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

Vera Novak,[1] William Milberg,[2] Ying Hao,[1,3] Medha Munshi,[1,4] Peter Novak,[5] Andrew Galica,[1] Bradley Manor,[1] Paula Roberson,[6] Suzanne Craft,[7] and Amir Abduljalil[8]

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 ($R^2_{adjusted} = 0.44$, $P = 0.0098$) and in the verbal fluency test in the control group ($R^2_{adjusted} = 0.64$, $P = 0.0087$) 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.

Diabetes Care 2014;37:751–759 | DOI: 10.2337/dc13-1672

[1]Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA
[2]New England Geriatric, Research, Education and Clinical Center–Boston Division, VA Boston Healthcare, and Department of Psychiatry, Harvard Medical School, Boston, MA
[3]Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China
[4]Joslin Diabetes Center, Boston, MA
[5]Department of Neurology, University of Massachusetts Medical School, Worcester, MA
[6]Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR
[7]Division of Gerontology and Geriatric Medicine, Wake Forest University School of Medicine, Winston-Salem, NC
[8]Department of Radiology, Ohio State University, Columbus, OH

Corresponding author: Vera Novak, vnovak@bidmc.harvard.edu.

Received 16 July 2013 and accepted 1 October 2013.

Clinical trial reg. no. NCT01206322, clinicaltrials.gov.

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, 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 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 CO_2 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 >5 years and treated with oral anti-DM agents. Control subjects 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,

Table 1—Demographic characteristics of the DM and control groups

	DM	Control	*P*
Age (years)	62.0 ± 7.9	60.1 ± 9.9	0.7
Sex (men/women)	8/7	4/10	0.2*
Race (white/AA/Asian)	10/3/2	13/1/0	0.2*
Education (years)	14.3 ± 3.8	17.1 ± 3.2	0.04
DM duration (years)	11.3 ± 4.7		
HbA_{1c} (%)	7.4 ± 1.4	5.6 ± 0.2	<0.0001
HbA_{1c} (mmol/mol)	57 ± 13	38 ± 1.95	
Fasting glucose	131.9 ± 37.7	87.9 ± 9.7	0.0002
Systolic BP (mmHg)	128.6 ± 15.1	125.5 ± 14.3	0.6
Diastolic BP (mmHg)	73.5 ± 8.7	72.1 ± 10.9	0.7
Hematocrit (%)	40.3 ± 3.5	40.2 ± 2.3	0.9
Hyperlipidemia (yes/no)	10/5	2/12	0.004
Total cholesterol (mg/dL)	161.0 ± 35.6	213.1 ± 45.6	0.002
Triglycerides (mg/dL)	132.1 ± 75.9	108.8 ± 47.4	0.3
Urinary albumin (mg/dL)	26.5 ± 37.9	7.0 ± 5.8	0.07
Microalbumin-to-creatinine ratio	26.3 ± 45.8	7.5 ± 7.2	0.1
Hypertension (%)	47	0	0.003*
MMSE	28.3 ± 1.7	28.8 ± 1.6	0.6$
Hopkins Verbal Learning-Delayed Recall T Score	54.5 ± 8.5	41.8 ± 9.1	0.008
Trail-Making Part B T Score	38.5 ± 12.9	52.1 ± 11.5	0.005
Rey-Osterrieth Complex Figure Delayed Recall T Score	43.4 ± 15.1	45.0 ± 19.2	0.9
Global gray matter volume (cm^3)	598.5 ± 25.1	691.3 ± 27.5	0.02

Data are means ± SD unless otherwise indicated. Between-group comparisons. ANOVA, unadjusted. AA, African American. *Pearson χ^2 test, inclusion criteria: normotensive control subjects. $LS 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 (≥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 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) insonation 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} ≥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 regiment. 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) (TCD 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 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% 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 nonlinearly 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 hypercapnia 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

	DM			Control			Ins vs.	Ins vs.	Ins and PL dif.:
Protocol and variables	Placebo	Insulin	Difference	Placebo	Insulin	Difference	PL: DM	PL: control	DM vs. control
Baseline									
Glucose IV (mg/dL)	165.3 ± 77.2	161.3 ± 61.8	3.5 ± 28.0	99.0 ± 8.8	100.7 ± 8.0	0.33 ± 6.3	0.4	0.4	0.7
Glucose FS (mg/dL)	154.1 ± 71.0	167.6 ± 71.7	1.9 ± 11.9	95.8 ± 9.9	96.5 ± 10.1	0.71 ± 6.7	0.9	0.1	0.8
Heart rate (bpm)	68.5 ± 10.5	68.3 ± 10.5	0.11 ± 3.8	67.4 ± 10.8	66.1 ± 11.3	−1.36 ± 6.1	0.4	0.5	0.5
Systolic BP (mmHg)	126.8 ± 10.2	125.4 ± 12.1	−1.39 ± 9.8	117.9 ± 14.0	118.7 ± 12.8	0.77 ± 9.9	0.6	0.5	0.6
Diastolic BP (mmHg)	74.5 ± 9.4	75.9 ± 11.6	1.36 ± 6.1	72.2 ± 8.9	72.9 ± 9.3	0.76 ± 4.4	0.4	0.2	0.8
Mean MCA BFV (cm/s)	37.8 ± 12.6	36.9 ± 5.7	−0.85 ± 10.9	41.2 ± 9.4	39.4 ± 10.5	−1.13 ± 9.3	0.2	1.0	0.9
Insulin/placebo 0–5 min postadministration									
Mean MCA BFV (cm/s) ≥0 min*	31.6 ± 12.7	32.5 ± 5.5	0.89 ± 11.1	35.5 ± 8.7	35.5 ± 8.7	0.28 ± 8.4	0.4	0.8	0.9
Glucose IV (mg/dL), ≥2 min	160.9 ± 59.5	159.3 ± 56.0	1.57 ± 12.7	104.5 ± 9.5	103.3 ± 7.7	−1.08 ± 9.2	0.8	0.7	0.6
Glucose FS (mg/dL), ≥2 min	154.4 ± 61.7	157.6 ± 63.4	3.2 ± 17.3	99.2 ± 8.7	100.5 ± 7.0	1.31 ± 5.7	0.9	0.3	0.7
Mean MCA BFV (cm/s), ≥5 min	37.2 ± 10.5	37.6 ± 5.6	0.41 ± 8.8	40.7 ± 9.9	40.1 ± 9.4	0.06 ± 8.8	0.3	0.5	0.9
Heart rate (bpm)	69.8 ± 11.5	72.0 ± 11.7	2.01 ± 5.8	70.2 ± 11.9	69.0 ± 11.7	−0.23 ± 7.4	0.3	0.4	0.4
Systolic BP (mmHg)	126.6 ± 12.3	126.3 ± 14.1	−0.34 ± 13.9	118.4 ± 18.8	119.2 ± 14.3	1.24 ± 15.1	1.0	0.6	0.8
Diastolic BP (mmHg)	74.6 ± 9.8	76.9 ± 10.5	2.3 ± 11.5	73.0 ± 11.7	74.0 ± 9.3	0.40 ± 7.8	0.3	0.5	0.6
MRI: perfusion 10–60 min postadministration									
Glucose IV (mg/dL), ≥10 min	156.1 ± 63.8	160.9 ± 57.1	4.7 ± 11.6	104.5 ± 8.7	100.2 ± 11.3	−2.7 ± 10.8	0.06	0.1	0.1
Glucose IV (mg/dL), ≥40 min	153.8 ± 63.5	143.5 ± 38.7	2.7 ± 13.3	102.3 ± 5.3	100.7 ± 6.6	−2.6 ± 6.0	0.9	0.2	0.3
Glucose IV (mg/dL), ≥60 min	150.7 ± 59.1	139.8 ± 38.8	0.5 ± 17.5	105.4 ± 9.4	99.8 ± 8.1	−4.8 ± 9.3	0.4	0.3	0.4
Heart rate (bpm)	68.9 ± 10.7	66.5 ± 10.3	−2.3 ± 3.2	63.2 ± 10.5	64.0 ± 10.0	0.88 ± 8.6	0.4	0.6	0.2
Systolic BP (mmHg)	128.8 ± 14.0	124.9 ± 20.1	−3.7 ± 8.4	122.5 ± 13.5	124.0 ± 15.0	1.48 ± 6.1	0.1	0.1	0.08
Diastolic BP (mmHg)	75.8 ± 9.6	75.5 ± 13.3	−0.4 ± 7.7	75.0 ± 6.8	73.7 ± 9.0	−1.24 ± 6.8	0.3	0.9	0.8
Perfusion whole brain (mL/100 g/min)	43.3 ± 2.8	43.4 ± 3.6	0.06 ± 1.5	45.8 ± 1.4	46.3 ± 2.4	0.55 ± 1.6	1.0	0.7	0.8
Right insular cortex perfusion (mL/100 g/min)	39.2 ± 3.3	46.4 ± 3.6	7.1 ± 1.8	40.3 ± 2.4	36.9 ± 2.5	−3.32 ± 1.8	0.001	0.09	0.0003
Vasodilatation MCA (mL/100 g/min/mmHg)	0.02 ± 0.35	−0.29 ± 0.5	−0.38 ± 0.62	0.62 ± 0.2	0.10 ± 0.47	−0.47 ± 0.56	0.6	0.4	0.9
Cognitive testing ≥60 min postadministration									
Cognitive testing start, postadministration (min)	77.8 ± 4.4	77.9 ± 7.8	0.07 ± 5.1	80.9 ± 8.7	78.6 ± 6.4	−2.29 ± 8.7	0.5	0.5	0.4
BVMT T2 T Score	38.5 ± 8.3	41.8 ± 8.9	3.4 ± 11.8	46.5 ± 13.5	51.7 ± 11.5	5.2 ± 10.5	0.2	0.08	0.7
BVMT total recall T Score	39.5 ± 8.9	41.2 ± 9.9	1.8 ± 10.9	43.1 ± 17.3	50.9 ± 9.7	7.8 ± 14.0	0.3	0.06	0.3
D-KEFS verbal fluency FAS T Score	51.0 ± 13.6	50.1 ± 13.7	−0.9 ± 4.9	63.9 ± 8.1	62.3 ± 8.8	−1.6 ± 5.8	0.5	0.3	0.7
Verbal fluency category T Score	49.3 ± 11.5	50.9 ± 15.3	1.6 ± 14.0	58.1 ± 14.4	58.1 ± 11.7	−0.1 ± 9.0	0.3	0.5	0.7

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).
*BFV MCA comparison with baseline insulin administration, control subjects on insulin $P = 0.001$, control subjects on placebo $P = 0.052$, DM subjects on insulin $P = 0.01$, and DM subjects on placebo $P = 0.003$.

was calculated as a slope of regression between baseline, hypocapnia, 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 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 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, nonparametric tests, and the least square (LS) models. 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, 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 among perfusion, vasoreactivity, and cognition. LS models were calculated separately within group and condition (e.g., DM group on insulin) for each variable to minimize multiple-comparison 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 CO_2 were also tested. Conservatively, we selected models with $R^2 > 0.25$, and $P < 0.05$. Here, we present $R^2_{adjusted}$ (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 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, $R^2_{adjusted} = 0.31$, $P = 0.008$; retention, $R^2_{adjusted} = 0.21$, $P = 0.046$, and $R^2_{adjusted} = 0.1$ recognition, $P = 0.038$), processing speed (Trail Making Test A, $R^2_{adjusted} = 0.2$, $P = 0.01$) and executive function (Trail Making Test B, $R^2_{adjusted} = 24$, $P = 0.005$) (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 $R^2_{adjusted} = 0.14$, $P = 0.029$; control subjects on insulin compared with DM group on placebo $P < 0.01$), T3 ($R^2_{adjusted} = 0.14$, $P = 0.026$), and total recall ($R^2_{adjusted} = 0.18$, $P = 0.02$).

These effects remained similar after adjustment for potential confounding effects of education on immediate recall T2 ($R^2_{adjusted} = 0.12$, $P = 0.017$) and T3 ($R^2_{adjusted} = 0.1$, $P = 0.029$) (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 $R^2_{adjusted} = 0.26$, $P = 0.0045$; LS model

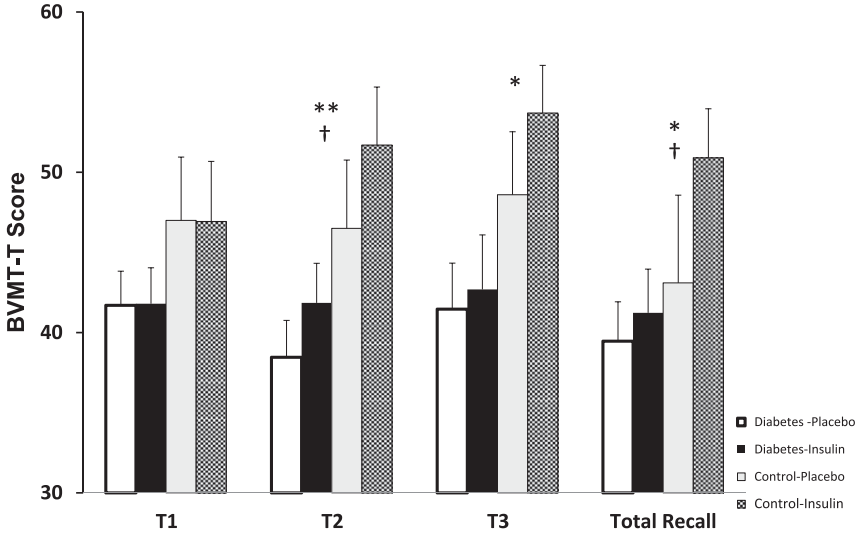

Figure 1—Brief visual memory scores for immediate recall trials 1–3 (T1–T3) and total recall for the DM and control groups. Overall, control subjects on insulin performed better than the DM group on insulin and on placebo; $*P < 0.03$ and $**P < 0.01$ control subjects on insulin vs. DM group on placebo (LS models adjusted for age). For the whole cohort, performance on insulin improved compared with placebo for † T2, $P = 0.04$, and was borderline for total recall, $P = 0.052$ (paired *t* test).

adjusted for age and education ($R^2_{adjusted} = 0.25$, $P = 0.018$), switching ($R^2_{adjusted} = 0.2$, $P = 0.006$; $R^2_{adjusted} = 0.17$, $P = 0.012$), and composite verbal fluency ($R^2_{adjusted} = 0.12$, $P = 0.02$; $R^2_{adjusted} = 0.11$, $P = 0.049$). On placebo, control subjects were better only on FAS—not other verbal fluency measures (LS model adjusted for age and education, $R^2_{adjusted} = 0.27$, $P = 0.019$). The effect of education was not significant in the models. There was no difference in performance comparing insulin with 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 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. 2*A*; 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 ($R^2_{adjusted} = 0.28$, $P = 0.04$) and also with vasodilatation in the insular cortex ($R^2_{adjusted} = 0.22$, $P = 0.04$) (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 ($R^2_{adjusted} = 0.43$, $P = 0.01$), BVMT T3 ($R^2_{adjusted} = 0.39$, $P = 0.035$), and total recall ($R^2_{adjusted} = 0.44$, $P = 0.0098$) (LS models adjusted for age, sex, and vasodilatation in leptomeningeal MCA territory) (Fig. 2*B*). These relationships were not observed after placebo administration, as shown in Fig. 2*C* for total recall ($R^2_{adjusted} = -0.14$, $P = 0.34$) (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 ($R^2_{adjusted} = 0.4$, $P = 0.035$). This relationship between visuospatial memory and vasodilatation was not observed after placebo administration in either group.

In control subjects on insulin, FAS score ($R^2_{adjusted} = 0.39$, $P = 0.04$) and the composite verbal fluency measure ($R^2_{adjusted} = 0.18$, $P = 0.045$) 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 vasodilation in the left MCA ($P = 0.024$) ($R^2 = 0.75$, $R^2_{adjusted} = 0.64$, $P = 0.0087$, LS model adjusted for age and sex) (Fig. 2*D*) and also greater left-right difference in vasodilatation in the insular cortex ($R^2 = 0.75$, $R^2_{adjusted} = 0.68$, $P = 0.0023$). 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 ($R^2_{adjusted} = 0.26$, $P = 0.04$, 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

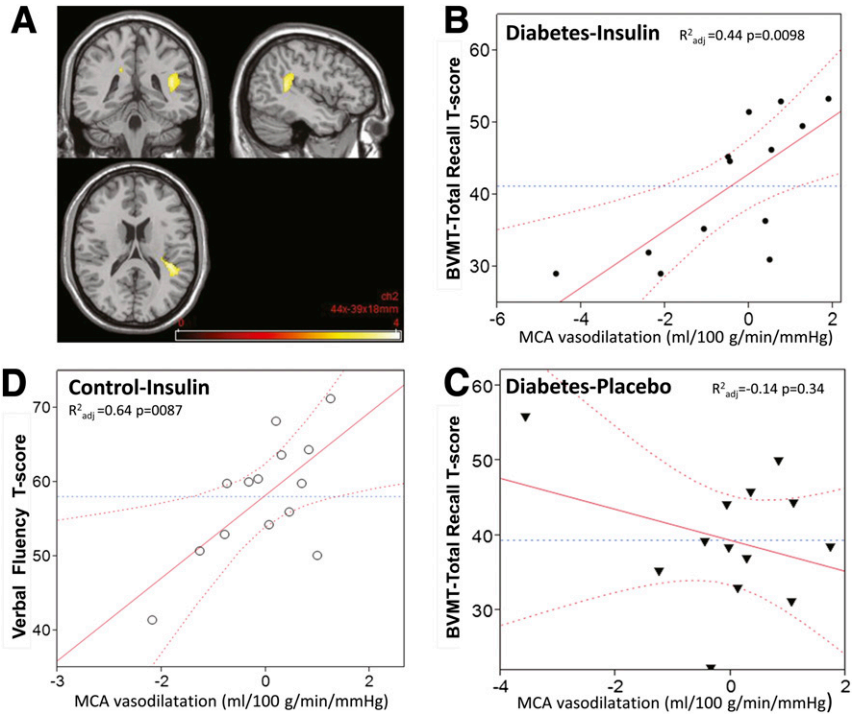

Figure 2—Voxel-based analysis demonstrates that within the DM group, intranasal administration of insulin induced more increased perfusion compared with placebo in the right insular cortex (independent Student *t* test applied to the subtraction result between conditions, voxel-level uncorrected $P < 0.001$) (*A*). In the DM group, the BVMT T score after insulin administration was related to vasodilatation in the MCA territory ($R^2 = 0.58$, $R^2_{adjusted} = 0.44$, $P = 0.0098$) (*B*). This relationship was not observed after placebo administration ($R^2 = 0.14$, $R^2_{adjusted} = -0.14$, $P = 0.34$, 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 ($R^2 = 0.75$, $R^2_{adjusted} = 0.64$, $P = 0.0087$, $P = 0.024$, LS regression models adjusted for age and sex) (*D*).

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 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.

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 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 (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

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 ε4 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 nondemented 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.

Acknowledgments. The authors acknowledge contributions of Clinical Research Center nursing and MRI staff.

Funding. V.N. has received grants from the National Institutes of Health (NIH)–National Institute of Diabetes and Digestive and Kidney Diseases (5R21-DK-084463-02) and the 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 grant support from the China Scholarship Council (201206010220). S.C. has received a grant from the NIH-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, NIH award 8KL2TR000168-05). W.M. was also supported by the Translational Research Center for Traumatic Brain Injury (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. 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, NIH 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 NIH.

Duality of Interest. M.M. received a research grant from Sanofi. S.C. has served as a Scientific Advisory Board member for Eli Lilly and has received a donation of insulin. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. V.N. designed the study and protocol and oversaw all aspects of study conduct, experiments, and manuscript preparation. W.M. designed and oversaw cognitive testing. Y.H. performed MRI analyses. M.M. and 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. V.N. 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.

Prior Presentation. Parts of this study were presented in abstract form at the 73rd Scientific Sessions of the American Diabetes Association, Chicago, Illinois, 21–25 June 2013.

References

1. Xu WL, Qiu CX, Wahlin A, Winblad B, Fratiglioni L. Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. Neurology 2004; 63:1181–1186
2. de Bresser J, Tiehuis AM, van den Berg E, et al.; Utrecht Diabetic Encephalopathy Study Group. Progression of cerebral atrophy and white matter hyperintensities in patients with type 2 diabetes. Diabetes Care 2010;33:1309–1314
3. van den Berg E, Reijmer YD, de Bresser J, Kessels RP, Kappelle LJ, Biessels GJ; Utrecht Diabetic Encephalopathy Study Group. A 4 year follow-up study of cognitive functioning in patients with type 2 diabetes mellitus. Diabetologia 2010;53:58–65
4. Moran C, Phan TG, Chen J, et al. Brain atrophy in type 2 diabetes: Regional distribution and influence on cognition. Diabetes Care. 12 August 2013 [Epub ahead of print]

5. Last D, Alsop DC, Abduljalil AM, et al. Global and regional effects of type 2 diabetes on brain tissue volumes and cerebral vasoreactivity. Diabetes Care 2007;30:1193–1199
6. Novak V, Zhao P, Manor B, et al. Adhesion molecules, altered vasoreactivity, and brain atrophy in type 2 diabetes. Diabetes Care 2011;34:2438–2441
7. Tiehuis AM, Vincken KL, van den Berg E, et al. Cerebral perfusion in relation to cognitive function and type 2 diabetes. Diabetologia 2008;51:1321–1326
8. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–1625
9. Gunning-Dixon FM, Raz N. The cognitive correlates of white matter abnormalities in normal aging: a quantitative review. Neuropsychology 2000;14:224–232
10. Benedict C, Dodt C, Hallschmid M, et al. Immediate but not long-term intranasal administration of insulin raises blood pressure in human beings. Metabolism 2005;54:1356–1361
11. Benedict C, Kern W, Schultes B, Born J, Hallschmid M. Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. J Clin Endocrinol Metab 2008;93:1339–1344
12. Craft S, Baker LD, Montine TJ, et al. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Arch Neurol 2012;69:29–38
13. Hallschmid M, Benedict C, Schultes B, et al. Towards the therapeutic use of intranasal neuropeptide administration in metabolic and cognitive disorders. Regul Pept 2008;149:79–83
14. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 2004;127:481–496
15. Hanson LR, Frey WH 2nd. Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. BMC Neurosci 2008;9(Suppl. 3):S5
16. Kerr D, Stanley JC, Barron M, Thomas R, Leatherdale BA, Pickard J. Symmetry of cerebral blood flow and cognitive responses to hypoglycaemia in humans. Diabetologia 1993;36:73–78
17. Reger MA, Watson GS, Green PS, et al. Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. J Alzheimers Dis 2008;13:323–331
18. Wang Z, Aguirre GK, Rao H, et al. Empirical optimization of ASL data analysis using an ASL data processing toolbox: ASLtbx. Magn Reson Imaging 2008;26:261–269
19. D'Agostino E, Maes F, Vandermeulen D, Suetens P. Atlas-to-image non-rigid registration by minimization of conditional local entropy. Inf Process Med Imaging 2007;20:320–332
20. MacIntosh BJ, Pattinson KT, Gallichan D, et al. Measuring the effects of remifentanil on cerebral blood flow and arterial arrival time using 3D GRASE MRI with pulsed arterial spin labelling. J Cereb Blood Flow Metab 2008;28:1514–1522
21. Zhao P, Alsop DC, Abduljalil A, et al. Vasoreactivity and peri-infarct hyperintensities in stroke. Neurology 2009;72:643–649
22. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: a transnasal approach to the human brain. Nat Neurosci 2002;5:514–516
23. Benedict RHB, Schretlen D, Groninger L, Dobraski M, Sphritz B. Revision of the Brief VisuospatilaaMemory test: Studies of normal performance, reliability and validity. Psychol Assess 1996;8:145–153
24. Yeudall LT, Fromm D, Reddon JR, Stefanyk WO. Normative data stratified by age and sex for 12 neuropsychological tests. J Clin Psychol 1986;42:918–946
25. Eckert MA, Menon V, Walczak A, et al. At the heart of the ventral attention system: the right anterior insula. Hum Brain Mapp 2009;30:2530–2541
26. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888–1904
27. Cranston I, Marsden P, Matyka K, et al. Regional differences in cerebral blood flow and glucose utilization in diabetic man: the effect of insulin. J Cereb Blood Flow Metab 1998;18:130–140
28. Reger MA, Watson GS, Frey WH 2nd, et al. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. Neurobiol Aging 2006;27:451–458
29. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. Endocr Rev 2007;28:463–491
30. Novak V, Hajjar I. The relationship between blood pressure and cognitive function. Nat Rev Cardiol 2010;7:686–698
31. Dufor O, Serniclaes W, Sprenger-Charolles L, Démonet JF. Top-down processes during auditory phoneme categorization in dyslexia: a PET study. Neuroimage 2007;34:1692–1707
32. Abboud H, Berroir S, Labreuche J, Orjuela K, Amarenco P; GENIC Investigators. Insular involvement in brain infarction increases risk for cardiac arrhythmia and death. Ann Neurol 2006;59:691–699
33. Sachdev PS, Lipnicki DM, Crawford J, et al.; Sydney Memory, Ageing Study Team. Factors predicting reversion from mild cognitive impairment to normal cognitive functioning: a population-based study. PLoS ONE 2013;8:e59649
34. Benedict C, Hallschmid M, Schultes B, Born J, Kern W. Intranasal insulin to improve memory function in humans. Neuroendocrinology 2007;86:136–142
35. Reger MA, Craft S. Intranasal insulin administration: a method for dissociating central and peripheral effects of insulin. Drugs Today (Barc) 2006;42:729–739
36. Shemesh E, Rudich A, Harman-Boehm I, Cukierman-Yaffe T. Effect of intranasal insulin on cognitive function: a systematic review. J Clin Endocrinol Metab 2012;97:366–376