Long-Acting Intranasal Insulin Detemir Improves Cognition for Adults with Mild Cognitive Impairment or Early-Stage Alzheimer's Disease Dementia

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Abstract. Previous trials have shown promising effects of intranasally administered insulin for adults with Alzheimer's disease dementia (AD) or amnestic mild cognitive impairment (MCI). These trials used regular insulin, which has a shorter half-life compared to long-lasting insulin analogues such as insulin detemir. The current trial examined whether intranasal insulin detemir improves cognition or daily functioning for adults with MCI or AD. Sixty adults diagnosed with MCI or mild to moderate AD received placebo ($n = 20$), 20 IU of insulin detemir ($n = 21$), or 40 IU of insulin detemir ($n = 19$) for 21 days, administered with a nasal drug delivery device. Results revealed a treatment effect for the memory composite for the 40 IU group compared with placebo ($p < 0.05$). This effect was moderated by APOE status ($p < 0.05$), reflecting improvement for APOE-ε4 carriers ($p < 0.02$), and worsening for non-carriers ($p < 0.02$). Higher insulin resistance at baseline predicted greater improvement with the 40 IU dose ($r = 0.54$, $p < 0.02$). Significant treatment effects were also apparent for verbal working memory ($p < 0.03$) and visuospatial working memory ($p < 0.04$), reflecting improvement for subjects who received the high dose of intranasal insulin detemir. No significant differences were found for daily functioning or executive functioning. In conclusion, daily treatment with 40 IU insulin detemir modulated cognition for adults with AD or MCI, with APOE-related differences in treatment response for the primary memory composite. Future research is needed to examine the mechanistic basis of APOE-related treatment differences, and to further assess the efficacy and safety of intranasal insulin detemir.

Keywords: Alzheimer's disease, clinical trials, randomized, insulin, intranasal drug administration, mild cognitive impairment

INTRODUCTION

The importance of insulin in normal brain function is underscored by evidence that insulin dysregulation contributes to the pathophysiology of Alzheimer's disease (AD) [1–3]. Research in animals and humans has shown that AD pathology is associated with lower levels of insulin in the cerebrospinal fluid [4]. Insulin has a close relationship with amyloid-β (Aβ), the peptide produced by cleavage of the amyloid-β protein precursor. Aβ pathologically aggregates to form plaques in AD, and its oligomeric form is synaptotoxic even

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prior to plaque deposition [5]. *In vitro* and in animal models, insulin reduces Aβ oligomer formation and protects against Aβ-induced synaptotoxicty and long-term potentiation disruption [2, 6]. Consequently, disruptions in brain insulin signaling have been suggested as one of the primary pathophysiological factors in the development of AD. Clinical studies have documented substantial, progressive disturbances in brain glucose utilization and responsiveness to insulin and insulin-like growth factor stimulation that co-occur with progression of AD [7, 8]. Disruption in central insulin regulation (also referred to as brain insulin resistance) induces pathological features of AD, and can be caused by attenuated expression of insulin receptors and insulin-like growth factor, reduced brain insulin receptor sensitivity, or increased serine phosphorylation of downstream insulin signaling molecules [1, 9, 10]. Impaired transport of insulin across the blood-brain barrier may also result in deficient levels of insulin in the central nervous system (CNS). Thus, enhancing brain insulin may prevent AD-related pathological processes.

Recent clinical trials have yielded promising effects of intranasally administered insulin for adults diagnosed with mild AD dementia or amnestic mild cognitive impairment (MCI) [11–13]. In a pilot trial, adults diagnosed with mild AD or MCI who received a 20 IU daily dose of intranasal insulin improved in delayed memory, and participants receiving either a 20 or 40 IU dose improved on caregiver-rated functional ability [13]. Both doses of insulin were associated with preserved cognition for younger participants on the Alzheimer's Disease Assessment Scale-Cognitive subscale, and preserved glucose uptake was noted for participants taking intranasal insulin on FDG-PET scans in the parietotemporal, frontal, precuneus, and cuneus regions. The improvements in episodic memory were still present two months after cessation of treatment [13].

Prior studies have also demonstrated that treatment response to many AD therapies is moderated by carriage of the apolipoprotein E-ε4 (APOE-ε4) allele [14]. In particular, research has indicated that both peripheral and central insulin metabolism, as well as insulin-altering therapies, are modulated by the APOE-ε4 allele [4, 15–17]. The APOE-ε4 allele is known to increase the risk of developing sporadic AD, but the mechanism of action is not fully elucidated [14]. Previous studies have demonstrated APOE-ε4-related differences in response to various AD therapies, and in particular to the therapeutic effects of insulin [16, 18]. In two pilot studies, treatment response was strongest for APOE-ε4 negative older adults with mild memory problems or AD [11, 19], whereas in another study, APOE-ε4 positive adults showed greater sensitivity to insulin at lower doses [20]. Other studies have also found interactions between APOE-ε4 carriage and central insulin or glucose action [18, 21].

The previous clinical trials for AD described above have all used regular insulin, which has a relatively short half-life and mimics post prandial release and in general have observed the most reliable benefits with doses of 20 or 40 IU daily. The long-acting insulin analog insulin detemir, because of the acylation of a 14-carbon fatty acid to lysine at locus B29, displays increased self-association and reversible albumin binding [22], which delays absorption of the molecule and thereby reduces the risk of hypoglycemic episodes [23]). Due to its increased lipophilicity, detemir may reach higher concentrations in the cerebrospinal fluid and brain than regular insulin [24]. The issue of whether detemir crosses the blood-brain barrier (BBB) is controversial. Although it has been described to have greater capacity to cross the BBB with potential for correspondingly increased cognitive benefit [25], elegant work in rodents has suggested that detemir does not penetrate the BBB [26], and thus intranasal or intracerebroventricular administration may be needed to ensure delivery to the brain. Although this issue remains unresolved in humans or in diseases with impaired BBB permeability, detemir has been shown to be as effective or more so than regular insulin at reducing hyperglycemia and nocturnal hypoglycemic episodes [27]. *In vivo* studies have shown that detemir demonstrates increased insulin-signaling in the hypothalamus and cerebrocortical tissue compared to regular insulin [28], suggesting that detemir may have greater central action compared to regular insulin. Another study comparing euglycemic infusion of detemir with regular insulin reported that detemir triggered a larger shift in EEG DC-potential recordings in healthy men, supporting the hypothesis that detemir affects brain functions to a greater extent than does regular insulin [29]. The peripheral effects of detemir also appear to vary according to baseline metabolic status, with greater effects on weight and other metabolic outcomes observed for adults who are more insulin resistant or obese [25, 30].

Intranasal administration of detemir has not previously been tested in adults with neurodegenerative disease. Because it has a different structure than regular insulin, with more prolonged elevations and greater CNS penetration, its safety and efficacy may differ from regular insulin. In the current pilot study, we

examined the safety profile and efficacy of two doses of insulin detemir for treatment of adults diagnosed with AD or amnestic MCI compared with placebo, using a three week protocol that effectively revealed cognitive and safety profiles in early studies of regular insulin [12], and that would provide necessary information to design future longer trials. We hypothesized that, compared with placebo, intranasal detemir would be associated with improved performance on an episodic memory composite. Secondary hypotheses predicted that daily function and working memory would improve for adults with MCI or AD taking intranasal insulin detemir versus placebo. Given previous findings of APOE-related differences and metabolism-related differences in treatment response, APOE-ε4 carrier and peripheral metabolic status were examined as possible mediating factors in a priori secondary analyses.

MATERIALS AND METHODS

Participants

The trial was registered at clinicaltrials.gov (NCT01547169) and conducted over a 2-year period. The study was approved by the Institutional Review Boards 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 60 older adults enrolled in our study (39 participants with amnestic MCI and 21 participants with probable AD with Mini-Mental State Examination (MMSE) scores >15). 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 amnestic MCI [12, 31] and National Institute for Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria for AD [32]. For participants with amnestic MCI, 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 amnestic MCI, which was then determined by expert consensus using all available data, following published criteria [31].

Participants were free from psychiatric disorders, alcoholism, severe head trauma, hypoxia, neurologic disorders other than amnestic MCI or AD, renal or hepatic disease, diabetes mellitus, chronic obstructive pulmonary disease, and unstable cardiac disease. Participants and all study personnel involved in data collection were blinded to treatment assignment. Treatment groups did not differ significantly in terms of age, education, body mass index, general cognitive status as assessed by the modified iMMSE, gender, diagnosis, whether they received anticholinesterase inhibitors or memantine, or whether they carried the APOE-ε4 allele. Enrollment data are presented in Fig. 1, and baseline demographic information is presented in Table 1.

Procedures

Participants were randomly assigned to receive a daily dosage of 20 IU of insulin detemir (10 IU detemir b.i.d.), 40 IU of insulin detemir (20 IU detemir b.i.d.), or placebo (saline b.i.d.) for 21 days. Saline or insulin detemir (Levemir®; 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 cleft region to maximize transport to the CNS. This device released a metered dose of saline or detemir into a chamber covering the participant's nose over a 2-min period, which was inhaled by breathing regularly. The choice of the 20 and 40 IU doses was based on our prior work in which similar doses of regular insulin enhanced cognition [11–13]. Dosing of insulin analogues has been calibrated to regular insulin by the pharmaceutical industry such that IU measurements of regular insulin and insulin detemir are equivalent. As this pilot study represents the first attempt to administer detemir to older adults with neurodegenerative disease, we used 20 and 40 IU doses and a three-week treatment duration that in previous studies of regular insulin provided initial indications of safety and efficacy to support additional longer-term investigation.

Parallel versions of the cognitive and functional protocol were administered at baseline and after 21 days of 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 h prior to cognitive testing. The primary outcome measure was a verbal memory composite score calculated from the sum of z-scores from the following four measures:
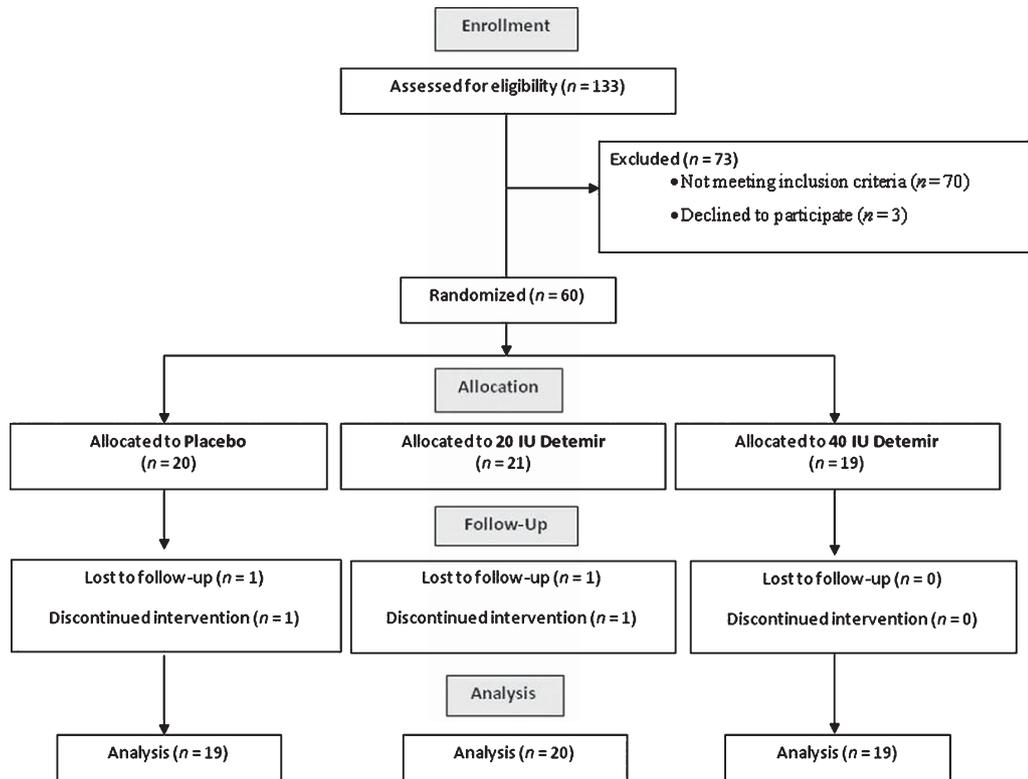
Fig. 1. Patient enrollment flowchart for the trial, which examines the effects of intranasal insulin detemir administration on cognition and function in adults with amnestic mild cognitive impairment or Alzheimer's disease.

immediate story recall, delayed story recall, immediate word list recall, and delayed word list recall. Immediate and delayed story recall [12] were 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 again after a 20-min delay. Immediate and delayed word list recall scores were derived from a 12-word Selective Reminding Word List task [33]. A higher score on the verbal memory composite indicates a better performance on these verbal memory measures. The four secondary outcome measures include tests of verbal working memory, visuospatial working memory, executive function, and caregiver-rated functional ability. Verbal working memory was measured by the Dot Counting N-back, in which participants were asked to count out loud the number of targets on consecutive computer displays. After n-number of displays, subjects recalled the number of targets presented on previous displays. Visuospatial working memory was assessed using the Benton Visual Retention Test (BVRT), Forms F and G, which is an object recognition memory paradigm [34]. For this task, subjects viewed a 2-D design and then identified this design included

in an array containing three additional, highly similar distractors. Executive functioning was determined with a computer-administered version of Stroop Color-Word Interference task, a test of selective attention and response inhibition. In this task, color names were presented on a computer screen in concordant or discordant font colors (e.g., the word "red" was presented in either red or green font). For each of four alternating trial blocks, participants either read the word or named the font color as quickly as possible, and response latency (voice onset) and content were recorded. The reaction time variable was determined by taking the average of the response latency from each correct trial. Each trial was preceded by a displayed reminder of task instruction to minimize memory load. Finally, caregiver-rated functional ability was measured by the Dementia Severity Rating Scale (DSRS) [35], in which the study partner rated the change in the participant's cognitive, social, and functional status over a specified period of time, with higher scores indicating greater impairment.

Cognitive scores for each of the two detemir dose groups were compared to the placebo group using repeated measures analysis of covariance (ANCOVA).

Age, diagnosis, MMSE score, gender, body mass index (BMI) and APOE-ε4 allele status were statistically examined as covariates.

Metabolic Measures

Participants underwent oral glucose tolerance testing (OGTT) 1–2 days prior to cognitive baseline testing and study drug initiation and 1–2 days after cognitive testing on day 21 but prior to study drug discontinuation. Blood was collected from fasting participants and they then consumed a drink containing 75 g glucose. Blood was collected at 15, 30, 60, 90, and 120 min after beverage consumption. Samples were immediately placed on ice and spun at 2,200 rpm in a cold centrifuge for 15 min, after which plasma, serum, lymphocytes, and red blood cells were aliquoted into separate storage tubes and flash frozen at –70°C. Blood glucose was measured via Accu-chek® glucose meters. Insulin resistance was calculated using the homeostatic model assessment of insulin resistance (HOMA-IR), a well-validated measure of insulin resistance calculated using fasting glucose and fasting insulin values obtained prior to administration of the OGTT beverage [36]. Another index of insulin resistance was derived from insulin and glucose levels during the OGTT: Insulin Area under the Curve (Insulin AUC) adjusted for glucose AUC was calculated using the trapezoidal rule in order to provide a secondary measure of insulin resistance that takes insulin response to a glucose challenge into account.

Safety and Compliance

Study partners supervised participants in the administration of intranasal treatment. Blood glucose levels were measured daily for the first week and then weekly thereafter; no episodes of hypoglycemia were observed. 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. Fasting plasma glucose values obtained from each study visit are reported in Supplementary Table 1.

Statistical Analyses

Data were analyzed with SPSS version 18. For the intent-to-treat sample, primary and secondary cognitive and functional outcome scores were log-transformed to normalize distributions. To test the

primary hypothesis that 21 days of treatment with detemir would improve cognition and daily function, the *a priori* analytic plan called for each of the insulin-treated groups to be compared with the placebo group. Outcomes scores were subjected to mixed-model repeated-measures analysis of covariance, including treatment group (placebo and 20 IU of detemir; placebo and 40 IU of detemir) as the between-subjects factor, and time (baseline and day 21) as the repeated factor, using the general linear models procedure, type III sums of squares. Age, diagnosis (MCI or AD), gender, APOE-ε4 carriage status (yes or no), BMI, baseline modified MMSE score, and years of education were also included as covariates. Nonsignificant covariates were dropped from the final model.

RESULTS

The three treatment groups did not differ at baseline on any outcome measure. There were also no group differences with respect to age, education, BMI, fasting insulin, fasting glucose, gender, APOE-ε4 carriage status, or MMSE score. For ease of interpretation, the change in adjusted means from Time 1 (pre-treatment) to Time 2 (post-treatment) is graphed to illustrate significant results (Figs. 2–5). Non-log transformed baseline and post-treatment group means for all measures are included in Supplementary Table 2 and are presented by diagnosis in Supplementary Table 3.

Primary Outcome

No significant overall effects for the verbal memory composite were observed for the 20 or 40 IU dose comparisons. However, a significant treatment by time by APOE-ε4 carriage interaction was observed for the 40 IU dose comparison ($p = 0.03$); interestingly, planned *post hoc* analyses revealed that APOE-ε4 positive carriers taking 40 IU intranasal detemir showed significant improvement in verbal memory ($p = 0.02$), whereas APOE-ε4 negative participants taking 40 IU intranasal detemir showed a significant decline in verbal memory ($p = 0.02$) (see Fig. 2). No effects were observed for other covariates.

Secondary Cognitive Outcomes

Overall analyses revealed significant improvement for the 40 IU group on both working memory tasks. For verbal working memory (Dot Counting N-back), a significant overall treatment group × time interaction was observed indicating improved verbal working memory
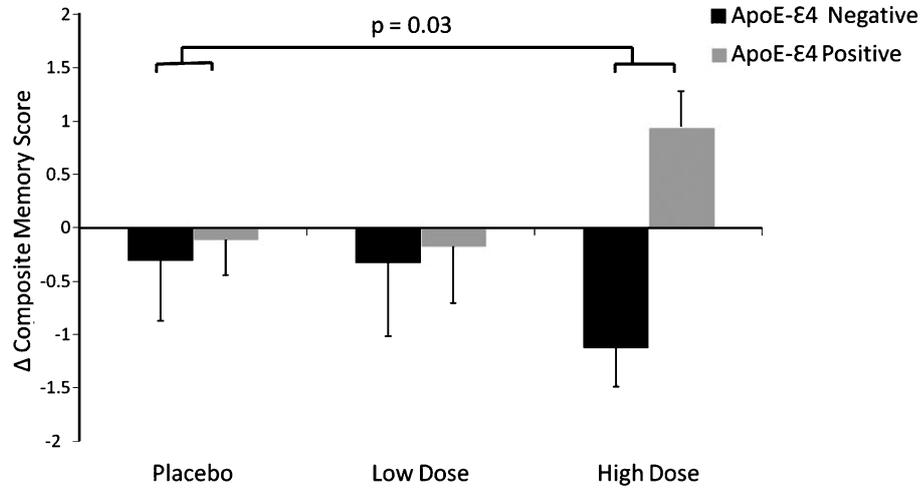
Fig. 2. Change in composite memory score from baseline to day 21, by treatment group and APOE-ε4 carriage.
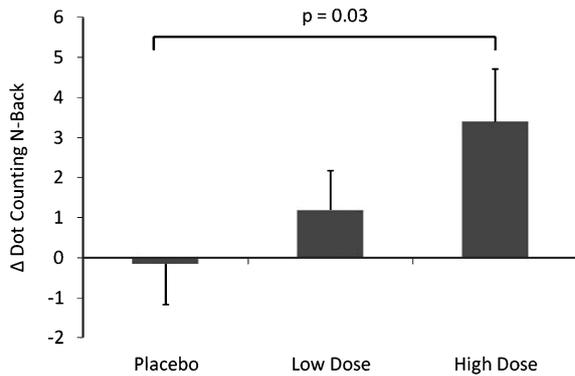

Fig. 3. Change in verbal working memory as measured by the Dot Counting N-Back Task from baseline to day 21, by treatment group.
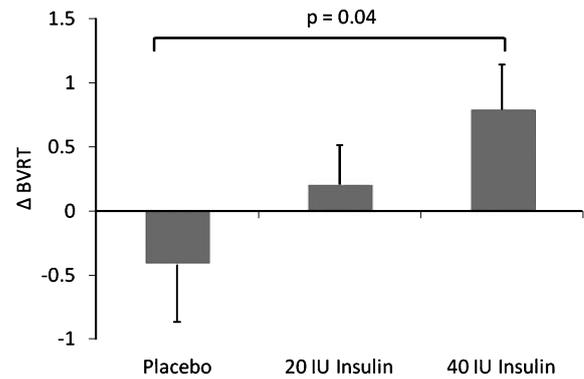

Fig. 4. Change in visuospatial working memory as measured by the BVRT from baseline to day 21, by treatment group.

in the 40 IU group versus the placebo group ($p = 0.03$; see Fig. 3). For visuospatial working memory (BVRT), a significant overall treatment group × time interaction was also observed ($p = 0.04$; see Fig. 4), indicating that subjects taking 40 IU of intranasal detemir showed improved visuospatial working memory versus those in the placebo group. No significant interactions were observed with APOE status or other covariates, and no significant differences were noted for subjects who received the 20 IU dose versus placebo. For tests of executive function (Stroop) and daily functioning (DSRS), there were no significant treatment × group effects for either dose comparison.

Metabolic Outcomes

As expected, higher baseline BMI was associated with higher baseline insulin resistance ($p < 0.01$).

In addition, APOE-ε4 carriage was associated with higher baseline Insulin AUC ($t = -2.05$; $p < 0.05$). There were no significant differences between APOE-ε4 carriers and non-carriers in the cholesterol profile (see Supplementary Table 4). For insulin resistance (HOMA-IR), the overall treatment × time effect was not significant. However, there was a treatment effect on insulin resistance with respect to APOE-ε4 carriage status for the 40 IU dose of detemir (treatment × time × APOE-ε4 interaction: $p < 0.01$ compared to the placebo group). For those with APOE-ε4 negative status, the higher dose of intranasal insulin detemir was associated with an increase in insulin resistance across 21 days. For those with APOE-ε4 positive status, taking intranasal detemir was associated with reduced insulin resistance across the 21 days (see Fig. 5). There were no significant treatment effects for Insulin AUC.
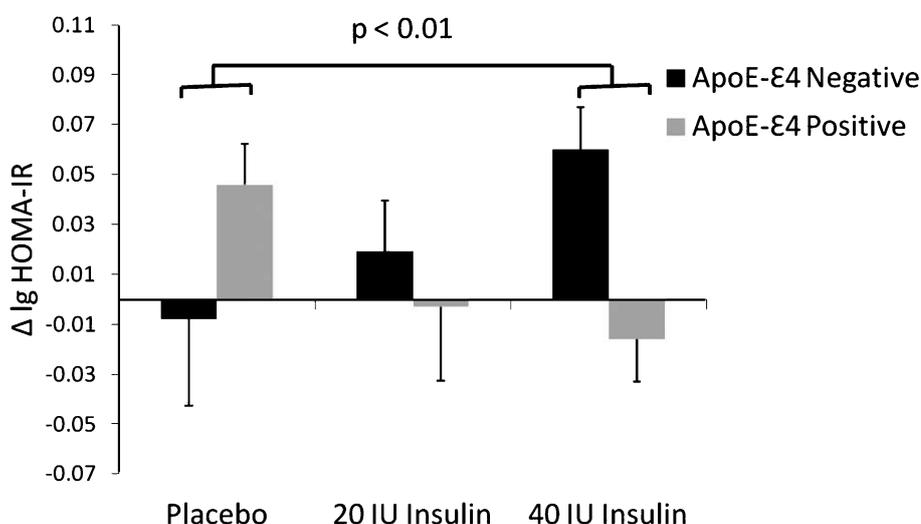
Fig. 5. Change in HOMA-IR from baseline to day 21, by treatment group and APOE-ε4 carriage.

Safety and Compliance

No treatment-related severe adverse events occurred during the study, and most adverse events were minor, such as dizziness or mild rhinitis. There were no episodes of hypoglycemia. The adverse events are listed in Table 3. The total number of adverse events was lower for the 20 IU detemir group compared with the placebo group ($p < 0.05$). Mean compliance (number of completed doses) was 98% and ranged from 89% to 100%. Compliance did not differ across treatment groups.

DISCUSSION

Treatment with 40 IU intranasal detemir was associated with improved verbal memory for adults with MCI and AD who were APOE-ε4 allele carriers, and improved visuospatial and verbal working memory for all participants. APOE-ε4 allele carriers taking the higher dose of intranasal detemir also experienced improvement in peripheral insulin resistance across three weeks of treatment. Conversely, APOE-ε4 negative individuals treated with the higher dose of detemir experienced increased peripheral insulin resistance across three weeks of treatment. No effect was shown for executive functioning or caregiver-rated daily functioning with 40 IU detemir, or for any of the cognitive outcomes after treatment with 20 IU detemir.

The current study marks the first time a long-acting insulin analogue has been administered intranasally to individuals with AD or amnestic MCI. This pilot study provides initial evidence that detemir can be safely administered to individuals with AD or MCI, and may enhance cognition for some groups. Of note, there were some key differences in the response to intranasal detemir compared with previous clinical trials that utilized regular insulin that support the possibility that insulin analogues may differ with respect to optimal therapeutic doses. Whereas a lower dose (20 IU daily) of regular insulin was effective for verbal memory in a previous study of individuals with AD or MCI, the 40 IU dose of intranasal detemir was most consistently associated with positive outcomes in the current study. It is possible that the different pharmacodynamic profiles of insulin formulations explain this finding. While detemir has a longer half-life that results in greater cumulative exposure, regular insulin mimics postprandial release and reaches a higher peak, and thus may activate mechanisms underlying episodic memory enhancement at lower doses. Interestingly, rapid acting insulin aspart, which achieves the highest peak concentration after administration, reportedly has greater memory-enhancing effects than regular insulin [37]. Detemir also has a lower affinity for insulin receptors than does regular insulin, which may induce different dose requirements [38].

Our results provide additional evidence that APOE genotype may influence response to intranasal insulin treatment in adults with MCI and AD. Interestingly, unlike previous studies with regular insulin, APOE-ε4 positive carriers showed greater improvement in verbal episodic memory with 40 IU intranasal detemir. Although the mechanisms underlying this effect cannot be determined from the present study, detemir's greater lipophilicity and albumin-binding properties that are

the basis of its protracted exposure profile may have played a role. Differences in amount or characteristics of albumin have been shown to modulate detemir's efficacy [39]. Albumin binding capacity and the albuminome are reportedly affected in various disease states such as cardiovascular and renal disease. Higher levels of post-translational glycation and nitration have reported in plasma and brain albumin in ADs patients that affect its ability to bind Aβ [40, 41]. APOE-ε4 carriers with AD have increased vulnerability to nitration [42], and thus may have a greater tendency for post-translational modifications of albumin, ultimately contributing to APOE-related differences in response to detemir [43]. These interesting possibilities require confirmation and further elucidation.

We also noted APOE-related differences in the effects of detemir on an index of insulin resistance, such that APOE-ε4 participants in the 40 IU group had improved insulin resistance whereas participants without the APOE-ε4 allele had worsened insulin resistance, changes which paralleled detemir effects on verbal episodic memory. Although peripherally-administered detemir has been shown to improve glucose regulation in adults with diabetes, some investigators have suggested that prolonged insulin exposure may promote insulin resistance in vulnerable individuals. For example, detemir has been shown to worsen insulin resistance in adults with particular metabolic profiles that may associate with APOE genotype, such as non-alcoholic steatosis ("fatty liver") [44–46]. Although there were no differences between APOE-ε4 carriers and non-carriers with respect to BMI or fasting glucose values, more in depth metabolic characteristics such as liver fat were not assessed.

It is also interesting that beneficial effects of 40 IU detemir on working memory were independent of APOE status. Working memory is preferentially mediated by the prefrontal and limbic systems [47], whereas verbal memory is associated with medial temporal/hippocampal circuits [48]. This pattern of results is consistent with previous clinical trials that show that different cognitive functions may have different dose response profiles [19], and underscore the importance of examining cognitive functions individually in addition to examining performance on global cognitive indicators.

An important goal of the present pilot study was to determine whether insulin detemir held sufficient promise as an AD treatment to support further investigation. Taken together, the benefits of detemir on memory and metabolic status provide a strong rationale for further examination of its therapeutic potential in

APOE-ε4 carriers. In APOE-ε4 non-carriers, a mixed picture was obtained, with improved working memory but worsened episodic memory and metabolic status. Additional study is needed to confirm this APOE-related pattern, potentially in a future Phase II study of moderate duration. If confirmed, this pattern would represent an important pharmacogenomic advance for AD therapeutics.

In conclusion, AD is a devastating illness, for which even small therapeutic gains have the potential to improve quality of life and significantly reduce the overall burden for patients, families, and society. Previous work has suggested that intranasal insulin may be a safe and effective treatment for the cognitive decline associated with AD. The current pilot study provides preliminary evidence that 40 IU intranasal detemir may provide effective treatment for individuals diagnosed with MCI and AD dementia, and in particular for memory-impaired adults who are APOE-ε4 carriers, a subgroup of patients notoriously resistant to therapeutic intervention [49]. Future longer-term studies are warranted to confirm this pattern and further examine the safety and efficacy for this promising treatment.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-141791.

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