

ONLINE FIRST

Intranasal Insulin Therapy for Alzheimer Disease and Amnestic Mild Cognitive Impairment

A Pilot Clinical Trial

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Objective: To examine the effects of intranasal insulin administration on cognition, function, cerebral glucose metabolism, and cerebrospinal fluid biomarkers in adults with amnestic mild cognitive impairment or Alzheimer disease (AD).

Design: Randomized, double-blind, placebo-controlled trial.

Setting: Clinical research unit of a Veterans Affairs medical center.

Participants: The intent-to-treat sample consisted of 104 adults with amnestic mild cognitive impairment (n=64) or mild to moderate AD (n=40).

Intervention: Participants received placebo (n=30), 20 IU of insulin (n=36), or 40 IU of insulin (n=38) for 4 months, administered with a nasal drug delivery device (Kurve Technology, Bothell, Washington).

Main Outcome Measures: Primary measures consisted of delayed story recall score and the Dementia Severity Rating Scale score, and secondary measures included the Alzheimer Disease's Assessment Scale–cognitive subscale (ADAS-cog) score and the Alzheimer's Disease Cooperative Study–activities of daily living (ADCS-ADL) scale. A subset of participants underwent lumbar

puncture (n=23) and positron emission tomography with fludeoxyglucose F 18 (n=40) before and after treatment.

Results: Outcome measures were analyzed using repeated-measures analysis of covariance. Treatment with 20 IU of insulin improved delayed memory ($P<.05$), and both doses of insulin (20 and 40 IU) preserved caregiver-rated functional ability ($P<.01$). Both insulin doses also preserved general cognition as assessed by the ADAS-cog score for younger participants and functional abilities as assessed by the ADCS-ADL scale for adults with AD ($P<.05$). Cerebrospinal fluid biomarkers did not change for insulin-treated participants as a group, but, in exploratory analyses, changes in memory and function were associated with changes in the A β 42 level and in the tau protein-to-A β 42 ratio in cerebrospinal fluid. Placebo-assigned participants showed decreased fludeoxyglucose F 18 uptake in the parietotemporal, frontal, precuneus, and cuneus regions and insulin-minimized progression. No treatment-related severe adverse events occurred.

Conclusions: These results support longer trials of intranasal insulin therapy for patients with amnestic mild cognitive impairment and patients with AD.

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INSULIN HAS A NUMBER OF IMPORTANT functions in the central nervous system. Brain insulin receptors are densely localized in the hippocampus, the entorhinal cortex, and the frontal cortex and are found primarily in synapses, where insulin signaling contributes to synaptogenesis and synaptic remodeling.^{1,2} Insulin also modulates glucose utilization in the hippocampus and other brain regions and facilitates memory at optimal levels in normal

metabolism.³ The importance of insulin in normal brain function is underscored by evidence that insulin dysregulation contributes to the pathophysiology of Alzheimer disease (AD), a disorder characterized in its earliest stages by synaptic loss and memory impairment. Insulin levels and insulin activity in the central nervous system are reduced in AD.^{4,5} Insulin has a close relationship with the β -amyloid peptide, a toxic peptide produced by endoproteolytic cleavage of the amyloid

precursor protein.² Insoluble A β deposits in the brain's parenchyma and vasculature in AD. Soluble A β species, particularly oligomers of the 42 amino acid species (A β 42), also have synaptotoxic effects.⁶ Insulin modulates the levels of A β and protects against the detrimental effects of A β oligomers on synapses.⁷⁻⁹

Thus, reduced levels of insulin and of insulin activity may contribute to a number of pathological processes that characterize AD. Restoring insulin to normal levels in the brain may therefore provide therapeutic benefit to adults with AD. Peripheral administration of insulin is not viable owing to the risk of hypoglycemia or induction and/or exacerbation of peripheral insulin resistance. In contrast, intranasal administration of insulin provides rapid delivery of insulin to the central nervous system via bulk flow along olfactory and trigeminal perivascular channels, and slower delivery via olfactory bulb axonal transport, without adversely affecting blood insulin or glucose levels. In rodent models, intranasally administered insulin binds to receptors in the hippocampus and the frontal cortex within 60 minutes.¹⁰ In human studies,^{11,12} intranasal insulin increases insulin levels in cerebrospinal fluid (CSF) within a similar time frame and acutely enhances memory. Furthermore, a 3-week trial of daily administration of intranasal insulin improved delayed story recall and caregiver-rated functional status in a small sample of adults with AD and in adults with amnesic mild cognitive impairment (aMCI),¹³ a condition thought to represent prodromal AD in most cases.

Our study examines the effects of longer-term insulin administration on primary outcome measures determined from the 3-week trial and on measures of global cognition and function used in traditional clinical trials in adults with aMCI or AD. In a subset of participants, we also examined changes in CSF-related AD biomarkers (A β 42 level and tau protein-to-A β 42 ratio) and changes in the cerebral metabolic rate of glucose (CMRGlc) utilization assessed by use of positron emission tomography (PET) with fludeoxyglucose F 18 (FDG). Our results showed that the administration of intranasal insulin stabilized or improved cognition and function and preserved CMRGlc in regions affected by AD.

METHODS

PARTICIPANTS

The trial was registered at clinicaltrials.gov (NCT00438568) and conducted over a 4-year period. Our study was approved by the Human Subjects Review Committees of the University of Washington and the Veterans Affairs Puget Sound Health Care System and was conducted in the Veterans Affairs Clinical Research Unit. Written informed consent was obtained from all participants. A total of 104 older adults enrolled in our study (64 participants with aMCI and 40 participants with probable AD who had Clinical Dementia Rating scores of 0.5-1 and Mini-Mental State Examination scores of >15). Sample composition (combined aMCI and AD) and power estimates to determine sample size were based on a previous 3-week trial of intranasal insulin.¹³ Forty participants (15 participants received placebo, 13 participants received a 20-IU dose of insulin, and 12 participants received a 40-IU dose of insulin) who completed the main study also completed the PET substudy. Twenty-three partici-

pants (8 participants received placebo and 15 participants received insulin) who completed the main study also completed the lumbar puncture substudy. Diagnoses and eligibility were determined by consensus of expert physicians and neuropsychologists following cognitive testing, evaluation of medical history, physical examination, and clinical laboratory screening using modified Petersen criteria for the diagnosis of aMCI^{13,14} and National Institute for Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria for AD. For participants with aMCI, cognitive scores were compared with an age- and education-adjusted estimate of the participant's premorbid ability (Shipley Vocabulary test). Participants whose delayed memory scores deviated at least 1.5 SDs from this estimate were considered for the diagnosis of aMCI, which was then determined by expert consensus using all available data, following published criteria.¹⁴

Participants were free from psychiatric disorders, alcoholism, severe head trauma, hypoxia, neurologic disorders other than aMCI or AD, renal or hepatic disease, diabetes mellitus, chronic obstructive pulmonary disease, and unstable cardiac disease. Participants, study partner, and all study personnel involved in data collection were blinded to treatment assignment. Treatment groups did not differ significantly in terms of education, body mass index, general cognitive status as assessed by the modified Mini-Mental State Examination, sex, diagnosis, whether they received cholinesterase inhibitor treatment, or whether they carried the apolipoprotein E (APOE) ϵ 4 allele. The participants who received 40 IU of insulin were younger than the placebo-assigned participants ($P=.02$), whereas no differences were observed between the placebo group and the group who received 20 IU of insulin (age was included as a covariate in all analyses). Enrollment data are presented in **Figure 1**, and demographic information is presented in **Table 1**.

PROCEDURES

A nurse unaffiliated with the trial used a table of random numbers to randomly assign participants to receive a daily dosage of 20 IU of insulin (ie, 36 participants received 10 IU of insulin twice a day), 40 IU of insulin (ie, 38 participants received 20 IU of insulin twice a day), or placebo (ie, 30 participants received saline twice a day) for 4 months. Participants were stratified by whether or not they were carriers of the APOE ϵ 4 allele. Saline or insulin (Novolin R; Novo Nordisk, Princeton, New Jersey) was administered after breakfast and dinner with a ViaNase nasal drug delivery device (Kurve Technology, Bothell, Washington) designed to deliver drugs to the olfactory region to maximize transport to the central nervous system. This device released a metered dose of insulin into a chamber covering the participant's nose, which was then inhaled by breathing regularly for 2 minutes until the prescribed dose was delivered.

Parallel versions of the cognitive and functional protocol were administered at baseline, months 2 and 4 of treatment, and 2 months after treatment. Testing occurred in the morning after a standard meal. Participants were instructed to skip their morning dose on the day of testing and thus had received their last dose more than 12 hours prior to cognitive testing. The coprimary outcome measures were the delayed story recall score and the Dementia Severity Rating Scale (DSRS) score, both of which had previously demonstrated the beneficial effects of insulin.¹³ The protocol consisted of the following measures: (1) The delayed story recall score¹³ was determined after a story containing 44 informational bits was read a single time to participants who were then asked to recall the story immediately and after a 20-minute delay. (2) The DSRS score¹⁵ was determined after a questionnaire was completed by the study partner; this questionnaire was used to rate the change in the participant's cog-

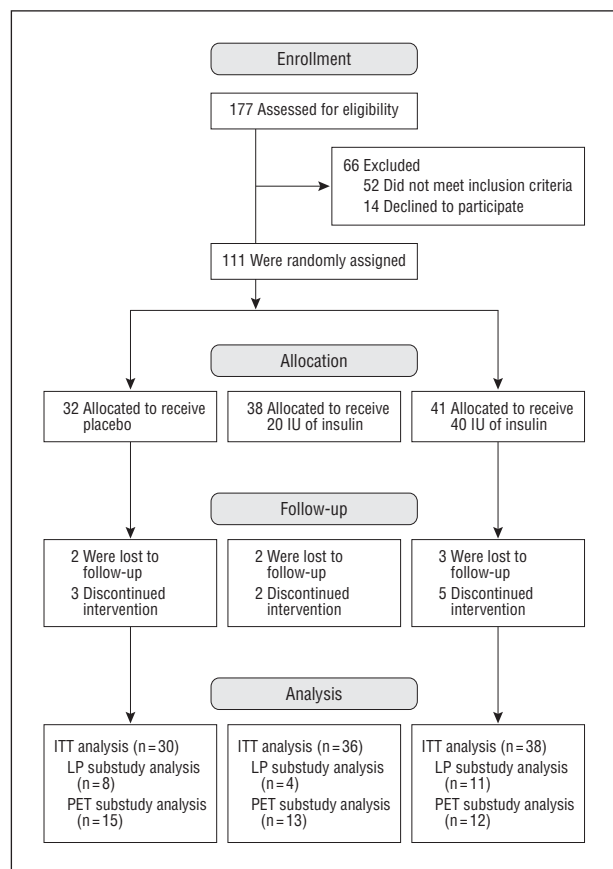


Figure 1. Patient enrollment flowchart for our trial, which examines the effects of intranasal insulin administration on cognition, function, cerebral glucose metabolism, and cerebrospinal fluid biomarkers in adults with amnesic mild cognitive impairment or Alzheimer disease. ITT indicates intent-to-treat sample; LP, lumbar puncture; PET, positron emission tomographic.

nitive, social, and functional status over a specified period of time, with higher scores indicating greater impairment. (3) The Alzheimer Disease's Assessment Scale–cognitive subscale (ADAS-cog)¹⁶ includes measures of memory, praxis, orientation, and language, with higher scores indicating greater impairment. (4) The Alzheimer's Disease Cooperative Study–activities of daily living (ADCS-ADL) scale¹⁷ was completed by the study partner and used to rate the participant's ability to perform daily activities within the past month, with lower scores indicating greater impairment.

LUMBAR PUNCTURE

After a 12-hour fast, an intravenous catheter was inserted, and the L4-5 interspace was infiltrated with lidocaine, 1%, for anesthesia. Using a 24-gauge Sprotte spinal needle (B. Braun Medical, Bethlehem, Pennsylvania), 30 mL of CSF was withdrawn into sterile syringes, aliquoted into prechilled polyethylene tubes, frozen immediately on dry ice, and stored at -70°C until testing.

AD BIOMARKERS

Levels of A β 1-42, tau protein, and P181-tau in CSF were measured with the multiparameter bead-based immunoassay (INNO-BIA AlzBio3; Innogenetics NV, Gent, Belgium). The CSF A β 40 level was measured by sandwich enzyme-linked immunosorbent assays using 6E10-coated plates (Signet Laboratories, Dedham, Massachusetts) in conjunction with biotinylated anti-A β 40 as previously described.¹⁸ The limit of detection was 15 pg/mL.

Table 1. Demographics of Intent-to-Treat Sample of 104 Adults With aMCI or Mild to Moderate AD

Demographic	Treatment Group		
	Placebo (n=30)	20 IU of Insulin (n=36)	40 IU of Insulin (n=38)
Age, mean (SEM), y	74.9 (1.6)	72.8 (1.5)	69.9 (1.4) ^a
Education, mean (SEM), y	15.3 (0.6)	15.5 (0.5)	16.2 (0.5)
3MSE score, mean (SEM)	84.2 (2.7)	83.7 (2.5)	84.3 (2.4)
BMI, mean (SEM)	27.4 (0.8)	26.7 (0.8)	26.9 (0.7)
Sex, % of patients			
Male	56.7	61.1	52.6
Female	43.3	38.9	47.4
AChEI treatment, % of patients			
No	60.0	72.2	65.8
Yes	40.0	27.8	34.2
APOE ϵ 4 carriers, % of patients			
No	55.2	50.0	57.9
Yes	44.8	50.0	42.1
Diagnosis, % of patients			
aMCI	70.0	55.6	60.5
AD	30.0	44.4	39.5

Abbreviations: AChEI, acetylcholinesterase inhibitor; AD, Alzheimer disease; aMCI, amnesic mild cognitive impairment; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); 3MSE, modified Mini-Mental State Examination.

^aThe participants who received 40 IU of insulin were younger than the placebo-assigned participants ($P=.02$).

PET WITH FDG

Positron emission tomographic imaging was obtained using a GE Advance PET scanner (GE Medical Systems, Milwaukee, Wisconsin). The participants were kept in a quiet, dimly lit room with their eyes open. Following an injection of 10 mCi of FDG, participants were monitored for 35 minutes, after which they were then moved to the scanner table, and a transmission scan and a brief emission scan were obtained in 2-dimensional data acquisition mode for 25 and 5 minutes, respectively, for postinjection attenuation correction. The final emission scan was obtained for 15 minutes in 3-dimensional data acquisition mode. Attenuation-corrected transaxial image sets were reconstructed using a filter-backprojection method. Reconstructed attenuation-corrected 3-dimensional emission images yield an in-plane spatial resolution of approximately 5 mm.

SAFETY AND COMPLIANCE

Study partners supervised participants in the administration of intranasal treatment. The blood glucose level was measured daily for the first week and weekly thereafter; no group changes were observed during the study period. Compliance was monitored by quantifying unused medication and via self-report. Safety data were reviewed semiannually by a data safety monitoring board. Adverse event reporting followed standard guidelines.

STATISTICAL ANALYSES

Data were analyzed with SAS version 9.2 (SAS Institute, Cary, North Carolina). For the intent-to-treat sample, coprimary and secondary cognitive and functional outcome scores (coprimary, delayed story recall and DSRS scores; secondary, ADAS-cog and ADAS-ADL scores) received identical analytic treatment. All scores were log-transformed to normalize distributions. To test the primary hypothesis that 4 months of insulin treat-

ment would improve delayed memory recall and daily function, the a priori analytic plan called for each of the insulin groups to be compared with the placebo group. However, to provide the most conservative approach, scores were first subjected to mixed-model repeated-measures analysis of covariance, including all treatment groups (placebo, 20 IU of insulin, and 40 IU of insulin), as the between-subjects factor, and time (baseline to month 4), as the repeated factor, using the general linear models procedure, type III sums of squares. After a significant ($P < .05$) time $\times$ treatment group interaction reflecting a different pattern of change, each of the 2 insulin groups was compared separately with the placebo group using repeated-measures analyses of covariance. Effect sizes (Cohen f) were calculated for all significant effects. Age was included as a covariate in all analyses. Diagnosis (aMCI or AD), sex, APOE $\epsilon 4$ carriage status (yes or no), baseline modified Mini-Mental State Examination score, and years of education were also included as covariates. Nonsignificant covariates were dropped from the model, and the SAS least squares means option was used to calculate means adjusted for significant covariates. Significant relationships with covariates were explored with the Pearson correlation coefficient (continuous variables) or with follow-up analyses of variance (class variables). Missing values (3% of all cognitive and functional outcome data) were treated with multiple imputation.¹⁹ Exploratory analyses were undertaken using the above-mentioned analytic strategy to determine whether changes in primary outcome measures were apparent 2 months after treatment initiation and whether they remained apparent 2 months after treatment cessation.

For exploratory biomarker analyses, because only a subset of participants elected to undergo lumbar puncture, and because no differences were observed between the 2 insulin groups, these groups were combined into a single insulin-treated group to maximize power. Biomarkers were then analyzed with the repeated-measures analysis of covariance strategy, and owing to the small sample size, exploratory Spearman rank correlations were calculated to examine relationships among changes in biomarkers and outcome measures.

Only participants who completed our study underwent a posttreatment PET-FDG scan. For FDG-PET analysis, pretreatment and posttreatment scans were coregistered for each participant and anatomically standardized to Talairach and Tournoux stereotactic coordinates.^{20,21} Pixel intensity was normalized to cerebellar and pontine values, for which the regional CMRGlc is known to be less affected in the course of AD.²² Interval and regional CMRGlc changes within groups were assessed using voxelwise 1-sample t statistics (pretreatment and posttreatment pair), and probability integrals were converted to z scores.²³ Interval changes in the regional CMRGlc were then compared between (1) the group receiving 20 IU of insulin vs the placebo group and (2) the group receiving 40 IU of insulin vs the placebo group. Based on the number of voxels and smoothness of the statistical map, a type I error rate was controlled at .05 to account for multiple comparison.²³ The resulting statistical maps were visualized in 3-dimensional stereotactic surface projections. To confirm voxelwise analyses, an independent analysis using stereotactically predefined volumes of interest was also performed.²³ In this analysis, stereotactically predefined regions shown to be abnormal in AD (medial and lateral frontal, parietal, and temporal association cortices and precuneus/posterior cingulate) were applied to the standardized and normalized image data sets, and averaged counts within each region were normalized to those of reference regions as described previously.²³ The volume-of-interest values in each region were subjected to repeated-measures analysis of variance using the identical strategy and the same covariates described above for cognitive measures, with an additional within-subjects factor of side (right or left).

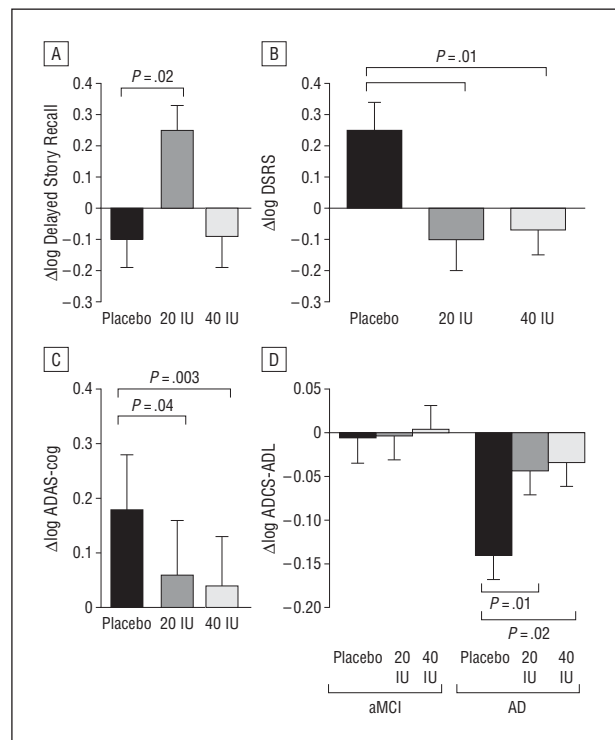


Figure 2. Log mean (A) delayed story recall, (B) Dementia Severity Rating Scale (DSRS), (C) Alzheimer Disease's Assessment Scale–cognitive subscale (ADAS-cog), and (D) Alzheimer's Disease Cooperative Study–activities of daily living (ADCS-ADL) scale change scores (from baseline to month 4) with standard errors of the mean (error bars) for placebo, 20-IU dose insulin, and 40-IU dose insulin groups. All scores are adjusted for age; ADAS-cog scores are further adjusted for the interaction of age with treatment group, and ADCS-ADL scale scores are further adjusted for diagnosis. AD indicates Alzheimer disease; aMCI, amnesic mild cognitive impairment.

RESULTS

COGNITIVE AND FUNCTIONAL OUTCOME MEASURES

The 3 treatment groups did not differ at baseline on any outcome measure; change from baseline is represented in **Figure 2** for ease of interpretation, and baseline and posttreatment group adjusted means for all measures are included in **Table 2**. Non-log-transformed values are included in eTable 1 (<http://www.archneuro.com>). A significant overall treatment group $\times$ time interaction was observed for delayed story recall ($P = .01$). Compared with the placebo group, the group that received 20 IU of insulin showed improved delayed story recall (Figure 2A) (treatment group $\times$ time interaction: $P = .02$, Cohen $f = 0.36$), whereas no improvement was observed for the group that received 40 IU of insulin. A significant overall treatment group $\times$ time interaction was also observed for study partner–rated function on the DSRS ($P = .008$). Compared with the placebo group, DSRS scores were preserved for both insulin groups (Figure 2B; treatment group $\times$ time interaction: $P = .01$ for both insulin groups and Cohen $f = 0.38$ and 0.41 for the 20-IU and 40-IU dose insulin groups, respectively).

In secondary analyses, significant effects were observed for the ADAS-cog (overall treatment $\times$ time in-

Table 2. Least Squares Mean Log-Transformed Scores^a for Cognitive and Functional Outcome Measures

Measure	Mean (SE)		
	Placebo Group	20-IU Dose Insulin Group	40-IU Dose Insulin Group
Delayed story recall score			
Baseline	2.25 (0.19)	1.86 (0.17)	1.99 (0.17)
2 mo	2.16 (0.20)	2.13 (0.18) ^b	1.94 (0.17)
4 mo	2.14 (0.20)	2.11 (0.18) ^c	1.90 (0.18)
6 mo	1.87 (0.21)	2.10 (0.19) ^d	1.95 (0.19) ^e
DSRS score			
Baseline	1.64 (0.19)	1.72 (0.16)	1.78 (0.17)
2 mo	1.71 (0.19)	1.65 (0.18) ^b	1.79 (0.18)
4 mo	1.89 (0.20)	1.62 (0.17) ^c	1.72 (0.18) ^c
6 mo	1.70 (0.22)	1.71 (0.20)	1.71 (0.20)
ADAS-cog score			
Baseline	1.93 (0.13)	2.21 (0.12)	2.26 (0.12)
2 mo	2.09 (0.12)	2.21 (0.11)	2.21 (0.11)
4 mo	2.11 (0.13)	2.27 (0.11) ^c	2.31 (0.11) ^c
6 mo	2.14 (0.14)	2.18 (0.12)	2.21 (0.12)
ADCS-ADL scale score			
Baseline	3.75 (0.03)	3.79 (0.03)	3.77 (0.03)
2 mo	3.72 (0.03)	3.78 (0.03)	3.78 (0.03)
4 mo	3.68 (0.04)	3.77 (0.03)	3.76 (0.03)
6 mo	3.72 (0.03)	3.78 (0.03)	3.78 (0.03)

Abbreviations: ADAS-cog, Alzheimer Disease's Assessment Scale–cognitive subscale; ADCS-ADL, Alzheimer's Disease Cooperative Study–activities of daily living; DSRS, Dementia Severity Rating Scale.

^aAll means are adjusted for age. The ADAS-cog scores are further adjusted for interaction between age and treatment group, and the ADCS-ADL scale scores are further adjusted for interaction between diagnosis and treatment group.

^bSignificant interaction ($P < .05$) between treatment group and time for baseline vs month 2 comparison with placebo group.

^cSignificant interaction ($P < .05$) between treatment group and time for baseline vs month 4 comparison with placebo group.

^dTrend interaction ($P < .10$) between treatment group and time for month 4 vs month 6 comparison with placebo group.

^eSignificant interaction ($P < .05$) between treatment group and time for month 4 vs month 6 comparison with placebo group.

teraction: $P = .004$). Both insulin groups had less decline in cognition compared with the placebo group on the ADAS-cog (treatment group $\times$ time interaction: $P = .04$ for the 20-IU dose insulin group and $P = .002$ for the 40-IU dose insulin group; Cohen $f = 0.27$ for the 20-IU dose insulin group and Cohen $f = 0.40$ for the 40-IU dose insulin group) (Figure 2C). Treatment effects on the ADAS-cog interacted with age (overall treatment $\times$ time $\times$ age interaction: $P = .01$; individual treatment $\times$ time $\times$ age interactions for the 20-IU and 40-IU dose insulin groups compared with the placebo group: $P = .04$ and $.003$, respectively, and Cohen $f = 0.26$ and 0.39 , respectively), such that, for the placebo group, younger age was associated with greater decline (increased score) on the ADAS-cog ($r = -0.40$, $P = .02$), whereas, for the 40-IU dose insulin group, greater improvement (lowered score) tended to be associated with younger age ($r = 0.31$, $P = .06$). For the ADCS-ADL scale, no overall effects of treatment on daily function were observed. However, a significant overall interaction with diagnosis was observed for this measure (overall treatment $\times$ time $\times$ diagnosis interaction: $P = .02$). Participants with AD receiving either dose of insulin had preserved function compared with placebo-

assigned participants with AD who showed a slight decline, whereas participants with aMCI showed no change regardless of treatment assignment (individual interactions for the participants with AD in the 20-IU and 40-IU dose insulin groups compared with the placebo group: $P = .01$ and $.02$, respectively, and Cohen $f = 0.45$ and 0.43 , respectively) (Figure 2D). Adjustment for APOE $\epsilon 4$ status, baseline modified Mini-Mental State Examination score, whether they received cholinesterase inhibitor treatment, sex, and education did not affect the pattern of any result.

Exploratory analyses were conducted to determine whether changes in primary outcome measures were apparent 2 months after treatment initiation. For delayed story recall and the DSRS, significant improvement was observed for the 20-IU dose insulin group ($P = .01$ and $.03$, respectively, and Cohen $f = 0.34$ and 0.34 , respectively) (Table 2). No improvement was observed for the 40-IU dose insulin group at the 2-month time point on either measure. Similar exploratory analyses were conducted to determine whether treatment effects persisted 2 months after treatment cessation. Delayed story recall scores tended to be higher for the 20-IU dose insulin group, and they were also elevated for the 40-IU dose insulin group, compared with the placebo group (treatment group $\times$ time interaction: $P = .07$ and $.04$ and Cohen $f = 0.23$ and 0.27 for month 4 vs month 6 comparisons, respectively). No differences were observed between groups at month 6 for study partner-rated function on the DSRS.

AD BIOMARKERS

The CSF A β 42 levels were slightly though not significantly lower for the insulin-treated groups at both baseline and month 4 ($P = .14$ and $.12$, respectively) (eTable 2). The concentrations of A β 42, A β 40, and tau protein in CSF did not change for the placebo or insulin-treated groups as a whole. In exploratory analyses, however, increased CSF A β 42 concentrations were associated with better delayed story recall and daily function on the ADAS-ADL scale, whereas decreased CSF A β 42 concentrations were associated with worse performance (Spearman rank correlation $\rho = 0.59$ and $P = .02$ and $\rho = 0.60$ and $P = .02$ for combined 20-IU and 40-IU dose insulin groups, respectively). Similarly, decreased tau protein-to-A β 42 ratios during the 4-month study period were correlated with improved delayed story recall and better daily function on both ADCS-ADL and DSRS for insulin-treated participants, whereas increased ratios were associated with worse performance ($\rho = -0.52$ and $P = .05$, $\rho = -0.50$ and $P = .07$, and $\rho = 0.53$ and $P = .05$ for the combined groups, respectively). No relationships between biomarkers and cognitive and/or functional outcome measures were observed for the placebo group.

FDG-PET CMRglc

Compared with placebo-assigned participants, the 20-IU dose insulin group showed reduced progression of hypometabolism in bilateral frontal, right temporal, bilateral occipital, and right precuneus and/or cuneus regions during the 4-month treatment period (Table 3 and

Table 3. Data on Areas of Reduced Progression for 20-IU and 40-IU Dose Insulin Groups Compared With the Placebo Group

Comparison	z Score	Stereotactic Coordinates ^a		
		x	y	z
20-IU dose insulin group vs placebo group				
Inferior occipital cortex (left)	4.3	19	-62	-7
Lateral temporo-occipital cortex (right)	3.9	-39	-80	2
Precuneus (right)	3.8	-3	-73	23
Superior temporal cortex (right)	3.7	-53	-24	2
Lateral occipital cortex (left)	3.5	6	-87	9
Orbital frontal cortex	3.2	-1	48	-16
40-IU dose insulin group vs placebo group				
Orbital frontal cortex	5.8	1	23	-18
Inferior occipital cortex (left)	5.3	21	-64	-9
Inferior parietal cortex (left)	4.1	35	-40	47
Precuneus and cuneus regions (right)	4.1	3	-80	18
Lateral occipital cortex (left)	3.7	26	-85	11
Medial frontoparietal cortex (left)	3.7	10	-19	41
Caudate (right)	3.6	-12	3	20

^aA positive value on the x coordinate indicates the left hemisphere; a positive value on the y coordinate indicates the anterior part of the brain; and a positive value on the z coordinate indicates the superior part of the brain.²¹

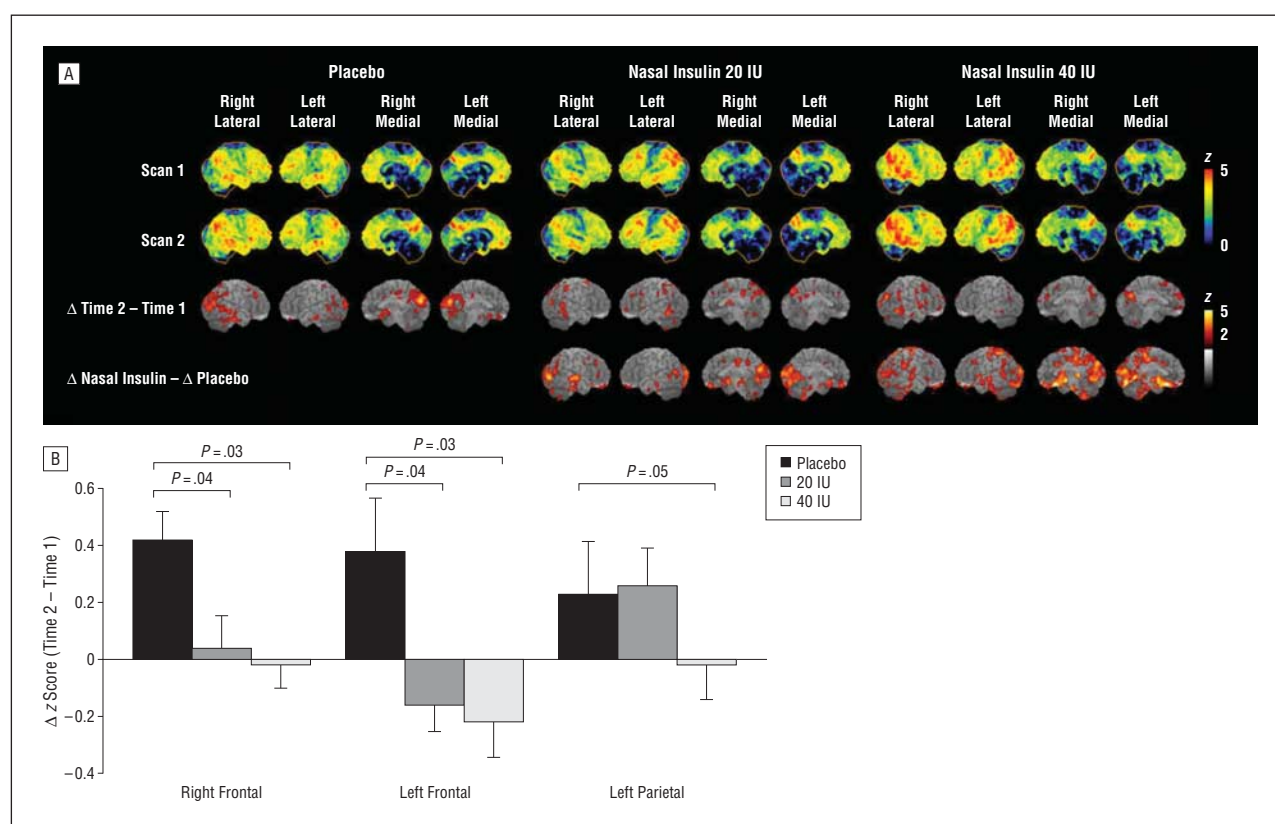


Figure 3. A, Areas of hypometabolism at baseline (scan 1) and month 4 (scan 2), along with changes in hypometabolism (Δ time 2–time 1) within each group, and differences in change between the placebo group and the 20-IU or 40-IU dose insulin group (Δ nasal insulin– Δ placebo). The red and orange colors, compared with the green and blue colors, indicate areas of greater hypometabolism from time 1 to time 2, and from placebo group to insulin groups. B, Change in mean regional z scores with standard errors of mean (error bars) for the right and left frontal regions and the left parietal region for the placebo, 20-IU dose insulin, and 40-IU dose insulin groups. For the right and left frontal volume-of-interest (VOI) values, placebo-assigned participants had reduced activity during the 4-month period, whereas the 20-IU and 40-IU dose insulin groups had preserved or slightly increased activity (treatment group $\times$ time interaction: $P = .04$ for comparison between placebo group and 20-IU dose insulin group; $P = .03$ for similar comparison between placebo group and 40-IU dose insulin group). Similar analyses for left medial parietal VOI values revealed reduced activity over time for the placebo group compared with the 40-IU dose insulin group (time $\times$ treatment group interaction: $P = .05$).

Figure 3A). The 40-IU dose insulin group also showed less progression of hypometabolism in these regions (except for the temporal cortex) and in the left parietal cor-

tex. The results of volume-of-interest analyses were generally consistent with these results. Repeated-measures analyses of variance for the right and left frontal volume-

Table 4. Data on Adverse Events and Percentage of Intent-to-Treat Sample for All Adverse Events Occurring for at Least 5% of the Participants in Any Treatment Group

Adverse Event	Treatment Group					
	Placebo		20 IU of Insulin		40 IU of Insulin	
	Events, No.	Sample, %	Events, No.	Sample, %	Events, No.	Sample, %
Light-headedness and/or dizziness	3	10.0	3	8.3	5	13.2
Headache not related to lumbar puncture	1	3.3	4	8.3	2	5.3
Nose bleed	0	0.0	6	8.3	3	2.6
Rhinitis	1	3.3	8	16.7	4	7.9
Upper respiratory tract infection	2	6.7	2	5.6	1	2.6
Fall	2	6.7	1	2.8	1	2.6
Rash	2	6.7	1	2.8	2	2.6
Other	16	46.7	30	58.3	33	60.5
Total	27	56.7	55 ^a	72.2	51 ^b	68.4

^a $P < .05$ for comparison of 20-IU dose insulin group vs placebo group.

^b $P < .10$ for comparison of 40-IU dose insulin group vs placebo group.

of-interest values revealed that placebo-assigned participants had reduced activity during the 4-month period, whereas the 20-IU and 40-IU dose insulin groups had preserved or slightly increased activity (treatment group $\times$ time interaction: $P = .04$ and $.03$ for comparison between placebo vs 20-IU and 40-IU dose groups, respectively) (Figure 3B). Similar analyses for the left medial parietal volume-of-interest values revealed reduced activity over time for the placebo group compared with the 40-IU dose insulin group (treatment group $\times$ time interaction: $P = .05$) (Figure 3B). In both medial frontal and parietal analyses, only the diagnostic group was a significant covariate, with treatment effects being most apparent for participants with AD (medial frontal analysis of treatment group $\times$ time interaction: $P = .02$ and $.02$ for placebo group vs the 20-IU and 40-IU dose insulin groups, respectively; left medial parietal analysis of treatment group $\times$ time interaction: $P = .05$ for placebo group vs 40-IU dose insulin group) and nonsignificant for participants with aMCI.

SAFETY AND COMPLIANCE

No treatment-related severe adverse events occurred during our study, and most adverse events were minor, such as mild rhinitis. The adverse events that occurred more than 5% of the time in any group are listed in **Table 4**. The mean (SE) total number of adverse events was higher for the 20-IU dose insulin group (1.44 [0.20]) compared with the placebo group (0.80 [0.22]) ($P = .04$). A similar trend was noted for the comparison between the 40-IU dose insulin group and the placebo group (mean [SE] total number of adverse events, 1.21 [0.16] vs 0.80 [0.22]; $P = .10$). Mean compliance (number of completed doses) ranged from 95% to 97% and did not differ across treatment groups.

COMMENT

Our results suggest that the administration of intranasal insulin may have a therapeutic benefit for adults with aMCI or AD. Compared with the participants in the placebo group, participants treated with the 20-IU dose of

insulin showed improved delayed memory, and both insulin doses (ie, 20 and 40 IU) preserved the study partner-rated ability to perform daily functions. General cognitive abilities, as assessed with the ADAS-cog, were also preserved by both doses of intranasal insulin. In exploratory analyses, changes in CSF A β 42 levels and tau protein-to-A β 42 ratios were associated with cognitive and functional changes for insulin-treated participants. Placebo-assigned participants showed decreased CMRGlc values in several brain regions, including the frontal, temporal, and parietal cortices as well as the precuneus and/or cuneus regions during the 4-month period, whereas insulin-treated participants showed no significant decline. Finally, no treatment-related severe adverse events occurred. These promising results provide an impetus for longer-term trials of intranasal insulin therapy in adults with aMCI or AD.

INTRANASAL INSULIN EFFECTS ON COGNITION AND FUNCTION

The primary outcome measure of delayed story recall was improved for participants receiving the 20-IU dose of insulin but not for participants receiving the 40-IU dose of insulin. In contrast, beneficial effects were observed for both doses of insulin on the coprimary measure (the DSRS) as well as on the ADAS-cog. We have demonstrated previously in an acute-dosing study¹² that the insulin-dose response curve for memory is characterized by a $\cap$ -shaped function, in which beneficial effects are observed at optimal levels, and null or negative effects are observed when levels are too low or too high. It is possible, therefore, that, in the present study, the 40-IU dose of insulin exceeded the optimal dose for memory but not for other aspects of cognition or daily function. Failure to find significant dose-related effects on this or other measures may also be due to the relatively short time period over which treatment was administered, the lack of power, or the variability in test performance. In addition, on some measures, optimal performance may have been derived with the 20-IU dose of insulin, such that higher levels did not offer additional benefit.

Group performance on several measures was characterized by a pattern of decline in the placebo group and preservation or slight improvement in both insulin-treated groups. The pattern of decline in the placebo group during the 4-month period with regard to the ADAS-cog score (with a mean raw change for the placebo group of 0.95 points) was consistent with results obtained from other clinical trials.²⁴ Our ability to detect a decline in the placebo group and differences between groups, despite the relatively brief duration of the trial, may be enhanced because of its single-site nature, which may have reduced variability in administration and scoring of cognitive and functional outcome measures.²⁵ The validity of cognitive and functional decline in the placebo group is supported by the observation that these participants also showed a reduced CMRGlc in several brain regions during the 4-month period, whereas the insulin-treated participants did not. Finally, it should be noted that, although we achieved statistical significance for most cognitive and functional outcome measures, the observed effects were small in absolute terms, as might be expected from this relatively brief intervention, and thus their long-term clinical significance is unclear.

The effect of intranasal insulin therapy on the ADAS-cog was mediated by age, with younger participants showing greater decline in the placebo group and greater benefit in the 40-IU dose insulin group. In previous work,²⁶ we have observed age-related effects of increasing doses of insulin on memory, although the underlying mechanisms are unknown. Additionally, insulin's functional benefit as assessed by the ADCS-ADL scale was apparent only for participants with AD and not for participants with aMCI. This pattern is not surprising, given that we used the ADCS-ADL version designed to assess daily function in AD, and given that adults with aMCI, by definition, have no or mild functional deficits. We were, however, able to detect the beneficial effects for insulin-treated participants with either AD or aMCI on our primary functional outcome measure, the DSRS, which has detected similar changes in a previous study.¹³ The DSRS is a simpler measure than the ADCS-ADL scale, and study partners reported anecdotally that it was easier to complete, which may have contributed to greater reliability and sensitivity.

Previous studies documented a relationship between APOE genotype and response to acute intranasal insulin administration, such that APOE $\epsilon 4$ carriers showed no memory facilitation, and adults without the $\epsilon 4$ allele showed robust facilitation.¹² Our study was not powered to examine APOE genotype as an independent predictor of treatment response, but $\epsilon 4$ carriage status was considered as a covariate in all analyses and was not related to any treatment effect. A larger trial will be necessary to definitively determine the relationship of APOE $\epsilon 4$ carriage and insulin treatment response.

PRESERVATION OF CMRGlc IN AD-RELATED BRAIN REGIONS WITH INSULIN TREATMENT

Progressive hypometabolism over time has been well documented in AD and aMCI in a number of brain regions, including the precuneus and cuneus regions and

the parietal, temporal, frontal, and occipital cortices.²⁷ In particular, reduced glucose metabolism in the precuneus and cuneus regions, which receive afferent projections from multiple brain areas, can be observed at the earliest stages of AD.²⁸ Participants in both insulin treatment groups showed reduced progression of hypometabolism in the precuneus region, as well as in the frontal and occipital cortices, compared with the placebo group. Participants in the 20-IU dose insulin group also showed less progression of temporal hypometabolism, whereas participants in the 40-IU dose group showed less parietal hypometabolism. Volume-of-interest analyses supported these findings and suggested that effects may be stronger for insulin-treated participants with AD. This pattern is not surprising, given that patients with AD show a faster progression on FDG-PET scans compared with adults with aMCI.²⁷ Although these results are promising, caution is needed in their interpretation, given that imaging was only performed for participants who completed our study.

COGNITIVE EFFECTS CORRELATED WITH CHANGES IN AD BIOMARKERS FOR INSULIN-TREATED PARTICIPANTS

The CSF biomarkers of A β 42 level, tau protein level, and the tau protein-to-A β 42 ratio are related to fundamental pathophysiological characteristics of AD. Typically, adults with aMCI or AD show lowered CSF A β 42 concentrations and elevated tau protein-to-A β 42 ratios.^{29,30} We did not observe treatment-related changes in biomarker values for the insulin-treated groups as a whole. In exploratory analyses, however, we observed that changes in the A β 42 level and the tau protein-to-A β 42 ratio were correlated with cognitive and functional changes for insulin-treated participants. No similar associations were observed for the placebo group, suggesting that these correlations were not due to general factors such as disease progression. However, these results must be interpreted with caution, owing to the exploratory nature of these analyses. Similarly, only a subset of participants completed the lumbar puncture, so it is possible that selection bias may have affected the pattern of results.

All postbaseline outcome measures were obtained at least 12 hours after dosing occurred. Thus, the observed results were not due to the acute effects of insulin. Although it is not possible to specify the mechanisms through which the administration of intranasal insulin may affect cognition, CMRGlc, and correlated changes in cognition and biomarkers, several potential mechanisms have been suggested by animal studies. In rodent models, intranasal insulin binds to receptors in the hippocampus and frontal cortex, and in a diabetic rodent model, 8 months of intranasal insulin treatment reduced diabetes-related cerebral atrophy and preserved memory and central nervous system insulin signaling.¹⁰ Insulin treatment prevents A β -induced dendritic spine and synapse loss and A β -disrupted long-term potentiation, which may enhance memory.^{7,9} Finally, in AD rodent models, insulin treatment reduced A β deposition and tau hyperphosphorylation.³¹

STUDY LIMITATIONS

Our study had several limitations. The 40-IU dose insulin group was younger than the placebo group; however, age was a covariate in all analyses, and significant differences were observed between the placebo group and the 20-IU dose insulin group, both of which were comparable with respect to age. The CSF and FDG-PET data were collected for only a subset of participants, and thus these results may be subject to sampling biases. We did not verify increased insulin levels in CSF directly after insulin administration, because we chose to examine CSF changes that were not due to acute insulin effects. Owing to the paucity of safety data available for intranasal insulin treatment in adults with AD, we administered insulin for a relatively short, 4-month period, which limited our ability to determine long-term cognitive, functional, and safety effects. For example, although we achieved statistical significance for our primary outcome measures, the observed effects were small in absolute terms, and thus their clinical significance is unclear. Our trial was a small, single-site pilot study, which presents special challenges in the interpretation of results; clearly, a longer, larger, multisite trial is needed to confirm and extend our findings.

In conclusion, the results of our pilot trial demonstrate that the administration of intranasal insulin stabilized or improved cognition, function, and cerebral glucose metabolism for adults with aMCI or AD. Safety profiles and compliance were excellent for this short-term intervention. Taken together, these results provide an impetus for future clinical trials of intranasal insulin therapy and for further mechanistic studies of insulin's role in the pathogenesis of AD.

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