapnia (2 min) and hypocapnia (2 min). To induce hypercapnia, subjects breathed a mixture of 5% CO₂ and 95% air to increase CO₂ up to 45 mm Hg using a rebreathing circuit. To induce hypocapnia, subjects hyperventilated to reduce CO₂ to 25 mm Hg. Images were analyzed using tools developed in interactive data.
language (IDL, Research Systems, Boulder, Colorado, USA) and MATLAB (MathWorks, Natick, Massachusetts, USA).

Anatomical MR images (MP-RAGE) were co-registered non-linearly to the MNI152 standard template, co-registered with perfusion images and segmented to calculate regional gray and white matter and cerebrospinal fluid volumes and perfusion in anatomical regions and vascular territories (SPM, University College London, UK) (18),(19). Voxel-based analyses were conducted on baseline perfusion images using the spatial smoothing with a three-dimensional isotropic Gaussian kernel size (FWHM, 8 mm). Voxel-wise analyses (20) compared the subtraction results insulin and placebo administration for each subject, using an independent Student’s t-test. The significant threshold was set to uncorrected voxel-level $p < 0.001$ and the continuous voxel number >10. Vasoreactivity was assessed as vasodilatation, vasoconstriction and vasoreactivity rate. Vasodilatation was calculated as a change in perfusion between baseline and hypercapnia divided by change of CO$_2$; vasoconstriction was calculated as a change in perfusion between baseline and hypocapnia and vasoreactivity rate was calculated as a slope of regression between baseline, hypocapnia and hypercapnia for each subject within brain regions of interest (21),(6).

**Neuropsychological Assessment**

Baseline assessment included measures of verbal learning (Hopkins Verbal Learning Test-Revised (HVLT-R)), executive function (Trail-Making Tests A and B, Digit Span), visual memory (Rey-Osterrieth Complex Figure Test (ROCFT)) and MMSE. Testing on insulin vs. placebo (Day 2 and Day 3) had to be completed within a short time-frame of two hours after insulin
administration because of insulin pharmacokinetics (22),(10),(11). Therefore, we selected a brief battery of parallel versions of the Brief Visuospatial Memory Test-Revised (BVMT) and the verbal fluency measures (FAS, Category, and Switching conditions) of the Delis-Kaplan Executive Function System (D-KEFS) assessment, which have previously shown sensitivity to cognitive changes in similar populations (23),(24).

Data and Statistical Analysis

All variables were summarized using descriptive statistics and compared between groups using one-way ANOVA, non-parametric tests and the least square models (LS). Insulin and placebo conditions were compared within each group and within the entire cohort using a paired t-test. Dependent BVMT variables reported as age-adjusted T scores were performances on each of the three immediate recall trials (T1, T2 and T3), the total learning score across the three immediate recall trials (Total Recall), delayed recall (Delayed Recall), and the change in performance from immediate recall to delayed recall trials (Learning). Performances on the FAS, Category and Switching verbal fluency trials were also reported as age and education-adjusted T scores. A composite verbal fluency score was created by averaging the T scores of the three trials (JMP Pro, 10.0.0 SAS Institute Cary NC). LS models were also used to evaluate the relationships between perfusion and vasoreactivity and cognition. LS models were calculated separate within group and condition (e.g. diabetes group on insulin) for each variable to minimize multiple comparisons effects. BVMT and verbal fluency T scores were included as dependent variables and model effects included age, sex and regional perfusion or vasoreactivity. Education and the order of insulin/placebo administration were investigated as potential covariates. Specific to perfusion models, the effects of hematocrit and CO₂ were also
tested. Conservatively, we selected models with $R^2 > 0.25$, and $p < 0.05$. In the paper we present $R^2_{adj}$ adjusted for model covariates. Nominal observed $p$-values are reported without adjustment for multiple testing in this small proof of concept study.

Results

**Demographic and baseline cognitive characteristics**

Baseline group characteristics were similar per inclusion criteria (Table 1). Baseline cognitive testing conducted on Day 1 showed that the diabetic group performed worse than the control group on verbal learning measures (HVLT-R learning was borderline, $p = 0.052$; delayed recall, $R^2_{adj} = 0.31$ $p = 0.008$; retention, $R^2_{adj} = 0.21$ $p = 0.046$; and $R^2_{adj} = 0.1$ recognition, $p = 0.038$), processing speed (Trail Making Test A, $R^2_{adj} = 0.2$ $p = 0.01$) and executive function (Trail Making Test B, $R^2_{adj} = 24$ $p = 0.005$) (LS models adjusted for education years), had fewer years of education ($p = 0.04$) and lower global gray matter volume ($p = 0.02$).

**Safety monitoring and adverse events**

The protocol was well tolerated, and there were no serious adverse events. Six controls and 11 diabetic subjects received insulin on Day 2. There were no hypoglycemic episodes, nasal irritation or allergic reactions to insulin. Table 2 summarizes the time course of glucose (intravenous and fingerstick), cardiovascular vital signs between insulin vs. placebo conditions which were similar within each group. Glucose levels and vital signs were stable and similar across insulin and placebo conditions in both groups. The difference between insulin vs. placebo conditions was also similar for both groups. Blood sample collection times and cognitive testing...
administration times did not differ between insulin and placebo. Blood flow velocities in the ACA and MCA, measured by TCD, declined during administration in both insulin and placebo conditions for controls and diabetic subjects by 9% (p=0.05-0.001) but returned to baseline within 5 minutes after administration.

**Brief Visuospatial Learning and Memory Test Revised (BVMT)**

BVMT performances after insulin administration tended to be higher than on-placebo performances, and control subjects performed better than diabetic subjects. Overall, controls on insulin performed better than the diabetes group on insulin and on placebo, on measures of immediate recall Trials 2-3 (T2, T3) and total learning (Total Recall) (Figure 1). On the BVMT, control subjects on insulin were the highest-scoring subgroup, while diabetic subjects on placebo scored the lowest. This relationship was observed for immediate recall T2 (LS model adjusted for age $R^2_{adj} = 0.14, p=0.029$; controls on insulin compared to diabetes group on placebo $p<0.01$), T3 ($R^2_{adj} = 0.14, p=0.026$) and Total Recall ($R^2_{adj} =0.18, p=0.02$). These effects remained similar after adjusting for potential confounding effects of education on immediate recall T2 ($R^2_{adj}=0.12 p=0.017$) and T3 ($R^2_{adj}= 0.1 p=0.029$) (LS model age, education adjusted). The effect of education was not significant in these models. For the whole cohort, the performance on insulin improved as compared to placebo on T2 (p=0.04) and borderline for Total Recall (paired t-test, p=0.052). In both groups subjects were also better able to correctly identify target figures on insulin than on placebo (paired t-test, raw scores, p=0.02) and registered fewer false alarms (paired t-test, raw scores, p=0.05), though normative data for these measures was highly-skewed in the test population and no T scores were available.
Verbal Fluency

Verbal fluency performances after insulin administration tended to be higher than on-placebo performances. Controls on insulin performed better than diabetic subjects on insulin on FAS (LS model adjusted for age $R^2_{\text{adj}} = 0.26$, $p=0.0045$; LS model adjusted for age and education $R^2_{\text{adj}} = 0.25$, $p=0.018$); switching ($R^2_{\text{adj}} = 0.2$, $p=0.006$; $R^2_{\text{adj}} = 0.17$, $p=0.012$) and composite verbal fluency ($R^2_{\text{adj}} = 0.12$, $p=0.02$; $R^2_{\text{adj}} = 0.11$, $p=0.049$). On placebo, controls were better only on FAS, but not other verbal fluency measures (LS model adjusted age, education $R^2_{\text{adj}} = 0.27$, $p=0.019$). The effect of education was not significant in the models. There was no difference in performance comparing insulin to placebo conditions within groups.

Regional perfusion and vasoreactivity

Regionally, changes in perfusion and vasoreactivity after insulin administration were observed in the MCA territory which contains the insular cortex and integrative areas for learning, memory and language within the temporal and parietal lobes. Baseline perfusion was lower in the diabetes group in the insular cortex ($p=0.039$) as compared to controls (Table 2). In the diabetes group, perfusion in the right insular cortex increased after insulin administration ($p=0.001$) as compared to placebo. Voxel-based analyses have shown that increase of perfusion on insulin was greater in the diabetes group as compared to the control group ($p=0.0003$) (Figure 2A, Table 2). Perfusion did not differ in other regions.
Associations between perfusion, vasoreactivity and cognition

In the whole cohort, cognitive performance on the BVMT and verbal fluency measures upon insulin administration was related to perfusion and vasodilatation within the MCA territory, and specifically to the insular cortex that regulates attention-related task performance. Across all subjects, perfusion increases after insulin administration within the MCA territory were associated with an improvement of BVMT T3, and for the BVMT Delayed Recall in the right MCA territory ($R_{adj}^2 = 0.28$, $p=0.04$) and also in the insular cortex ($R_{adj}^2 =0.22$, $p=0.04$) (LS model age, sex, group). Following insulin administration in the diabetes group, better visuospatial memory correlated with vasodilatation in the MCA territory for immediate recall T2 ($R_{adj}^2=0.43$, $p=0.01$); BVMT T3 ($R_{adj}^2 =0.39$, $p=0.035$) and Total Recall ($R_{adj}^2=0.44$, $p=0.0098$) (LS models age, gender, vasodilatation in leptomeningeal MCA territory) (Figure 2B). These relationships were not observed following placebo administration, as shown in Figure 2C for Total Recall ($R_{adj}^2=-0.14$, $p=0.34$) (LS models age, gender, vasodilatation in leptomeningeal MCA territory).

A similar trend was observed between BVMT immediate recall (T2, T3) and Total Recall vasodilatation in the whole ACA territory ($p=0.05$-$0.08$). Following insulin administration within the control group, better performance on BVMT immediate recall T3 was also related to MCA vasodilatation ($R_{adj}^2=0.4$, $p=0.035$). This relationship between visuospatial memory and vasodilatation was not observed following placebo administration in either group.

In controls on insulin, FAS score ($R_{adj}^2=0.39$, $p=0.04$) and the composite verbal fluency measure ($R_{adj}^2=0.18$, $p=0.045$) were associated with greater vasodilatation in the right insular cortex (model adjusted for age). In controls on insulin, category performance was associated with greater vasodilatation in the right MCA ($p=0.027$) and decreased vasodilation in the left MCA ($p=0.024$) ($R^2 =0.75$, $R_{adj}^2=0.64$, $p=0.0087$, LS model age, gender adjusted) (Figure 2D), and
also greater left-right difference in vasodilatation in the insular cortex ($R^2 = 0.75$, $R^2_{adj} = 0.68$, $p=0.0023$). In the diabetes group on insulin, FAS scores were also associated with more vasodilatation in the left ($p=0.02$) and lesser vasodilatation in the right insular cortex ($R^2_{adj} = 0.26$, $p=0.04$, LS model age, gender adjusted).

**Discussion**

This proof-of-concept study evaluated the acute effects of a single dose of intranasal insulin as compared to placebo on vasoreactivity and cognition in older diabetic and control adults using a randomized cross-over design. The intranasal administration of insulin was safe, with no serious adverse events or hypoglycemic episodes and the protocol was feasible for participants. The diabetes group presented with mild cognitive deficits in learning, retention and executive function. Insulin administration improved visuospatial memory and verbal fluency for the entire cohort, but within the control and diabetes group differences between insulin and placebo were not significant, likely due to a relatively small sample size. Across both groups, these on-insulin improvements in cognitive performance were associated with greater vasodilatation in the MCA territory, and particularly within the right insular cortex. In diabetic subjects on insulin, baseline perfusion increased in the right insular cortex. Visuospatial performance after insulin administration in the diabetes group and verbal fluency performance in the control group were related to greater vasodilation in the MCA territory. These relationships were not observed for cognitive responses to placebo administration.
The MCA territory includes cortical areas representing learning and memory, as well as the insular cortex, which is an important relay region for autonomic functions, emotions and memory. In particular, the right insular cortex provides a link across systems that are selectively responsive to attention-related problem solving during conditions that require attention and coordination during a task performance (25). Our results suggest that improvement of cognitive performance on insulin may be related to regional perfusion and vasodilatation, and may specifically activate anterior regions that regulate attention-related task performance.

Diabetes is associated with lower baseline perfusion, blunted vasodilatation to hypercapnia and exaggerated vasoconstriction to hypocapnia, and the regions of altered vasoreactivity extend across ACA and MCA territories, and anatomically across frontal, parietal and occipital lobes (5),(6). Cerebral perfusion and vasoreactivity negatively correlate with the degree of insulin resistance, diabetes control, vascular inflammation and other indicators of cerebromicrovascular disease (3),(5),(6). The exact mechanisms by which intranasal insulin may affect regional perfusion are not known, but may include endothelium and nitric–oxide dependent vasodilatation and reduction of vasoconstriction by regulating secretion endothelin-1 (26). Vasodilatation-associated increases in blood flow via insulin-stimulated production of nitric-oxide in vascular endothelium have not been well studied in the human brain. Therefore, vasodilatation to hypercapnia, although not a specific measure of endothelial function, may serve as an effective proxy to neurovascular coupling within specific regions, as well as the ability to redistribute blood flow to those regions (21),(6). Therefore, we anticipate that intranasal insulin may have direct effects on neurovascular coupling, regional vascular tone and neuronal activity (27),(26),(28),(29). Cognitive performance correlates with blood flow and its redistribution to
areas with increased neuronal activity (7). Previous research has supported a link between vasoreactivity and cognitive performance (30). Decreased vasodilatation and increased vasoconstriction reactivity associated with diabetes have been linked with regional gray matter atrophy and worse functionality in older diabetic adults (6). Conversely, the relationship between improved vasodilatation on insulin with improved cognitive scores may suggest vasoreactivity as a potential diagnostic tool for determining responsiveness to intranasal insulin therapy. The relationship between vasodilatation in right insular cortex and performance to visuospatial task is intriguing. The activation of the right insular cortex has been linked to better performance on cognitive tasks that are challenging or require longer processing, and to simple tasks in older or impaired individuals (31), and to tasks that are associated with autonomic system arousal (32).

We cannot, however, refute the notion that intranasal insulin may interact with cerebral glucose metabolism and thus enhance the immediate recall and memory, as recently demonstrated in non-diabetic subjects with mild Alzheimer’s disease (12). Diabetes has been shown to accelerate brain aging by at least five years and to increase the risk of Alzheimer’s disease such that even younger diabetic patients have greater learning and memory deficits than age-matched controls. Reversion of cognitive decline may be possible. Therefore, targeting the population with diabetes and mild cognitive deficits may be useful for prevention of future cognitive decline and dementia later in life (33). Studies evaluating effects of intranasal insulin on cognition suggested potential benefits but have been limited to small sample sizes and healthy young and older adults, or non-diabetic adults with mild cognitive impairment or mild Alzheimer’s disease (34),(35),(17). The on-insulin improvements of delayed verbal recall in non-diabetic adults with cognitive impairment associated with mild Alzheimer’s disease were stronger
in apolipoprotein E (APOE) ε4 allele-negative subjects as compared to APOE positive subjects (28). Furthermore, preserved memory and functionality in these subjects was also associated with reduction of Aβ 42 levels in cerebrospinal fluid (12).

This pilot study evaluated the acute effects of a single dose of 40IU of intranasal insulin on two subsequent days, and therefore had several limitations. We have observed group-treatment effects between insulin and placebo conditions, but within the groups differences were limited due to the small sample size. Potential confounders such as increased familiarity with the environment and potential learning effects despite randomized treatment and parallel versions of tests may have affected the results. Our analyses accounted for these effects. Both groups performed better on the verbal and numeric tasks on Day 3 of testing, while the majority of participants in both groups received insulin on Day 2. This training effect therefore may potentially diminish the observed effects of insulin administration. Additionally, there were more women than men participants, which may have contributed to the presence of gender effects with verbal learning and memory. A possible reverse relationship between intranasal insulin dose and cognitive responses have been reported (36) (17), but an optimal dose for diabetic subjects is not known.

Finally, we tested only a single dose of insulin, and therefore it is unclear whether lower or higher doses could be more effective, and whether this dose may lead to long-term improvement of memory if administered over longer period of time.

This study provides preliminary evidence that intranasal insulin administration appears safe in older adults with type 2 DM, does not affect systemic glucose control, and may provide acute improvements in cognitive function in older non-demented diabetic and non-diabetic patients.
The link between cognitive improvement and vasodilation in anterior brain circulation, suggest that activation of anterior brain regions controlling visuospatial memory may be a potential mechanism of acute intranasal-insulin changes in cognitive performance. Shared central insulin-signaling in vascular and metabolic pathways, may provide new therapeutic targets to couple perfusion regulation with homeostasis to prevent brain atrophy and consequently cognitive decline in older diabetic people. However, larger and prospective studies are needed to determine the long-term safety and efficacy to prevent or slow down cognitive deterioration in older people with type 2 diabetes.
Figure Legend

**Figure 1**: Brief Visual Memory scores for immediate recall Trials 1-3 (T1-T3) and Total Recall for the diabetes and control groups. Overall, controls on insulin performed better than the diabetes group on insulin and on placebo; *p<0.03 and **p<0.01 controls on insulin vs. diabetes group on placebo (LS models adjusted for age). For the whole cohort, performance on insulin improved as compared to placebo for \( \Delta T2 \ p=0.04 \) and borderline for Total Recall \( p=0.052 \) (paired- t test).

**Figure 2**: Voxel-based analysis demonstrates that within the diabetes group, intranasal administration of insulin induced more increased perfusion, as compared to placebo, in the right insular cortex (independent Student’s t-test applied to the subtraction result between conditions, voxel-level uncorrected \( p<0.001 \)) (A). In the diabetes group, the Brief Visuospatial Memory Total Recall T score following insulin administration was related to vasodilatation in the middle cerebral artery territory \( (R^2=0.58, R^2_{adj}=0.44, p=0.0098) \) (B). This relationship was not observed following placebo administration \( (R^2=0.14, R^2_{adj}=-0.14, p=0.34, \) LS regression models age and gender adjusted) (C). In controls, following insulin administration, the Verbal Fluency Category T score was also related to vasodilatation in the right middle cerebral artery territory \( (R^2=0.75, R^2_{adj}=0.64, p=0.008, p=0.024, \) LS regression models age and gender adjusted) (D).
Author’s contributions:

V. N. designed the study and protocol and oversaw all aspects of study conduct, experiments, and manuscript preparation, and takes responsibility for the content of the article; W.M. designed and oversaw cognitive testing; Y.H. performed MRI analyses; M.M. oversaw clinical aspects of the study; P.N. oversaw clinical aspects of the study; A.G. contributed to data collection and statistical analyses; B.M. contributed to data collection and manuscript preparation; P.R. contributed to study design and oversaw statistical analyses; S.C. contributed to study design; A.A. contributed to MRI analysis. Preliminary results of this study were presented at the 73rd Scientific Sessions of the American Diabetes Association, June 21-25, 2013, in Chicago, IL, poster 978-P. Dr. Vera Novak is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest:

V.N. none; W.M. none; Y.H. none; M.M. received research grant from Sanofi; P.N. none; A.G. none; B.M. none; P.R. none; S.C. has served as a Scientific Advisory Board member for Eli Lilly and has received a donation of insulin and placebo for intranasal study; A.A. none

Funding acknowledgements:

V.N. has received grants from the NIH–National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (5R21-DK-084463-02) and the National Institutes of Health (NIH)–National Institute on Aging (NIA) (1R01-AG-0287601-A2) related to this study, and V.N, M.M., P.N., A.G., B.M., P.R and S.C. received salaries from these grants.
Y.H. received the grant support from China Scholarship Council (CSC) (No. 201206010220).
S.C. has received a grant from the National Institutes of Health (NIH)-National Institute on Aging (NIA) (R01-AG-027415). B.M. received a KL2 Medical Research Investigator Training (MeRIT) award (1KL2RR025757-04) from The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award 8KL2TR000168-05). W.M. was also supported by the Translational Research Center for TBI and Stress Disorders (TRACTS), a VA Rehabilitation Research and Development Traumatic Brain Injury Center of Excellence (B6796-C) and VA Merit Review Award to Regina McGlinchey. M.M. received a research grant from Sanofi. This work was conducted with support from Harvard Catalyst | The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award 8UL1TR000170-05 and financial contributions from Harvard University and its affiliated academic health care centers). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health. The authors acknowledge contributions of Clinical Research Center nursing and MRI staff.
Table 1: Demographic characteristics of the diabetes and control groups

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<tr>
<td>Age (years)</td>
<td>62.0±7.9</td>
<td>60.1±9.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>8,7</td>
<td>4,10</td>
<td>0.2*</td>
</tr>
<tr>
<td>Race (white/AA/Asian)</td>
<td>10, 3, 2</td>
<td>13, 1, 0</td>
<td>0.2*</td>
</tr>
<tr>
<td>Education (years)</td>
<td>14.3±3.8</td>
<td>17.1±3.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>11.3±4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.4±1.4</td>
<td>5.6±0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>57±13</td>
<td>38±1.95</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>131.9±37.7</td>
<td>87.9±9.7</td>
<td>0.0002</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>128.6±15.1</td>
<td>125.5±14.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>73.5±8.7</td>
<td>72.1±10.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.3±3.5</td>
<td>40.2±2.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Hyperlipidemia (yes/no)</td>
<td>10, 5</td>
<td>2, 12</td>
<td>0.004</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>161.0±35.6</td>
<td>213.1±45.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides (mg/DL)</td>
<td>132.1±75.9</td>
<td>108.8±47.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Urinary albumin (mg/DL)</td>
<td>26.5±37.9</td>
<td>7.0±5.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Microalbumin/creatinine ratio</td>
<td>26.3±45.8</td>
<td>7.5±7.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Hypertension N (%)</td>
<td>47%</td>
<td>0</td>
<td>0.003*</td>
</tr>
<tr>
<td>Mini-Mental State Examination</td>
<td>28.3±1.7</td>
<td>28.8±1.6</td>
<td>0.6³</td>
</tr>
<tr>
<td>Hopkins Verbal Learning- Delayed Recall T Score</td>
<td>54.5±8.5</td>
<td>41.8±9.1</td>
<td>0.008</td>
</tr>
<tr>
<td>Trail making Part B T Score</td>
<td>38.5±12.9</td>
<td>52.1±11.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Rey-Osterrieth Complex Figure Delayed Recall T Score</td>
<td>43.4±15.1</td>
<td>45.0±19.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Global Gray Matter volume (cm³)</td>
<td>598.5±25.1</td>
<td>691.3±27.5</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Between groups comparisons, ANOVA, unadjusted. Mean± SD

* Pearson’s chi-squared test, inclusion criteria: normotensive controls

³ LS model adjusted for education years AA African/American
Table 2: Protocol Flow and Administration Effects on Select Physiological, Perfusion, and Cognitive Measures

<table>
<thead>
<tr>
<th>Protocol &amp; Variables</th>
<th>Diabetes</th>
<th>Insulin</th>
<th>Difference</th>
<th>Control</th>
<th>Ins. vs. PL</th>
<th>Ins vs. PL</th>
<th>Diff. Ins-PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose IV (mg/dL)</td>
<td>165.3±77.2</td>
<td>161.3±61.8</td>
<td>3.5±28.0</td>
<td>99.0±8.8</td>
<td>100.7±8.0</td>
<td>0.33±6.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose FS (mg/dL)</td>
<td>154.1±71.0</td>
<td>167.6±71.7</td>
<td>1.9±11.9</td>
<td>95.8±9.9</td>
<td>96.5±10.1</td>
<td>0.71±6.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>68.5±10.5</td>
<td>68.3±10.5</td>
<td>0.11±3.8</td>
<td>67.4±10.8</td>
<td>66.1±11.3</td>
<td>-1.36±6.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>126.8±10.2</td>
<td>125.4±12.1</td>
<td>-1.39±9.8</td>
<td>117.9±14.0</td>
<td>118.7±12.8</td>
<td>0.77±9.9</td>
<td>0.6</td>
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<tr>
<td>Diamastolic BP (mmHg)</td>
<td>74.5±9.4</td>
<td>75.9±11.6</td>
<td>1.36±6.1</td>
<td>72.2±8.9</td>
<td>72.9±9.3</td>
<td>0.76±4.4</td>
<td>0.4</td>
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<tr>
<td>Mean MCA BFV (cm/s)</td>
<td>37.8±12.6</td>
<td>36.9±5.7</td>
<td>-0.85±10.9</td>
<td>41.2±9.4</td>
<td>39.4±10.5</td>
<td>-1.13±9.3</td>
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<td>Placebo</td>
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<tr>
<td>Glucose IV (mg/dL)</td>
<td>154.1±71.0</td>
<td>167.6±71.7</td>
<td>1.9±11.9</td>
<td>95.8±9.9</td>
<td>96.5±10.1</td>
<td>0.71±6.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Glucose FS (mg/dL)</td>
<td>154.4±61.7</td>
<td>157.6±63.4</td>
<td>3.2±17.3</td>
<td>99.2±8.7</td>
<td>100.5±7.0</td>
<td>1.31±5.7</td>
<td>0.9</td>
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<tr>
<td>Heart Rate (bpm)</td>
<td>69.8±11.5</td>
<td>72.0±11.7</td>
<td>2.01±5.8</td>
<td>70.2±11.9</td>
<td>69.0±11.7</td>
<td>-0.23±7.4</td>
<td>0.3</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>126.6±12.3</td>
<td>126.3±14.1</td>
<td>-0.34±13.9</td>
<td>118.4±18.8</td>
<td>119.2±14.3</td>
<td>1.24±15.1</td>
<td>1.0</td>
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<tr>
<td>Diamastolic BP (mmHg)</td>
<td>74.6±9.8</td>
<td>76.9±10.5</td>
<td>2.3±11.5</td>
<td>73.0±11.7</td>
<td>74.0±9.3</td>
<td>0.40±7.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose IV (mg/dL)</td>
<td>156.1±63.8</td>
<td>160.9±57.1</td>
<td>4.7±11.6</td>
<td>104.5±8.7</td>
<td>100.2±11.3</td>
<td>-2.7±10.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Glucose FS (mg/dL)</td>
<td>153.8±63.5</td>
<td>143.5±38.7</td>
<td>2.7±13.3</td>
<td>102.3±5.3</td>
<td>100.7±6.6</td>
<td>-2.6±6.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>68.9±10.7</td>
<td>66.5±10.3</td>
<td>-2.3±3.2</td>
<td>63.2±10.5</td>
<td>64.0±10.0</td>
<td>0.88±8.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128.8±14.0</td>
<td>124.9±20.1</td>
<td>-3.7±8.4</td>
<td>122.5±13.5</td>
<td>124.0±15.0</td>
<td>1.48±6.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Diamastolic BP (mmHg)</td>
<td>75.8±9.6</td>
<td>75.5±13.3</td>
<td>-0.4±7.7</td>
<td>75.0±6.8</td>
<td>73.7±9.0</td>
<td>-1.24±6.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose IV (mg/dL)</td>
<td>43.3±2.8</td>
<td>43.4±3.6</td>
<td>0.06±1.5</td>
<td>45.8±1.4</td>
<td>46.3±2.4</td>
<td>0.55±1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Right insular cortex perfusion (ml/100g/min)</td>
<td>39.2±3.3</td>
<td>46.4±3.6</td>
<td>7.1±1.8</td>
<td>40.3±2.4</td>
<td>36.9±2.5</td>
<td>-3.32±1.8</td>
<td>0.001</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
<td>-----------</td>
<td>-----------</td>
<td>----------</td>
<td>-------</td>
</tr>
<tr>
<td>Vasodilatation MCA (ml/100g/min/mmHg)</td>
<td>0.02±0.35</td>
<td>-0.29±0.5</td>
<td>-0.38±0.62</td>
<td>0.62±0.2</td>
<td>0.10±0.47</td>
<td>-0.47±0.56</td>
<td>0.6</td>
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<tr>
<td>Cognitive Testing Start, Post-Admin (min)</td>
<td>77.8±4.4</td>
<td>77.9±7.8</td>
<td>0.07±5.1</td>
<td>80.9±8.7</td>
<td>78.6±6.4</td>
<td>-2.29±8.7</td>
<td>0.5</td>
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<tr>
<td>BVMT Trial 2 T Score</td>
<td>38.5±8.3</td>
<td>41.8±8.9</td>
<td>3.4±11.8</td>
<td>46.5±13.5</td>
<td>51.7±11.5</td>
<td>5.2±10.5</td>
<td>0.2</td>
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<tr>
<td>BVMT Total Recall T Score</td>
<td>39.5±8.9</td>
<td>41.2±9.9</td>
<td>1.8±10.9</td>
<td>43.1±17.3</td>
<td>50.9±9.7</td>
<td>7.8±14.0</td>
<td>0.3</td>
</tr>
<tr>
<td>D-KEFS Verbal Fluency FAS T Score</td>
<td>51.0±13.6</td>
<td>50.1±13.7</td>
<td>-0.9±4.9</td>
<td>63.9±8.1</td>
<td>62.3±8.8</td>
<td>-1.6±5.8</td>
<td>0.5</td>
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<tr>
<td>Verbal Fluency Category T Score</td>
<td>49.3±11.5</td>
<td>50.9±15.3</td>
<td>1.6±14.0</td>
<td>58.1±14.4</td>
<td>58.1±11.7</td>
<td>-0.1±9.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

- Insulin vs. placebo comparisons within diabetes group (Insulin vs.PL, DM), matched pairs
- Insulin vs. placebo comparisons within control group (Insulin vs.PL, C), matched pairs
- Difference between insulin – placebo between DM and control groups (ANOVA)
- BFV MCA comparison baseline-insulin admin, controls on insulin p=0.001, controls on placebo p=0.052, diabetes–insulin p=0.01, diabetes–placebo p=0.003
Reference List


Figure 1
Figure 2

A

B

C

D

Diabetes-Insulin

R²_adj = 0.44 p = 0.0098

MCA vasodilatation (ml/100g/min/mmHg)

Diabetes-Placebo

R²_adj = 0.14 p = 0.34

MCA vasodilatation (ml/100g/min/mmHg)

Diabetes-Placebo

R²_adj = 0.14 p = 0.34

MCA vasodilatation (ml/100g/min/mmHg)

Diabetes-Placebo

R²_adj = 0.14 p = 0.34

MCA vasodilatation (ml/100g/min/mmHg)

Diabetes-Placebo

R²_adj = 0.14 p = 0.34

MCA vasodilatation (ml/100g/min/mmHg)

Diabetes-Placebo

R²_adj = 0.14 p = 0.34

MCA vasodilatation (ml/100g/min/mmHg)

Diabetes-Placebo

R²_adj = 0.14 p = 0.34

MCA vasodilatation (ml/100g/min/mmHg)

Diabetes-Placebo

R²_adj = 0.14 p = 0.34

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R²_adj = 0.14 p = 0.34

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Diabetes-Placebo

R²_adj = 0.14 p = 0.34

MCA vasodilatation (ml/100g/min/mmHg)