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Intranasal Insulin Enhanced Resting-State Functional Connectivity of Hippocampal Regions in Type 2 Diabetes

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Type 2 diabetes mellitus (T2DM) alters brain function and manifests as brain atrophy. Intranasal insulin has emerged as a promising intervention for treatment of cognitive impairment. We evaluated the acute effects of intranasal insulin on resting-state brain functional connectivity in older adults with T2DM. This proof-of-concept, randomized, double-blind, placebo-controlled study evaluated the effects of a single 40 IU dose of insulin or saline in 14 diabetic and 14 control subjects. Resting-state functional connectivity between the hippocampal region and default mode network (DMN) was quantified using functional MRI (fMRI) at 3Tesla. Following insulin administration, diabetic patients demonstrated increased resting-state connectivity between the hippocampal regions and the medial frontal cortex (MFC) as compared with placebo (cluster size: right, *P* = 0.03) and other DMN regions. On placebo, the diabetes group had lower connectivity between the hippocampal region and the MFC as compared with control subjects (cluster size: right, *P* = 0.02), but on insulin, MFC connectivity was similar to control subjects. Resting-state connectivity correlated with cognitive performance. A single dose of intranasal insulin increases resting-state functional connectivity between the hippocampal regions and multiple DMN regions in older adults with T2DM. Intranasal insulin administration may modify functional connectivity among brain regions regulating memory and complex cognitive behaviors.

Type 2 diabetes mellitus (T2DM) accelerates brain aging that manifests as a widespread generalized atrophy (1) and earlier onset of dementia and Alzheimer disease (AD) (2). Aging, diabetes, and AD alter insulin transport and utilization in the brain (3). Central insulin is a neuromodulator involved in the key processes underlying cognition (4,5), energy homeostasis (6), synapse formation, and neuronal survival (7).

Intranasal insulin administration delivers insulin directly to the brain (8), and therefore intranasal insulin administration is emerging as a promising tool to deliver therapeutics to the brain tissue (9). Intranasal insulin increases regional perfusion (10,11) and improves cognition and memory (hippocampal function) in healthy young and older people (12,13), as well as in patients with cognitive impairment or mild AD (14).

Our proof-of-concept pilot study demonstrated that a single intranasal insulin dose of 40 IU acutely improved visuospatial memory in older people with T2DM and

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healthy control subjects (10). In patients with diabetes, better cognitive performance following intranasal insulin administration correlated with regional vasodilatation in the middle cerebral artery territory and in the insular cortex. Still, the mechanisms for insulin-related improvement of memory (hippocampal function) remain unclear. Functional MRI (fMRI) studies have led to the characterization of a network, termed the default mode network (DMN), that is activated during wakeful rest and deactivated during the performance of cognitive tasks (15,16). Numerous brain regions within the DMN have been linked to higher cognitive processes (i.e., language and memory), including the medial temporal lobe, the medial prefrontal cortex, anterior (ACC) and posterior cingulate cortex (PCC), and the medial, lateral, and inferior parietal cortex (IPC) (16,17). Older people with diabetes have worse functional connectivity among these regions, as compared with healthy control subjects, and the abnormal neuronal connectivity may precede clinical manifestations of brain atrophy and cognitive impairment (18–20).

We hypothesized that intranasal insulin may acutely modify signaling between the hippocampus and the DMN regions that have been implicated in memory and cognitive processing. We acquired resting-state fMRI to identify functional connectivity between the hippocampus and DMN regions following the administration of intranasal insulin or placebo in older adults with and without T2DM.

RESEARCH DESIGN AND METHODS

Design

We conducted a pilot, randomized, double-bind, placebo-controlled study with crossover design of a single dose of intranasal insulin or sterile saline in T2DM and healthy older adults (FDA-IND 107690; www.clinicaltrials.gov NCT01206322). Details of the study protocol have been reported, and intranasal insulin administration was safe without affecting systemic glucose levels (10).

Subjects

The study was conducted at the Syncope and Falls in the Elderly (SAFE) Laboratory, the Center for Advanced MR Imaging, and the Clinical Research Center (CRC) at the Beth Israel Deaconess Medical Center (BIDMC). The protocol was approved by the BIDMC Committee on Clinical Investigation. Participants were recruited prospectively via advertisements in the local community. Diabetic participants were required to be diagnosed with T2DM for at least 5 years and treated with oral antidiabetic agents. Control subjects were required to be normotensive, have fasting blood glucose $<$100 mg/dL, and not be treated for any systemic disease. Of 262 participants who completed the phone screen, 64 were eligible and provided written informed consent. Twenty-nine participants completed the protocol, and data from 28 participants were included in the analyses: 14 diabetic (7 females, 61.7 $\pm$ 8.1 years) and 14 healthy subjects (10 females, 60.1 $\pm$ 9.9 years) (Table 1).

Thirty-six participants were excluded for the following reasons: consent withdrawal (n = 7), diagnosis of DM $<$5 years (n = 3), insulin treatment (n = 1), intranasal medication (n = 1), abnormal laboratory results (n = 3), control subjects with HbA_{1c} $>$6% (n = 4), uncontrolled hypertension (n = 4), subthreshold Mini–Mental State Exam (MMSE) scores ($\leq$24 on age-adjusted norms) (n = 2), psychiatric 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), lost to follow-up (n = 3), and poor fMRI data quality due to motion artifacts (n = 1).

On-site screening included the following: fasting laboratory chemistries, electrocardiogram, vital signs, detailed medical history and medication review, and anthropometric measurements. One control participant was excluded after randomization because of high blood pressure, and one subject's data were excluded from analyses due to motion artifacts on the MRI scan. All other exclusions occurred before randomization during the screening phase. Glycemic control and other prescribed medications were taken 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 days 2 and 3. Participants had current prescriptions of one or more medications: glycemic control agents (biguanides [metformin, n = 11], sulfonylureas [glyburide, n = 4; glipizide, n = 2], and thiazolidinediones [pioglitazone, n = 2]), antihypertensives (β blockers, n = 5), angiotensin II receptor blockers (n = 3), ACE inhibitors (n = 4), statins (n = 10; control subjects, n = 0), and hormone replacement (control subjects, n = 1). Women were required to be postmenopausal.

Protocol

Studies were conducted at the BIDMC CRC. On CRC admission day 1, participants completed a baseline cognitive assessment. On days 2 and 3, protocols included safety monitoring for glucose and cardiovascular vital signs, insulin/placebo administration, anatomical and resting-state fMRI, and cognitive assessment. Resting-state fMRI was performed 26.5 $\pm$ 9.3 min after intranasal insulin administration. Vitals signs were also monitored during MRI using a Medrad Veris MR Vital Signs Monitor (Warrendale, PA).

Insulin/Placebo Administration

Each participant was treated with 40 IU insulin (Novolin; Novo Nordisk) or sterile saline in a random order on days 2 and 3 using a ViaNase device (Kurve Technologies, Inc.). 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). The placebo contained an equivalent volume of sterile saline.

Anatomical and fMRI

Anatomical and functional studies were performed on a 3Tesla GE HDx MRI scanner (GE Medical Systems, Milwaukee, WI) using the three-dimensional magnetization-prepared

Table 1—Demographic characteristics of the diabetes and control groups

	Diabetes (n = 14)	Control (n = 14)	P
Age (years)	61.7 ± 8.1	60.1 ± 9.9	0.7
Sex (male/female)	8/6	4/10	NS
Race (white/AA/Asian)	9/3/2	13/1/0	
Education (years)	14.1 ± 3.9	17.1 ± 3.2	0.03
Diabetes duration (years)	11.6 ± 4.8		
HbA_{1c} (%)	7.4 ± 1.5	5.6 ± 0.2	<0.0001
HbA_{1c} (mmol/mol)	58.4 ± 16.8	38 ± 1.95	<0.0001
Fasting glucose	131.8 ± 30.1	87.9 ± 9.7	0.0004
Systolic blood pressure (mmHg)	126.69 ± 13.8	125.5 ± 14.3	NS
Diastolic blood pressure (mmHg)	73.2 ± 8.92	72.1 ± 10.9	NS
Hyperlipidemia (yes/no)	9/5	2/12	0.005*
Total cholesterol (mg/dL)	160.7 ± 37.0	213.1 ± 45.6	0.003
Hypertension, n (%)	6 (8)	0	N/A*
MMSE	28.2 ± 1.7	28.8 ± 1.6	0.6*
Hopkins Verbal Learning-Delayed Recall T Score	41.8 ± 9.1	54.5 ± 8.5	0.0018
Trail-Making Part B T Score	37.6 ± 12.9	52.1 ± 11.5	0.004
Global gray matter volume (cm^3)	635.5 ± 29.0	691.3 ± 27.5	0.03
Left hippocampus volume (cm^3)	5.92 ± 0.45	5.76 ± 0.47	0.59
Right hippocampus volume (cm^3)	5.69 ± 0.43	5.62 ± 0.42	0.55
Left MFC volume (cm^3)	21.2 ± 0.7	22.9 ± 1.0	0.08
Right MFC volume (cm^3)	21.8 ± 0.9	23.3 ± 1.3	0.18

Between-group comparisons, ANOVA, unadjusted. Data are mean ± SD. *Pearson χ^2 test, inclusion criteria: normotensive control subjects. AA, African American. *LS model adjusted for education years, race.

rapid gradient echo (MP-RAGE) (repetition time = 6.6 ms, echo time = 2.8 ms, flip angle = 15°, bandwidth = 31.25 kHz, field of view = 24, slice thickness = 3 mm, 52 slices, matrix = 192 × 256). Resting-state functional images were collected over a 5-min period using a gradient-echo planar imaging pulse sequence sensitive to blood oxygenation level–dependent (BOLD) contrast (repetition time = 3,000 ms, echo time = 27 ms, flip angle = 60°, field of view = 25, slice thickness = 5 mm, 30 slices, matrix = 64 × 64, number of excitations = 1).

Neuropsychological Assessment

Baseline assessment (day 1) included the MMSE and measures of verbal learning (Hopkins Verbal Learning Test [HVLT]-Revised) and executive function (Trail-Making Tests A and B, Digit Span). Cognitive assessment on insulin versus placebo (days 2 and 3) was performed after MRI scan and had to be completed within 2 h after drug administration because of insulin pharmacokinetics (8,21,22). These assessments included a brief battery of parallel versions of the Brief Visuospatial Memory Test-Revised (BVMT-R) and the Verbal Fluency Task (timed word generation using letters [FAS], category, and switching conditions) of the Delis-Kaplan Executive Function System assessment (23,24).

Statistical Analyses

Statistical parametric mapping (SPM8, Wellcome Department of Cognitive Neurology, London, U.K., www.fil.ion.ucl.ac.uk/spm) was used to preprocess the raw fMRI data, and resting-state fMRI data analysis (REST V1.8, www.restfmri.net) was used for the network correlation analysis.

The first two volumes of the scanning session were discarded to allow for T1 equilibration effects. The remaining images were corrected for timing differences between each slice using Fourier interpolation. The images were then corrected for motion effects, where the first volume of the scanning session was designated as the reference volume. One participant with head motion >2.0 mm maximum displacement in any direction of x, y, and z or 2.0 degree of any angular motion throughout the course of the scan was excluded from the analyses. The mean EPI images were coregistered to the T1 images. Coregistered T1 images were normalized to the Montreal Neurological Institute Atlas via SPM 8 tools. The resulting images were smoothed with a Gaussian kernel of 6 mm × 6 mm × 6 mm (full-width half-maximum). Linear trends were removed from the image time series, and data were band-pass filtered at 0.01–0.08 Hz.

A hypothesis-driven regions of interest approach was used to investigate the hippocampus and parahippocampus (hippocampal region) using the regions of interest from the Wake Forest University PickAtlas (25). Bilateral hippocampus and parahippocampus were selected as seed regions, and the correlations of time course between seed regions and the whole brain were calculated in a voxel-

wise manner for each subject and condition (e.g., DM-insulin, DM-placebo, control-insulin, control-placebo). The Fisher transformation (*r*-to-*z* transformation) was used to normalize distribution of the Pearson correlation coefficient values (*r*) to standard *z* scores to represent the strength of connectivity (26). One-sample Student *t* tests (uncorrected, voxels with $P < 1 \times 10^{-9}$ and cluster size $\geq$270 mm^3) were used to determine brain regions with significant connectivity to the seed regions in each state. Connectivity maps were compared between the insulin and placebo condition for each subject using a paired Student *t* test. Two-sample Student *t* tests were used to compare the diabetes and control groups. The threshold was corrected with Alphasim (AFNI, Bethesda, MD, http://afni.nimh.nih.gov/afni/) in paired and two-sample Student *t* tests ($P < 0.05$; minimum cluster size was set to 270 mm^3).

Performances on the BVMT-R were reported as age-adjusted T scores for the total learning score across the three immediate recall trials (Total Recall) and delayed recall (Delayed Recall). Performances on the FAS, category, and switching verbal fluency trials were also reported as age- and education-adjusted T scores. Composite general cognitive function scores were calculated as average T scores.

Least square (LS) models were used to evaluate the relationships between fMRI measures (regional *z* scores) and cognitive measures (verbal fluency and BVMT-R, as dependent variables) with age and sex as model effects. A LS model for MMSE was adjusted for education years and race. LS models were calculated separately within group and condition (e.g., diabetes group on insulin) for each variable to minimize the effects of multiple comparisons. Conservatively, we selected models with $R^2 > 0.25$ and $P < 0.05$, and 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 Characteristics

Demographic group characteristics were similar (Table 1), but diabetic subjects had lower global gray matter volume ($P = 0.03$), fewer years of education ($P = 0.03$), and worse executive function ($P = 0.004$) and verbal memory ($P = 0.002$). Hippocampal volumes were similar between the groups.

Resting-State Connectivity

Multiple regions within the DMN exhibited functional connectivity to the right and left hippocampal regions. Figure 1*A–F* depicts a summary of the DMN regions that

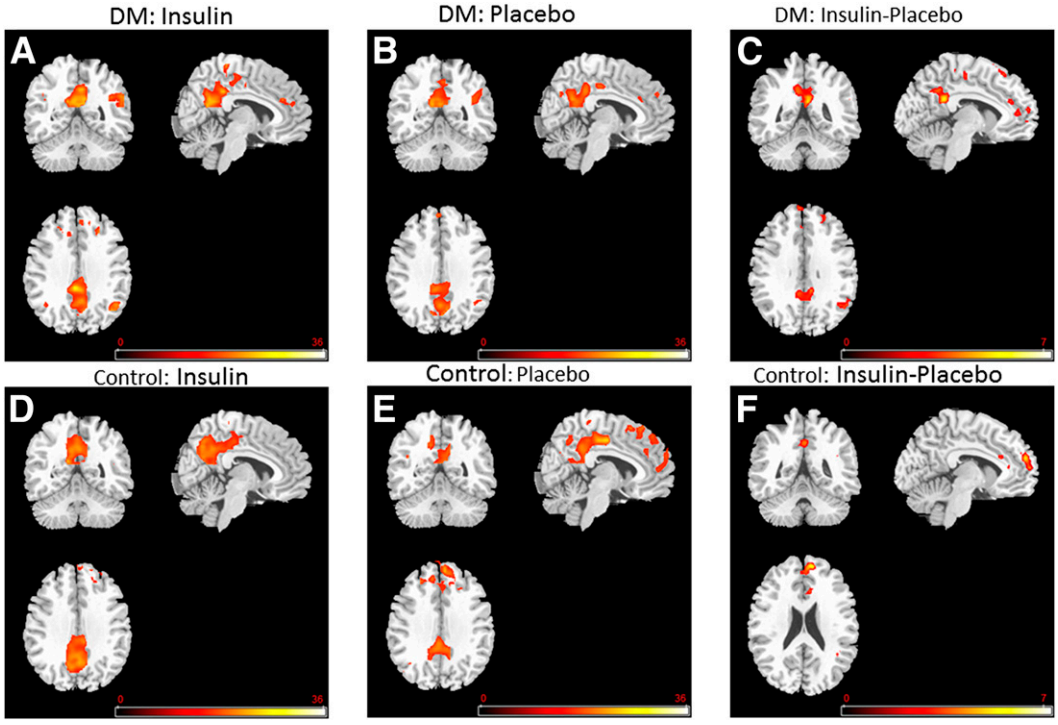

Figure 1—Resting-state functional network regions (MFC, PCC, IPC, and ACC) with significant connectivity (voxels with $|t| \geq 15.4$, cluster size $\geq$270 mm^3, and $P < 1 \times 10^{-9}$) to the right and left hippocampus in the diabetes and the control groups after intranasal insulin and placebo administration. *A*: Diabetes group, intranasal insulin administration. *B*: Diabetes group, placebo administration. *C*: Diabetes group, differences in functional connectivity between insulin and placebo administration. Intranasal insulin administration was associated with increased connectivity between hippocampal regions and MFC, R-IPC, and PCC, as compared with placebo (paired Student *t* test, voxel corrected within subject comparisons, cluster size $\geq$270 mm^3, $P < 0.05$). *D*: Age-matched healthy control subjects, intranasal insulin administration. *E*: Control group, placebo administration. *F*: Control group, differences in functional connectivity between insulin and placebo administration.

were significantly correlated (voxels with $|t| \geq 15.4$, cluster size ≥ 270 mm^3) to bilateral hippocampal regions following intranasal insulin and placebo administration in the diabetes group (Fig. 1*A* and *B*) and control subjects (Fig. 1*D* and *E*).

In the diabetes group, insulin increased connectivity between the medial frontal cortex (MFC), right (R)-IPC, PCC, and ACC and hippocampal regions, as compared with placebo (Fig. 1*A–C* and Table 2). The threshold was set at $P < 0.05$, voxel corrected; a minimum cluster size = 270 mm^3.

Similarly, in the control group, insulin increased connectivity in the MFC, PCC, and ACC (Fig. 1*D–F* and Table 2). Table 2 shows all regions connected to the right or left hippocampal regions.

In addition, we calculated the strength of ipsilateral connections and an average regional cluster size for each subject, and compared the insulin and placebo conditions within each group (Table 3). In the diabetes group, insulin administration increased the average cluster size within the MFC that was functionally connected to the right hippocampal region, as compared with placebo ($P = 0.03$). Following insulin administration, as compared with placebo, we also observed a trend toward an increase in cluster size within the left MFC that was functionally connected to the left hippocampal region ($P = 0.06$). The correlation between the right hippocampal region and the R-IPC also increased on insulin as compared with placebo (z value $P = 0.03$). The group average peak z value range for all regions was 0.76–1.69 following insulin administration and 0.71–1.55 following administration of the placebo.

In the control group, insulin administration increased the average cluster size within the left PCC that was functionally connected to the left hippocampal region, as compared with placebo ($P = 0.017$; z value $P = 0.056$). Correlations between the left ACC and the left hippocampus also tended to be stronger (z value $P = 0.056$). The group average peak z value range for all regions was for insulin 0.71–1.69 and for placebo 0.73–1.64.

Figure 2 maps the differences between the diabetes and the control groups after insulin (Fig. 2*A*) and placebo (Fig. 2*B*) administration. After insulin administration, the diabetes group still had worse functional connectivity in the MFC as compared with healthy control subjects (Fig. 2*A*), but these differences were less prominent than after the placebo administration (Fig. 2*B*) (the threshold was set as Alphasim corrected $P < 0.05$; a minimum cluster extent = 270 mm^3).

Ipsilateral comparisons indicated that after placebo administration, the diabetes group had a smaller cluster of voxels within the MFC that was functionally connected with the right hippocampus, as compared with control subjects (47% decrease; $P = 0.019$; z value $P = 0.31$). A similar trend was also observed for the connectivity between the MFC and the left hippocampus (58% reduction; $P = 0.058$; z value $P = 0.24$). However, the diabetes group had a larger cluster of connectivity between the right hippocampus and the PCC as compared with control subjects (29% increase; $P = 0.047$; z value $P = 0.09$), and a similar trend was observed for increased connectivity between the PCC and the left hippocampus (23% increase; $P = 0.1$; z value $P = 0.17$).

After insulin administration, the cluster size differences between the diabetes and the control groups decreased by 44% in the MFC and by 95% in the ACC.

Resting-State Connectivity and Cognition

Performances on verbal fluency and visuospatial memory (BVMT-R) tasks after insulin administration tended to be higher than on-placebo performances, and control subjects on insulin performed better than diabetic participants on insulin on FAS, switching, and composite verbal fluency, and BVMT-R T1–T3 trials and Total Recall (10).

In diabetic subjects on insulin, better performance on the verbal fluency category (naming all words in the same semantic category) was associated with stronger average connectivity (z value) between the right hippocampal region and the ACC ($R^2_{adj} = 0.28$; $P = 0.02$) (Fig. 3*A* and *B*). Verbal fluency category switching was associated with lower connectivity coefficient between the left hippocampal region and the MFC ($R^2_{adj} = 0.43$; $P = 0.04$) but not with cluster size. In control subjects on insulin, better scores on BVMT-R Delayed Recall tended to be associated with stronger average connectivity between the left hippocampal region and the PCC ($R^2_{adj} = 0.41$; $P = 0.07$).

In diabetic subjects on placebo, BVMT-R Total Recall scores were associated with lower average coefficients of connectivity between the left hippocampal region and the

Table 2—Insulin vs. placebo connectivity within diabetes and control groups

		Cluster size (mm^3)		Average t value		Peak t value	
Brain region	Brodmann area	Diabetes	Control	Diabetes	Control	Diabetes	Control
MFC	8//9	8,073	3,321	3.33	3.17	6.78	5.85
R-IPC	40	2,214	NS	3.33	NS	5.85	NS
PCC	23//31	1,404	1,188	3.49	2.84	5.53	4.17
ACC	24	4,752	972	3.22	3.13	6.34	5.28

Comparisons of connectivity between hippocampal regions and DMN regions in both hemispheres between insulin and placebo conditions in the diabetes and the control groups. Paired Student t tests were used to compare insulin vs. placebo conditions within the diabetes and control groups, $|t| > 2.16$ (Alphasim corrected $P < 0.05$).

Table 3—Insulin vs. placebo connectivity within diabetes and control groups

Diabetes group	Brodmann area	Cluster size (mm^3)		Peak *z* value		*P* value Insulin vs. placebo		Insulin *P* value DM vs. control	
		Insulin	Placebo	Insulin	Placebo	Cluster size	*z* value	Cluster size	*z* value
Brain region									
Left hippocampal regions									
MFC	8//9	4,900.5 ± 3,617.6	3,276.6 ± 2,703.2	0.97 ± 0.17	0.91 ± 0.23	0.06	0.16	0.18	0.48
L-IPC	39	345.2 ± 249.3	403.1 ± 318.8	1.65 ± 0.58	1.55 ± 0.78	0.21	0.3	0.11	0.47
R-IPC	40	779.8 ± 736.9	509.1.3 ± 636.0	0.76 ± 0.21	0.68 ± 0.21	0.095	0.096	0.31	0.29
PCC	23//31	1,824.4 ± 744.3	1,419.4 ± 1,060.3	1.07 ± 0.27	0.94 ± 0.33	0.32	0.38	0.49	0.27
ACC	24	2,009.6 ± 1,664.0	1,492.7 ± 1,289.3	0.91 ± 0.22	0.87 ± 0.24	0.24	0.40	0.35	0.26
Right hippocampal regions									
MFC	8//9	4,142.6 ± 3,857.0	2,684.6 ± 2,675.1	0.95 ± 0.24	0.90 ± 0.24	0.03	0.24	0.42	0.46
L-IPC	39	331.7 ± 278.9	435.9 ± 299.5	1.61 ± 0.85	1.69 ± 0.89	0.13	0.36	0.13	0.39
R-IPC	40	935.4 ± 825.5	779.1 ± 682.6	0.82 ± 0.25	0.71 ± 0.19	0.17	0.033	0.23	0.33
PCC	23//31	1,982.6 ± 809.8	1,776.2 ± 830.9	1.21 ± 0.27	1.11 ± 0.28	0.44	0.46	0.22	0.12
ACC	24	1,776.2 ± 1,496.8	1,018.3 ± 1,247.0	0.91 ± 0.23	0.79 ± 0.21	0.17	0.15	0.39	0.48

Control group								Placebo DM vs. control	
Brain region									
Left hippocampal regions									
MFC	8//9	6,665.1 ± 5,254.0	5,575.5 ± 4,496.5	0.97 ± 0.24	0.97 ± 0.22	0.10	0.16	0.058	0.24
L-IPC	39	439.7 ± 131.6	468.6 ± 99.3	1.67 ± 0.65	1.64 ± 0.47	0.21	0.44	0.24	0.35
R-IPC	40	636.4 ± 764.0	501.4 ± 734.9	0.71 ± 0.29	0.64 ± 0.28	0.31	0.29	0.33	0.28
PCC	23//31	1,695.2 ± 923.4	1,147.5 ± 1,026.2	0.98 ± 0.27	0.87 ± 0.30	0.017	0.052	0.099	0.17
ACC	24	2,131.1 ± 1,670.4	1,886.1 ± 1,925.9	0.95 ± 0.23	0.90 ± 0.25	0.24	0.056	0.40	0.42
Right hippocampal regions									
MFC	8//9	5,402.0 ± 4,961.7	5,691.2 ± 4,349.1	0.93 ± 0.25	0.94 ± 0.23	0.23	0.44	0.019	0.31
L-IPC	39	428.1 ± 131.4	459 ± 100.0	1.69 ± 0.69	1.65 ± 0.46	0.2	0.42	0.39	0.44
R-IPC	40	709.7 ± 784.4	692.4 ± 778.9	0.78 ± 0.24	0.73 ± 0.26	0.24	0.24	0.19	0.38
PCC	23//31	1,675.9 ± 755.6	1,373.1 ± 1,008.9	1.06 ± 0.29	1.00 ± 0.31	0.19	0.29	0.047	0.09
ACC	24	1,807.1 ± 1,690.2	1,940.1 ± 1,786.3	0.88 ± 0.22	0.88 ± 0.28	0.40	0.098	0.15	0.33

Comparisons of connectivity between right and left hippocampal regions and DMN regions in both hemispheres between insulin and placebo conditions within each group, and between the groups. Paired and two-tailed Student *t* test. L-IPC, left inferior parietal cortex.

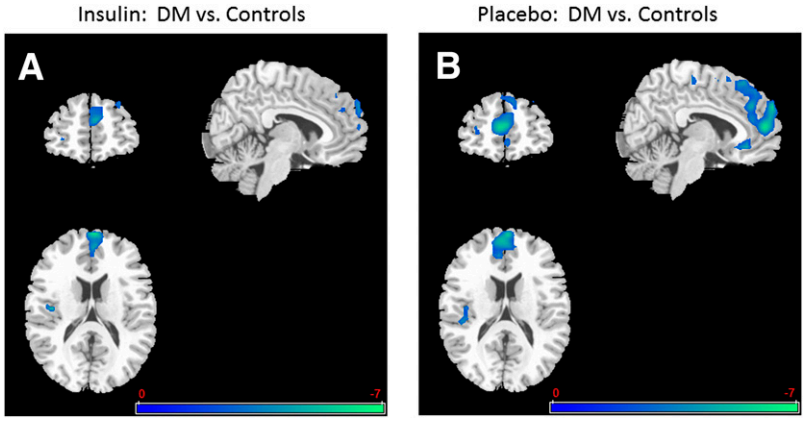

Figure 2—Differences in connectivity between the diabetes and the control groups after insulin (*A*) and placebo administration (*B*). After insulin administration, diabetic subjects had lower functional connectivity only in MFC as compared with control subjects. *B*: After placebo administration, diabetic subjects had larger areas or lower functional connectivity in multiple regions; the threshold was set as $P < 0.05$, a minimum cluster extent = 270 mm^3 (corrected).

ACC (R^2_{adj} = 0.45; P = 0.04), and the lower connectivity with the R-IPC (R^2_{adj} = 0.44; P = 0.03) (LS models were adjusted for age and sex). BVMT-R learning T scores were also associated with lower average coefficients of connectivity between the right hippocampal region and the IPC (R^2_{adj} = 0.60; P = 0.01) (Fig. 3*C* and *D*).

In control subjects on placebo, composite general cognitive function scores were also associated with lower average coefficients of connectivity between the right hippocampal region and the IPC (R^2_{adj} = 0.74; P = 0.007) (LS models adjusted for age and sex). HVLT-Recall T score was negatively associated with average connectivity (R^2_{adj} = 0.84; P = 0.01) and voxel size (R^2_{adj} = 0.84; P = 0.01) between the right hippocampus and the MFC and also between the left hippocampus and the MFC (R^2_{adj} = 0.81; P = 0.02), PCC (R^2_{adj} = 0.73; P = 0.04), and R-IPC (R^2_{adj} = 0.72; P = 0.04). These relationships were not observed after insulin administration in the diabetes and control groups.

Resting-State Connectivity and Glycemic Control

There was no significant relationship between HbA_{1c} and resting-state connectivity after insulin administration. In the control group, after placebo administration, HbA_{1c} was associated with stronger connectivity between the right and the left hippocampus and R-IPC (R^2_{adj} = 0.76; P = 0.03).

DISCUSSION

This study demonstrated that in diabetic and age-matched healthy subjects, intranasal administration of a single dose of insulin acutely increased resting-state functional connectivity between the hippocampal regions and multiple regions within the DMN (i.e., MFC, IPC, ACC, and PCC) that are linked to integrative higher cognitive functions. After placebo administration, connectivity between hippocampal regions and these DMN regions was lower in diabetic subjects as compared with healthy control subjects in several brain regions. After insulin administration, the cluster size differences between the diabetes and the control groups decreased by 44% in the MFC and by 95% in the ACC. After administration of intranasal insulin, the differences in functional connectivity between the diabetes and control groups were no longer significant.

These findings suggest that acute administration of insulin via intranasal delivery route may improve functional connections between brain regions involved in memory and cognitive processing in other domains.

The insulin resistance syndrome is associated with reduced brain insulin levels and sensitivity in age-related memory impairment and AD (5,27–29). Brain insulin plays an important role as a neuromodulator in cognition (4,5), energy homeostasis, food intake, sympathetic activity, neuron-astrocyte signaling, synapse formation, and neuronal survival (7,30). Insulin has been shown to reinforce signaling in the dopamine-mediated brain reward system and modulate food intake and responses to reward stimuli (31–33). Intranasal insulin increases rapidly in cerebrospinal fluid and binds to insulin receptors (34,35) in the olfactory bulb, several regions in the cerebral cortex including the autonomic network (e.g., insular cortex, dorsal root ganglia, nigro-striatal neurons), cerebellum (36–38), hypothalamus, and hippocampus (34,35,39).

T2DM is associated with impairment of hippocampus-dependent memory, and these effects are proportional to diabetes severity (2). Resting-state functional connectivity is also altered in T2DM subjects, and the severity of impairment correlates with the degree of insulin resistance (18,19). The effects of intranasal insulin on resting-state connectivity have not been studied. Diabetic subjects had worse baseline cognitive performance, especially in the memory and executive function domains. We have previously shown, in this cohort, that intranasal insulin may

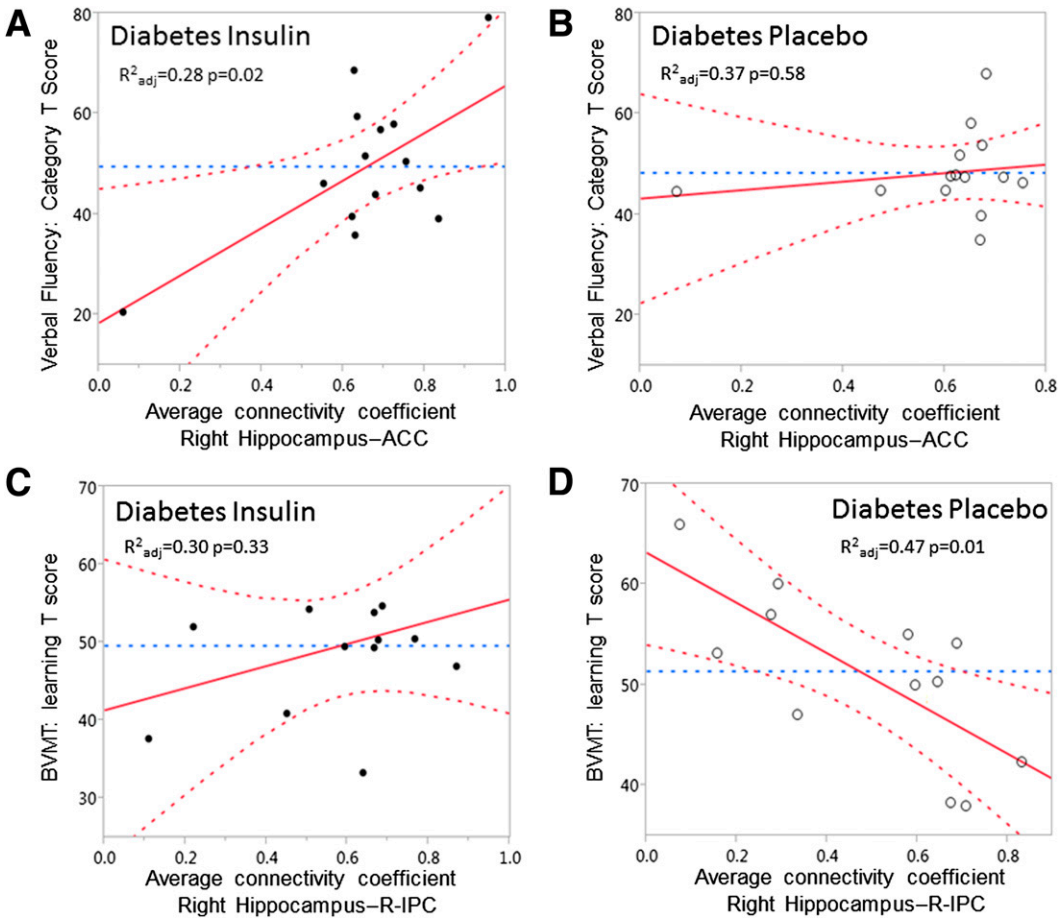

Figure 3—The relationship between functional connectivity measures and cognitive performance in the diabetes group after insulin and placebo administration. After insulin administration (*A*), the average coefficient of connectivity between the right hippocampus and ACC was associated with better verbal fluency score, but not after placebo administration (*B*). Brief visuospatial memory learning T score showed a positive trend with coefficient connectivity between right hippocampus and R-IPC after insulin administration (*C*) and a strong negative association after placebo administration (*D*).

acutely improve visuospatial memory in older diabetic and healthy adults, and that this improvement of memory and verbal learning may be dependent upon vasodilation response in the middle cerebral artery territory and in particular insular cortex (10). In diabetic subjects on insulin, better performance on the verbal fluency naming task was associated with stronger coefficient of connectivity between the right hippocampal region and ACC and lesser connectivity between the left hippocampal regions and the MFC for a more difficult category switching task. In control subjects on insulin, better performance on the visuospatial memory task (BVMT-R) tended to correlate with stronger connectivity between the left hippocampal region and PCC. Differences in relationships between cognition and connectivity between the right and left hippocampal regions are intriguing and reflect a complexity of the large-scale verbal fluency network that comprises of verbal fluency and orthographic discrimination subnetworks (40). Set switching is a complex operation involving a number of different brain structures that usually include various parts of the dorsolateral and dorsomedial prefrontal cortex, as well as temporal regions where hippocampus is located (41). Functional integration within the verbal fluency network declines with age and task difficulty. Low productive-difficult tasks are associated with significant decreases in connectivity. Therefore, the decreased connectivity between the left hippocampus and DMN regions may reflect inhibition of the left hippocampus as a result of the complex category switching process (42). After placebo administration, we have observed a "deactivation pattern" (15,16) that is characterized by task-related decreases in activity and connectivity among several DMN regions. In other words, during a task, a better task-related performance is associated with a decrease in functional connectivity within DMN.

In diabetic subjects, the worse performance on BVMT-R task was associated with stronger functional connectivity between the hippocampal regions and the ACC and IPC. Similarly in the control groups, negative associations were found between the general cognitive score and verbal learning performance and connectivity between

the hippocampal regions and the MFC, PCC, and IPC. It has been demonstrated using magnetoencephalography and a two-step hyperinsulimic clamp that resting-state activity correlates with insulin disposal (43). Furthermore, intranasal insulin may improve peripheral insulin sensitivity; insulin sensitization was associated with increased hypothalamic blood flow and parasympathetic heart rate variability (44,45). Intranasal insulin also diminished saliva cortisol and stress-induced responsiveness along the hypothalamus-pituitary axis (46,47). These findings may suggest that intranasal insulin administration may enhance functional connectivity between DMN and other brain regions and may modulate central autonomic responses to stress.

This pilot study has several limitations. The small sample size may have limited the ability to observe the full extent of functional connectivity. Cognitive testing was performed after completion of fMRI scan, and therefore we could not assess acute responses in functional connectivity to different cognitive tasks that may involve different brain regions and range of difficulty. Eleven of 14 diabetic participants were treated with metformin, which may be associated with worse cognitive performance (48). Women were required to be postmenopausal, and only one participant received hormone replacement therapy, which minimized potential effects of estrogen levels on functional connectivity (49). Furthermore, the optimal dose of intranasal insulin to modulate brain function remains unknown, as no dose-response studies have been completed to date within this population. Larger and/or more frequent doses may thus optimize the effects of intranasal insulin on brain function. Longer-term studies are also warranted to evaluate the potential for intranasal insulin for neuroprotection and improvement of cortical connectivity.

Conclusion

This study provided preliminary evidence that intranasal insulin may acutely increase functional connectivity between the hippocampal regions and the DMN in older adults with T2DM and age-matched healthy subjects. Furthermore, differences in postinsulin connectivity between diabetic and control subjects diminished. Cognitive performance on insulin was associated with regional changes in functional connectivity. Our findings provide insights into how intranasal insulin acutely modulates resting-state brain activity and its relationship to performance on higher cognitive tasks. Therefore, enhancement of functional connectivity may serve as a potential mechanism of acute intranasal insulin effect in the brain. However, larger prospective studies are needed to determine long-term safety and efficacy for prevention of cognitive decline in older people with T2DM.

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Author Contributions. H.Z. and Y.H. performed MRI analyses and contributed to manuscript preparation. B.M. contributed to study conduct and manuscript preparation. P.N. oversaw clinical aspects of the study. W.M. designed and oversaw cognitive testing and contributed to manuscript preparation. J.Z. and J.F. oversaw MRI analyses. V.N. designed the study and oversaw study conduct, data collection, analyses, and manuscript preparation. 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.

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