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

OBJECTIVE
To determine acute effects of intranasal insulin on regional cerebral perfusion and cognition in older adults with type 2 diabetes mellitus (DM).

RESEARCH DESIGN AND METHODS
This was a proof-of-concept, randomized, double-blind, placebo-controlled intervention evaluating the effects of a single 40-IU dose of insulin or saline on vasoreactivity and cognition in 15 DM and 14 control subjects. Measurements included regional perfusion, vasodilatation to hypercapnia with 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 ($P \leq 0.05$). In the DM group, an increase of perfusion after insulin administration was greater in the insular cortex compared with the control group ($P = 0.0003$). Cognitive performance after insulin administration was related to regional vasoreactivity. Improvements of visuospatial memory after insulin administration in the DM group ($R^2_{adj} = 0.44, P = 0.0098$) and in the 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, does not affect systemic glucose control, and may provide acute improvements of cognitive function in patients with type 2 DM, 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.

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Type 2 diabetes mellitus (DM) is a major risk factor for Alzheimer disease and vascular dementia. Associated brain atrophy is widespread and generalized, advancing brain age (1) and accelerating cognitive decline in older DM populations (2–4). 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 (5–7), and neurotoxicity (8). Typically, vasodilatory responses to hyperventilation 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 and 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-DM people with both acute and chronic intranasal administration (10–12). 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 DM in a proof-of-concept, double-blind, placebo-controlled, crossover study. We hypothesized that intranasal insulin acutely improves regional perfusion and that improvement of cognition may be dependent upon regional vasoreactivity in older DM adults compared with non-DM adults and compared with placebo treatment.

**RESEARCH DESIGN AND METHODS**

This was a single-center, randomized, double-blind, placebo-controlled safety and efficacy pilot intervention with crossover assignment [Food and Drug Administration Investigational New Drug Application (FDA-IND) 107690] to evaluate acute effects of intranasal insulin on regional vasoreactivity and cognition in older DM and non-DM adults. Primary end points were insulin-related changes in regional perfusion, vasoreactivity to CO₂ challenges, and cognitive exam scores in the DM group compared with placebo and with the control group. As no preliminary data on the effects of intranasal insulin on these end points in DM 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-DM 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, \( \alpha = 0.05 \). Studies were conducted at the Syncope and Falls in the Elderly 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 Committee on Clinical Investigation. 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 DM and 14 control subjects) completed the protocol (Table 1), 28 were excluded, and 7 withdrew consent.

DM participants were included if they were diagnosed with type 2 DM for \( \geq 5 \) years and treated with oral anti-DM agents. Control subjects were required to be normotensive, have fasting blood glucose \( < 100 \text{ mg/dL} \), and not be treated for any systemic disease, including hypertension. Exclusion criteria were type 1 DM, insulin treatment or allergy, or any systemic disease, including hypertension. Exclusion criteria were type 1 DM, insulin treatment or allergy, and chronic intranasal administration.

**Table 1—Demographic characteristics of the DM and control groups**

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>Control</th>
<th>( p )</th>
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</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>62.0 ± 7.9</td>
<td>60.1 ± 9.9</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Sex (men/women)</strong></td>
<td>8/7</td>
<td>4/10</td>
<td>0.2*</td>
</tr>
<tr>
<td><strong>Race (white/AA/Asian)</strong></td>
<td>10/3/2</td>
<td>13/1/0</td>
<td>0.2*</td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
<td>14.3 ± 3.8</td>
<td>17.1 ± 3.2</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>DM duration (years)</strong></td>
<td>11.3 ± 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>7.4 ± 1.4</td>
<td>5.6 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>HbA1c (mmol/mol)</strong></td>
<td>57 ± 13</td>
<td>38 ± 1.95</td>
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<tr>
<td><strong>Fasting glucose</strong></td>
<td>131.9 ± 37.7</td>
<td>87.9 ± 9.7</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>128.6 ± 15.1</td>
<td>125.5 ± 14.3</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>73.5 ± 8.7</td>
<td>72.1 ± 10.9</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Hemoglobin (%)</strong></td>
<td>40.3 ± 3.5</td>
<td>40.2 ± 2.3</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Hypertension (%)</strong></td>
<td>47</td>
<td>0</td>
<td>0.003*</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>28.3 ± 1.7</td>
<td>28.8 ± 1.6</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Hopkins Verbal Learning-Delayed Recall T Score</strong></td>
<td>54.5 ± 8.5</td>
<td>41.8 ± 9.1</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Trail-Making Part B T Score</strong></td>
<td>38.5 ± 12.9</td>
<td>52.1 ± 11.5</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Rey-Osteriell Complex Figure Delayed Recall T Score</strong></td>
<td>43.4 ± 15.1</td>
<td>45.0 ± 19.2</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Global gray matter volume (cm³)</strong></td>
<td>598.5 ± 25.1</td>
<td>691.3 ± 27.5</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated. Between-group comparisons. ANOVA, unadjusted. AA, African American. *Pearson χ² test, inclusion criteria: normotensive control subjects. SLS model adjusted for education years.
hypoglycemia, intranasal medications, clinically significant heart disease, arrhythmias, nephropathy, malignancies, strokes, major surgery within 6 months, uncontrolled hypertension, subthreshold Mini-Mental State Examination (MMSE) scores ($\geq$ 3 points below the comparative normal value for the subject’s age-group and education level or $\leq$ 24), current recreational drug or alcohol abuse, morbid obesity (BMI $\geq$ 40 kg/m$^2$), claustrophobia, or 3T magnetic resonance imaging (MRI)-incompatible metal implants, pacemakers, or arterial stents.

On-site screening included fasting laboratory chemistries, electrocardiogram, vital signs, detailed medical history and medication review, anthropometric measurements, and transcranial Doppler (TCD) isonsonation assessment. Of 64 subjects who completed the screening visit, 7 participants withdrew consent and 27 participants were found ineligible, and 1 control subject presented with elevated blood pressure (BP) 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), control status with HbA$_{1c}$ $\geq$ 6% ($n$ = 4), uncontrolled hypertension ($n$ = 4), subthreshold 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 loss to follow-up ($n$ = 3).

Studies were conducted at the CRC at BIDMC. DM subjects monitored their BP and glucose via finger stick four times daily for 3 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 DM diet and fasted 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 MRI; 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, and glimepiride], and thiazolidinediones [pioglitazone]) and 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-min intervals during the protocol with insulin or placebo administration and before each meal afterward. Electrocardiogram, BP using both sphygmomanometer and beat-to-beat (Portapres, Finapres Medical Systems, Amsterdam, the Netherlands) instrumentation, end tidal CO$_2$ (Capnomac Ultima; Datex-Ohmeda, Madison, WI) and blood flow velocities in the anterior (ACA) and middle cerebral arteries (MCAs) (TCF System Spencer Technologies, Seattle, WA) were continuously monitored during a 10-min baseline period, throughout insulin/placebo administration, and for 5 min postadministration. Vitals signs were also monitored during MRI using a Medrad Veris MR Vital Signs Monitor (Warrendale, PA).

**Insulin/Placebo Administration**

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

**MRI**

Anatomical and perfusion studies were performed on a 3-Tesla GE HDx MRI scanner (GE Medical Systems, Milwaukee, WI) using the three-dimensional magnetization-prepared rapid gradient echo (MP-RAGE) and three-dimensional continuous arterial spin labeling (CASL). After a localization scan, perfusion scans were taken during normocapnia (6 min and 2 min), hypocapnia (2 min), and hyperventilation (2 min). To induce hypercapnia, subjects breathed a mixture of 5% CO$_2$ and 95% air to increase CO$_2$ up to 45 mmHg using a rebreathing circuit. To induce hypocapnia, subjects hyperventilated to reduce CO$_2$ to 25 mmHg. Images were analyzed using tools developed in interactive data language (IDL; Research Systems, Boulder, CO) and MATLAB (MathWorks, Natick, MA).

Anatomical magnetic resonance images (MP-RAGE) were coregistered nonlinearily to the MNI152 standard template (CASL), coregistered 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, London, U.K.) (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 of insulin and placebo administration for each subject, using an independent Student 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 hyperventilation divided by change of CO$_2$; vasoconstriction was calculated as a change in perfusion between baseline and hypocapnia, and vasoreactivity rate...
Table 2—Protocol flow and administration effects on select physiological, perfusion, and cognitive measures

<table>
<thead>
<tr>
<th>Protocol and variables</th>
<th>DM</th>
<th>Control</th>
<th>Ins vs. PL: DM</th>
<th>Ins vs. PL: control</th>
<th>Ins and PL dif.: DM vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Insulin</td>
<td>Difference</td>
<td></td>
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</tr>
<tr>
<td>Glucose IV (mg/dL), 2 min</td>
<td>154.1 ± 71.0</td>
<td>167.6 ± 71.7</td>
<td>13.5 ± 11.9</td>
<td>95.8 ± 9.9</td>
<td>965.0 ± 10.1</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>68.5 ± 10.5</td>
<td>68.3 ± 10.5</td>
<td>0.2 ± 3.8</td>
<td>67.4 ± 10.8</td>
<td>66.1 ± 11.3</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>126.8 ± 10.2</td>
<td>125.4 ± 12.1</td>
<td>−1.3 ± 9.8</td>
<td>117.9 ± 14.0</td>
<td>118.7 ± 12.8</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.5 ± 9.4</td>
<td>75.9 ± 11.6</td>
<td>1.4 ± 6.1</td>
<td>72.2 ± 8.9</td>
<td>72.9 ± 9.3</td>
</tr>
<tr>
<td>Mean MCA BFV (cm/s)</td>
<td>37.8 ± 12.6</td>
<td>36.9 ± 5.7</td>
<td>−0.8 ± 10.9</td>
<td>41.2 ± 9.4</td>
<td>39.4 ± 10.5</td>
</tr>
</tbody>
</table>

*BFV MCA comparison with baseline insulin administration, control subjects on placebo. Insulin vs. placebo comparisons within DM group, matched pairs. Insulin vs. placebo comparisons within control group, matched pairs. Difference between insulin and placebo between DM and control groups (ANOVA).

**BFV, blood flow velocity; C, control; dif., difference; D-KEFS, Delis-Kaplan Executive Function System; FS, interstitial glucose using a finger stick; Ins, insulin; IV, intravenous; PL, placebo. Insulin vs. placebo comparisons within DM group, matched pairs. Insulin vs. placebo comparisons within control group, matched pairs. Difference between insulin and placebo between DM and control groups (ANOVA).
was calculated as a slope of regression between baseline, hypopcapnia, and hypercapnia for each subject within brain regions of interest (6,21).

Neuropsychological Assessment
Baseline assessment included measures of verbal learning (Hopkins Verbal Learning Test-Revised), executive function (Trail-Making Tests A and B; Digit Span), visual memory (Rey-Osterrieth Complex Figure Test), and MMSE. Testing on insulin versus placebo (day 2 and day 3) had to be completed within a short time-frame of 2 h after insulin administration because of insulin pharmacokinetics (10,11,22). Therefore, we selected a brief battery of parallel conditions were compared within each group and within the entire cohort using a paired t test. Dependent BVMT scores were included as data and statistical analysis.

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 DM group performed worse than the control group on verbal learning measures (Hopkins Verbal Learning Test-Revised learning was borderline, \( P = 0.052 \); delayed recall, \( R^2_{\text{adjusted}} = 0.31, P = 0.008 \); retention, \( R^2_{\text{adjusted}} = 0.21, P = 0.046 \), and \( R^2_{\text{adjusted}} = 0.1 \) recognition, \( P = 0.038 \)), processing speed (Trail Making Test A, \( R^2_{\text{adjusted}} = 0.2, P = 0.01 \) and executive function (Trail Making Test B, \( R^2_{\text{adjusted}} = 24, P = 0.005 \)) (LS models adjusted for education years) and 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 control and 11 DM 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 finger stick) and cardiovascular vital signs between insulin versus 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 and 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 (BFVs) in the ACA and MCA, measured by TCD, declined during administration in both insulin and placebo conditions for control and DM subjects by 9% (\( P = 0.05–0.001 \)) but returned to baseline within 5 min after administration.

BVMT Revised
BVMT performances after insulin administration tended to be higher than on-placebo performances, and control subjects performed better than DM subjects. Overall, control subjects on insulin performed better than the DM group on insulin and on placebo on measures of immediate recall trials 2 and 3 (T2 and T3) and total learning (total recall) (Fig. 1). On the BVMT, control subjects on insulin were the highest-scoring subgroup, while DM subjects on placebo scored the lowest. This relationship was observed for immediate recall T2 (LS model adjusted for age \( R^2_{\text{adjusted}} = 0.14, P = 0.029 \); control subjects on insulin compared with DM group on placebo \( P < 0.01 \), T3 (\( R^2_{\text{adjusted}} = 0.14, P = 0.026 \), and total recall (\( R^2_{\text{adjusted}} = 0.18, P = 0.02 \)).

These effects remained similar after adjustment for potential confounding effects of education on immediate recall T2 (\( R^2_{\text{adjusted}} = 0.12, P = 0.017 \) and T3 (\( R^2_{\text{adjusted}} = 0.1, P = 0.029 \)) (LS model age, education adjusted). The effect of education was not significant in these models. For the whole cohort, the performance on insulin improved compared with placebo on T2 (\( P = 0.04 \)) and was 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. Control subjects on insulin performed better than DM subjects on insulin on FAS (LS model adjusted for age \( R^2_{\text{adjusted}} = 0.26, P = 0.0045 \); LS model
Intranasal Insulin Effects on Cognition

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 DM group in the insular cortex (P = 0.039) as compared with control subjects (Table 2). In the DM group, perfusion in the right insular cortex increased after insulin administration (P = 0.001) compared with placebo. Voxel-based analyses have shown that increase of perfusion on insulin was greater in the DM group compared with the control group (P = 0.0003) (Fig. 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 (R² adjusted = 0.28, P = 0.04) and also with vasodilatation in the insular cortex (R² adjusted = 0.22, P = 0.04) (LS model adjusted for age, sex, and group). After insulin administration in the DM group, better visuospatial memory correlated with vasodilatation in the MCA territory for immediate recall T2 (R² adjusted = 0.43, P = 0.01), BVMT T3 (R² adjusted = 0.39, P = 0.035), and total recall (R² adjusted = 0.44, P = 0.0098) (LS models adjusted for age, sex, and vasodilatation in leptomeningeal MCA territory) (Fig. 2B). These relationships were not observed after placebo administration, as shown in Fig. 2C for total recall (R² adjusted = −0.14, P = 0.34) (LS models adjusted for age, sex, and vasodilatation in leptomeningeal MCA territory).

A similar trend was observed between BVMT immediate recall (T2 and T3) and total recall vasodilatation in the whole ACA territory (P = 0.05–0.08). After insulin administration within the control group, better performance on BVMT immediate recall T3 was also related to MCA vasodilatation (R² adjusted = 0.4, P = 0.035). This relationship between visuospatial memory and vasodilatation was not observed after placebo administration in either group.

In control subjects on insulin, FAS score (R² adjusted = 0.39, P = 0.04) and the composite verbal fluency measure (R² adjusted = 0.18, P = 0.045) were associated with greater vasodilatation in the right insular cortex (model adjusted for age). In control subjects on insulin, category performance was associated with greater vasodilatation in the right MCA (P = 0.027) and decreased vasodilatation in the left MCA (P = 0.024) (R² = 0.75, R² adjusted = 0.64, P = 0.0087, LS model adjusted for age and sex) (Fig. 2D) and also greater left-right difference in vasodilatation in the insular cortex (R² = 0.75, R² adjusted = 0.68, P = 0.0023). In the DM group on insulin, FAS scores were also associated with more vasodilatation in the left (P = 0.02) and lesser vasodilatation in the right (R² adjusted = 0.26, P = 0.04, LS model adjusted for age and sex) insular cortex.

CONCLUSIONS

This proof-of-concept study evaluated the acute effects of a single dose of intranasal insulin compared with placebo on vasoreactivity and cognition in older DM and control adults using a randomized crossover design. The intranasal administration of insulin was safe, with no serious adverse events or hypoglycemic episodes, and the protocol was feasible for participants. The DM 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 DM group differences between insulin administration, as shown in Fig. 2C for total recall (R² adjusted = −0.14, P = 0.34) (LS models adjusted for age, sex, and vasodilatation in leptomeningeal MCA territory).
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 DM subjects on insulin, baseline perfusion increased in the right insular cortex. Visuospatial performance after insulin administration in the DM group and verbal fluency performance in the control group were related to greater vasodilatation 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.

DM 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, DM control, vascular inflammation, and other indicators of cerebrovascular 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 (NO)-dependent vasodilatation and reduction of vasoconstriction by regulating secretion of endothelin-1 (26). Vasodilatation-associated increases in blood flow via insulin-stimulated production of NO 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 (6,21). Therefore, we anticipate that intranasal insulin may have direct effects on neurovascular coupling, regional vascular tone, and neuronal activity (26–29). 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 DM have been linked with regional gray matter atrophy and worse functionality in older DM 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 of a 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, 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

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**Figure 2**—Voxel-based analysis demonstrates that within the DM group, intranasal administration of insulin induced more increased perfusion compared with placebo in the right insular cortex (independent Student t test applied to the subtraction result between conditions, voxel-level uncorrected P < 0.001) (A). In the DM group, the BVMT T score after insulin administration was related to vasodilatation in the MCA territory (R² = 0.58, R² adjusted = 0.44, P = 0.0098) (B). This relationship was not observed after placebo administration (R² = 0.14, R² adjusted = 0.14, P = 0.34, LS regression models adjusted for age and sex) (C). In control subjects, after insulin administration the verbal fluency category T score was also related to vasodilatation in the right MCA territory (R² = 0.75, R² adjusted = 0.64, P = 0.0087, P = 0.024, LS regression models adjusted for age and sex) (D).
non-DM subjects with mild Alzheimer disease (12). DM has been shown to accelerate brain aging by at least 5 years and to increase the risk of Alzheimer disease such that even younger DM patients have greater learning and memory deficits than age-matched control subjects. Reversion of cognitive decline may be possible. Therefore, targeting the population with DM 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-DM adults with mild cognitive impairment or mild Alzheimer disease (17,34,35). The on-insulin improvements of delayed verbal recall in non-DM adults with cognitive impairment associated with mild Alzheimer disease were stronger in ApoE4 e4 allele-negative subjects compared with 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 40 IU 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 owing 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 sex effects with verbal learning and memory. A possible reverse relationship between intranasal insulin dose and cognitive responses has been reported (17,36,37), but an optimal dose for DM 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 a 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 DM and non-DM patients. The link between cognitive improvement and vasodilation in anterior brain circulation suggests 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 people with DM. 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 DM.

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