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## Sex and ApoE Genotype Differences in Treatment Response to Two Doses of Intranasal Insulin in Adults with Mild Cognitive Impairment or Alzheimer’s Disease

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### Abstract

A previous clinical trial demonstrated that four months of treatment with intranasal insulin improves cognition and function for patients with Alzheimer’s disease (AD) or mild cognitive impairment (MCI), but prior studies suggest that response to insulin treatment may differ by sex and ApoE  $\epsilon$ 4 carriage. Thus, responder analyses using repeated measures analysis of covariance were completed on the trial’s 104 participants with MCI or AD who received either placebo or 20 or 40 IU of insulin for 4 months, administered by a nasal delivery device. Results indicate that men and women with memory impairment responded differently to intranasal insulin treatment. On delayed story memory, men and women showed cognitive improvement when taking 20 IU of intranasal insulin, but only men showed cognitive improvement for the 40 IU dose. The sex difference was most apparent for ApoE  $\epsilon$ 4 negative individuals. For the 40 IU dose, ApoE  $\epsilon$ 4 negative men improved while ApoE  $\epsilon$ 4 negative women worsened. Their ApoE  $\epsilon$ 4 positive counterparts remained cognitively stable. This sex effect was not detected in functional measures. However, functional abilities were relatively preserved for women on either dose of intranasal insulin compared with men. Unlike previous studies with young adults, neither men nor women taking intranasal insulin exhibited a significant change in weight over 4 months of treatment.

### Keywords

Alzheimer’s disease; insulin; intranasal drug administration; mild cognitive impairment; randomized clinical trials

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## INTRODUCTION

Insulin is a prolific hormone whose roles within the central nervous system (CNS) have been elaborated over the past several decades. Insulin receptors in the brain are heavily represented in the olfactory bulb, cerebral cortex, hippocampus, hypothalamus, amygdala, and septum [1, 2], areas that influence declarative memory, maintain energy homeostasis, and influence central control of weight [3]. Acutely raising peripheral insulin levels results in elevated insulin levels in the brain and cerebrospinal fluid (CSF), whereas chronically elevated peripheral insulin levels results in a downregulation of insulin transport across the blood-brain barrier that may lead to deficient CNS insulin [4]. Data from animal and human studies have linked peripheral insulin abnormalities with cognitive decline and Alzheimer's disease (AD) pathology [5–7]. Specifically, disorders of chronic hyperinsulinemia and insulin resistance are associated with less effective insulin within the CNS, which may then lead to impaired neuronal function in memory-dependent areas of the brain and promote AD pathology [8–11].

Evidence from animal and human studies suggests that by supplementing insulin levels in the CNS, the cognitive decline associated with AD is slowed and markers of AD pathology such as amyloid- $\beta$  (A $\beta$ ) levels may be modulated. Long-Evans rats underwent intracerebroventricular (ICV) administration of insulin, heat-deactivated insulin, or saline after a passive avoidance task. Rats who had taken insulin performed with increased latency on the following day, consistent with the theory that CNS insulin improves memory function [12]. When insulin is administered to humans intranasally, insulin is thought to rapidly access the brain, circumventing the blood-brain barrier and bypassing the periphery (see Benedict et al. [13] for a review of intranasal delivery of insulin to the brain). In healthy adults, insulin administered intranasally was reliably detected in CSF, peaking at 30 minutes after administration, with CSF insulin levels still elevated 80 minutes later [14]. In studies of older adults with AD, mild cognitive impairment (MCI), or normal memory, intranasal insulin has been shown to reliably increase verbal memory for memory-impaired adults when dosed acutely [15] and when taking intranasal insulin daily across 21 days [16]. In a follow-up study of older adults with early AD or MCI [17], subjects received intranasally-administered placebo, 20 IU of insulin, or 40 IU of insulin daily for 4 months. Subjects treated with either dose of insulin improved in caregiver-rated daily functioning, and those taking the lower dose (20 IU) improved on delayed verbal memory. Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) [18] scores improved on both doses of insulin, with greater effects observed for younger subjects. These analyses indicate that the intranasal insulin treatment may be a viable treatment for memory disorders, yet some people may benefit more than others. Identification of characteristics that predict which subjects are most likely to respond to treatment and at what doses would greatly facilitate the development of tailored therapeutic regimens.

Animal and human studies utilizing mostly young adults provide some evidence that intranasal insulin treatment response may differ by sex. For instance, male and female rats were found to have differential sensitivity to ICV leptin and insulin [19]. Human studies suggest sex differences in both cognitive and anorexigenic effects of intranasal insulin. Hallschmid et al. [20] administered placebo or 160 IU (4 $\times$ 40 IU) intranasal insulin per day

to 40 healthy young adults for 8 weeks each. Men receiving intranasal insulin lost weight and body fat, waist circumference decreased, and plasma leptin levels decreased 27%; alternatively, women gained weight, which the authors attributed to an increase in extracellular water. They posited that CNS insulin acts as a negative feedback signal in the regulation of adiposity in a sex-specific manner. Benedict et al. [21] reported that women, but not men, noted significant improvement on memory and working memory tasks after acute dosing with intranasal insulin. Alternatively, only men had reduced food consumption following intranasal insulin. To explain this finding, Krug et al. [22] studied 14 postmenopausal women in the same study design in order to determine whether low estrogen levels were a prerequisite for the anorexigenic effects of intranasal insulin. The results were identical to those reported by Benedict [21]; thus it was concluded that the differential findings based on sex were not mediated by estrogen.

Previous studies have also shown treatment response to be moderated by ApoE  $\epsilon$ 4 carriage [15, 23, 24]. It has been found that the link between AD pathology and insulin abnormalities is strongest for ApoE  $\epsilon$ 4 negative individuals [25]. Accordingly, treatment response was strongest for ApoE  $\epsilon$ 4 negative individuals in a previous study of intranasal insulin treatment for older adults with mild memory problems or AD [15]. Other studies have found interactions between ApoE  $\epsilon$ 4 carriage and central insulin or glucose action [25, 26].

This paper examines each of these previously reported moderators of treatment response in secondary analyses of a recently conducted intranasal insulin trial [17]. First, we examine whether cognition is differentially facilitated in males versus females, as some previous evidence suggests that females are more sensitive to the cognitive effects of insulin. Then, we examine whether the weight of males or females significantly changes over the course of 16 weeks of intranasal insulin treatment, as some previous evidence suggests that males are more sensitive to the anorexigenic effects of insulin. Finally, we explore whether ApoE  $\epsilon$ 4 carriage predicts treatment response to intranasal insulin for participants who completed our previous clinical trial, as some evidence suggests treatment response is strongest in ApoE  $\epsilon$ 4 negative individuals.

## MATERIALS AND METHODS

### Participants

Methods have been detailed in a previous publication [17]. The primary trial was conducted over a 4-year period and approved by the Institutional Review Boards at the University of Washington and the VA Puget Sound. Informed consent was obtained from all participants. A total of 104 older adults enrolled in the study (64 with amnesic MCI and 40 with mild-moderate AD, Mini-Mental Status Exam (MMSE)  $>15$ ). Eligibility was determined by a consensus of expert physicians and neuropsychologists following cognitive testing, evaluation of medical history, clinical laboratory screening, and a physical examination. Cognitive diagnoses were made according to modified Petersen criteria for the diagnosis of amnesic MCI [16, 27] and National Institute for Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRA) criteria for AD [28]. For participants with amnesic 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 SD from this estimate were considered for the diagnosis of amnesic MCI, which was then determined by expert consensus using all available data, following published criteria. Participants were free from psychiatric disorders, alcoholism, severe head trauma, hypoxia, neurologic disorders other than MCI or AD, renal or hepatic disease, diabetes, chronic obstructive pulmonary disease, and unstable cardiac disease. Participants, study partners, and all study personnel involved with data collection or analysis were blinded to treatment assignment.

## Procedures

Participants were randomly assigned to receive a daily dosage of 20 IU insulin (n = 36), 40 IU insulin (n = 38), or placebo (n = 30) administered intranasally for 4 months. Participants were stratified by whether or not they were carriers of the ApoE  $\epsilon$ 4 allele, a known risk factor for AD that has predicted insulin response in previous studies. ApoE  $\epsilon$ 4 negative status was defined as the absence of an  $\epsilon$ 4 allele (95% of subjects who were ApoE  $\epsilon$ 4 negative were homozygous for  $\epsilon$ 3, and 5% had one  $\epsilon$ 2 allele); ApoE  $\epsilon$ 4 positive status was defined as being homozygous (17%) or heterozygous (83%) for the  $\epsilon$ 4 allele. Saline or insulin (Novolin R; Novo Nordisk, Princeton, New Jersey) was administered after breakfast and again after dinner with a ViaNase® nasal drug delivery device (Kurve Technology, Bothell, Washington). The device was designed to deliver drugs to the olfactory region to maximize transport into the CNS. It released a metered dose of the study medication into a chamber covering the participant's nose, which was then inhaled as the participant breathed regularly for two minutes until the prescribed dose was delivered.

Parallel versions of the cognitive testing were administered at baseline, 2 and 4 months of treatment, and 2 months post treatment. The primary analysis compared changes from baseline to 4 months, which will comprise the timeframe for our analyses. Testing was completed in the morning after participants consumed a standard meal. The 4 month testing was completed the morning after the participants' last dose of study medication, thereby ensuring at least 12 hours had passed since their last dosing. The cognitive protocol contained two co-primary outcome measures: delayed story recall, and the Dementia Severity Rating Scale (DSRS). In delayed story recall [24, 29, 30], participants listened to a recording of a story that contained 44 units of information. They were asked to repeat the story both immediately and after a 20-minute delay. The delayed story recall score was obtained as a sum of the informational bits that were recalled verbatim at the 20-minute delay. The DSRS [31] score was determined after a questionnaire was completed by the study partner. The questionnaire was used to rate the change in the participant's cognitive, functional, and social status over a specific period of time, with higher scores indicating greater impairment. Two secondary measures were administered: the ADAS-Cog and the Alzheimer's Disease Cooperative Study-Activities of Daily Living Subscale (ADCS-ADL). The ADAS-Cog [18] includes measures of memory, praxis, orientation, and language, with higher scores indicating greater impairment. The ADSC-ADL scale [32] 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.

## Statistical analyses

Data were analyzed with SPSS version 18. Primary (delayed story recall and DSRS) and secondary (ADAS-Cog and ADCS-ADL) cognitive and functional outcome scores were analyzed; thus, there were four outcomes measured at baseline and after 4-months of treatment. Non-normal outcome distributions were log-transformed. Age, diagnosis (MCI or AD), education, and Modified Mini-Mental State Exam (3MSE) were included as covariates in all analyses; only significant covariates were included in the final models.

To test the hypothesis that treatment response would be moderated by sex, each outcome was subjected to mixed-model repeated-measures analysis of covariance (ANCOVA), including all treatment groups (placebo, 20 IU of insulin, or 40 IU of insulin) and sex groups (male or female) as between-subjects factors and time (baseline and 4 months) as the repeated factor, using the general linear model procedure, type III sums of squares. If a significant 3-way interaction between treatment group, sex, and time emerged, each of the two insulin groups was compared in separate models with the placebo group using repeated measures ANCOVA. ApoE  $\epsilon$ 4 allele carriage and body mass index (BMI) were explored as potential moderators for treatment response and also as potential moderators for the sex interaction. For BMI, *t*-tests were utilized to determine whether baseline sex differences were present. Chi-square analyses were utilized for ApoE 4 carriage. Then, ApoE  $\epsilon$ 4 carriage and BMI were entered into mixed-model ANCOVAs identical to the ones described above.

## RESULTS

The means and standard deviations of demographic variables and cell sizes for each group are reported in Table 1. *T*-tests revealed sex differences for age and BMI, in that men were older and had higher BMIs than women. Age and BMI were examined as covariates in all analyses. 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. 1–3). The means and standard deviations of the cognitive outcome measures are reported in Supplementary Table 1.

### Sex and treatment response

For delayed story recall, a treatment by sex interaction was observed ( $p = 0.02$ ; see Fig. 1), along with the previously reported overall treatment effect ( $p = 0.04$ ). The lower and higher insulin dose groups were then each compared to placebo. Those receiving the low dose of insulin demonstrated improved story recall over time compared with placebo-assigned participants ( $p = 0.02$ ) and this pattern did not differ for males and females ( $p = 0.59$ ). In contrast, when the high dose group was compared with placebo, there was no overall treatment effect ( $p = 0.78$ ), but there was a significant sex interaction ( $p = 0.05$ ) such that men on the high dose improved, but not women (Fig. 1). For partner-rated functional scores on the DSRS, there were marginally significant treatment effects ( $p = 0.05$  for low dose,  $p = 0.07$  for high dose) but no sex interactions ( $p = 0.92$  for low dose,  $p = 0.81$  for high).

In secondary analyses, no significant sex interactions were observed for the ADAS-Cog ( $p = 0.47$ ). For the ADCS-ADL scale, there was no overall treatment effect ( $p = 0.29$ ), but a significant treatment by sex interaction was observed ( $p < 0.01$ , see Fig. 2). Follow-up analyses reveal the sex interaction when each insulin group is compared to placebo ( $p = 0.01$  for low dose,  $p < 0.01$  for high). Females but not males benefitted from intranasal insulin.

### **Apolipoprotein $\epsilon 4$ (ApoE $\epsilon 4$ )**

ApoE  $\epsilon 4$  carriage was not a significant predictor of treatment response for any cognitive or functional outcome. Next, we examined whether the ApoE  $\epsilon 4$  carriage interacted with sex to predict study outcomes. Frequency of ApoE  $\epsilon 4$  carriage did not differ between males and females (27 of 59 men and 20 of 45 women were  $\epsilon 4$  positive). A significant treatment by sex by ApoE  $\epsilon 4$  carriage interaction was observed for delayed story recall ( $p = 0.04$ ; Fig. 3). Follow-up analyses indicate that the sex/allele carriage interaction was specific to the high dose group. ApoE  $\epsilon 4$  negative males benefitted from the high dose of insulin, whereas ApoE  $\epsilon 4$  negative females declined over time on the high dose of insulin ( $p < 0.01$ ). In co-primary and secondary outcome analysis, ApoE  $\epsilon 4$  carriage did not moderate the treatment response or the treatment by sex interaction for the DSRS, ADAS-Cog, or ADCS-ADL.

### **Weight and BMI**

Four months of treatment with intranasal insulin did not significantly alter subjects' weight, regardless of sex ( $p = 0.12$ ; Table 1). BMI did not moderate treatment response or the treatment by sex interaction for any cognitive or functional outcome.

### **Safety**

As previously reported, there were no treatment related serious adverse events (SAEs) in the trial, and most AEs were minor, such as mild rhinitis and infrequent nose bleeds [17].

## **DISCUSSION**

Our results indicate that there may be differences in how men and women respond to intranasal insulin treatment for adults with AD and MCI, differences that appear to be dose-dependent. On delayed story memory, both men and women showed cognitive improvement when taking the low dose (20 IU) of intranasal insulin daily for 16 weeks, but only men showed cognitive improvement on the high dose (40 IU). The sex difference was especially distinct for ApoE  $\epsilon 4$  negative individuals. On the high dose, ApoE  $\epsilon 4$  negative men improved while their ApoE  $\epsilon 4$  positive counterparts did not. The opposite was true for women in the high dose group; ApoE  $\epsilon 4$  negative women had the poorest cognitive outcome, while ApoE  $\epsilon 4$  positive women remained stable. ApoE  $\epsilon 4$  positive men and women remained stable on the high dose, without significant improvement or decline. This sex effect was not detected in functional measures. However, functional abilities were relatively preserved for women on either dose of intranasal insulin versus that of men. Unlike previous studies on young adults, neither men nor women taking intranasal insulin exhibited a significant change in weight over 4 months of treatment.

This study has provided additional evidence that the cognitive benefits of intranasal insulin may differ between sexes. Sex-specific findings varied between outcomes, possibly because the cognitive and functional outcome measures tap into different aspects of brain anatomy and function. When taking the high dose of intranasal insulin, men outperformed women on delayed story recall. This primary cognitive outcome is sensitive to hippocampal degeneration [33, 34] and is therefore sensitive to the cognitive decline associated with amnesic MCI and early AD. Sex differences were not observed on the ADAS-Cog, which measures a wider variety of cognitive functions. Women taking intranasal insulin tended to perform better versus placebo on the functional scales, which are most sensitive to later stages of the disease process, when functional changes are observed with greater frequency. Thus, different optimal doses of intranasal insulin may selectively benefit specific brain regions in men versus women. A larger study including brain imaging is needed to speak definitively to these sex differences.

Another interpretation of these data is that a fundamental difference in central insulin sensitivity exists between men and women. The many actions of CNS insulin are fairly recently elucidated and sex differences remain largely unexplored, but there is evidence of sex differences in peripheral insulin metabolism from which we can posit. Although whole-body insulin clearance is greater in women than men, splanchnic insulin extraction is greater in men. Therefore, greater insulin clearance in other peripheral organs accounts for women's greater whole-body insulin clearance [35]. This finding has led to speculation that women more effectively regulate insulin metabolism in peripheral organs, which is consistent with epidemiological evidence that despite the higher rates of obesity in females, men have higher rates of impaired glucose tolerance and higher incidence of diabetes [36]. There is also evidence that men are more sensitive to the cognitive consequences of peripheral insulin abnormalities. In a recent epidemiological study on individuals with MCI, Cholerton et al. [37] found a greater association between hyperinsulinemia and MCI status in men than in women. Thus, not only do men have more insulin abnormalities, but they are also more sensitive to the cognitive effects of such abnormalities, and therefore these differences may explain why men benefit from the corrective actions of higher doses of intranasal insulin.

There are other health-related factors that may have differed on average between men and women in the study that could account for the sex/dose interaction found in this study. Men were heavier on average, although BMI did not explain the sex differences noted in treatment response on the high dose of insulin. Other possible factors that may vary by sex include the distance from nasal cavity to brain, undetected metabolic differences, and brain size.

In previous work, ApoE  $\epsilon$ 4 status has been an important predictor of intranasal insulin response [15]. There has been evidence that ApoE  $\epsilon$ 4 negative individuals are more sensitive to the cognitive consequences of insulin resistance [15, 24] and that a relationship between insulin resistance and dementia risk may be most commonly observed for adults without an  $\epsilon$ 4 allele [38, 39]. Our current results further extend this finding to suggest that the ApoE  $\epsilon$ 4 gene interacts with sex to moderate treatment response. As noted above, men have a stronger relationship between insulin abnormalities and MCI status [37], and men have less efficient peripheral insulin clearance than women [35]. Therefore it is possible that the

ApoE  $\epsilon$ 4 negative males benefited from the higher dose of intranasal insulin because they were both more sensitive to intranasal insulin and had greater pre-existing insulin abnormalities. The ApoE  $\epsilon$ 4 negative females were less sensitive to changes in CNS insulin levels and had fewer pre-existing insulin abnormalities; therefore ApoE  $\epsilon$ 4 negative women did not benefit from the high dose of intranasal insulin.

There is growing evidence that ApoE  $\epsilon$ 4 carriers are different than non-carriers in how they respond to treatments such as exercise [40], insulin-sensitizing drugs [41], and many other health-related interventions [42]. However, many studies examining the ApoE  $\epsilon$ 4 gene do not examine sex as a potential modifier, but instead sex is only utilized as a control variable. Unfortunately, the current pilot trial was underpowered to fully examine these questions; a larger trial is necessary to really be able to speak to treatment response interactions based upon the ApoE  $\epsilon$ 4 allele.

Subjects' weight did not significantly change throughout the duration of the treatment. This is an important finding, as several studies administering ICV insulin to rats [19] and intranasal insulin to healthy young adults have found insulin to have an anorexigenic effect, especially for men [20, 21]. This finding is consistent with the work of Hallschmid et al. [43], who determined that obesity creates central resistance to the adiposity signal in intranasal insulin. Thus, this central resistance was likely the reason that older and more overweight subjects would be less likely to respond to the body weight regulating aspects of insulin. It is also possible that the dosage utilized in the current study did not meet the minimum threshold required to significantly alter subjects' weight. The studies that have noted weight changes in healthy younger adults typically used doses of intranasal insulin exceeding 100 IU per day, which was well above those dosages used in this study.

This study should be viewed in light of its limitations. As a pilot study, the sample size was small, which made interactions more difficult to detect due to lack of power. These analyses were *post-hoc* and exploratory, and due to small cell sizes (see Table 1), the findings should be interpreted with caution. Finally, although this is the longest trial for intranasal insulin to date, 16 weeks remains a relatively brief duration of treatment and thus it is uncertain what the long-term effects of intranasal insulin administration would be.

## CONCLUSIONS

There has been evidence that intranasal insulin is a safe and effective treatment for the memory loss associated with MCI and AD. The current paper suggests that treatment response may vary by sex and ApoE  $\epsilon$ 4 carriage. The current study justifies further examination by these variables in future work. Learning about differences in treatment response not only provides information that may be helpful in specifying which individuals will be most likely to benefit from this intervention, but it also provides additional information about the possible mechanisms of action for intranasal insulin.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.



## Acknowledgments

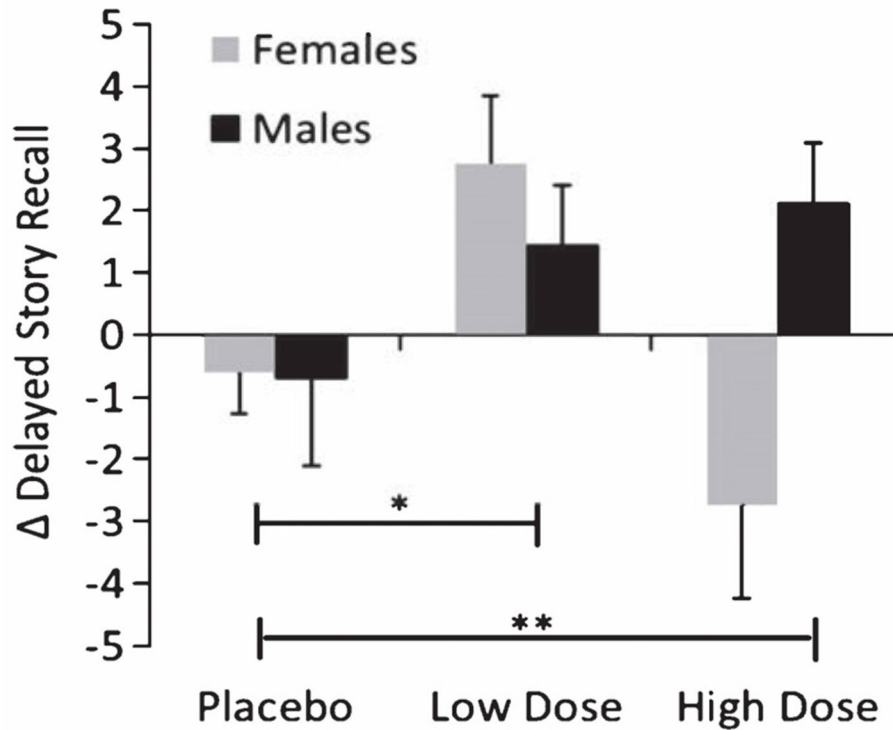
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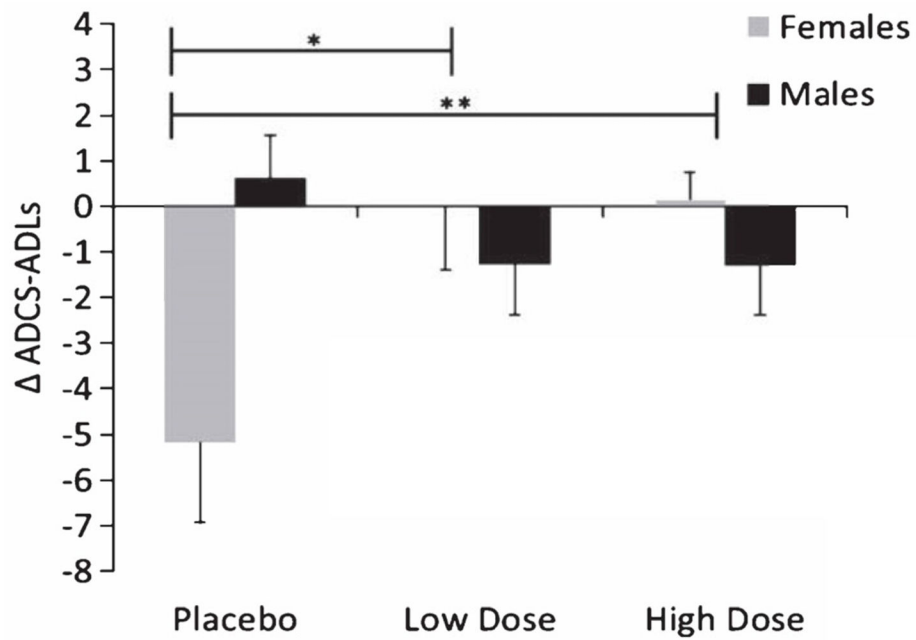
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**Figure 1.**

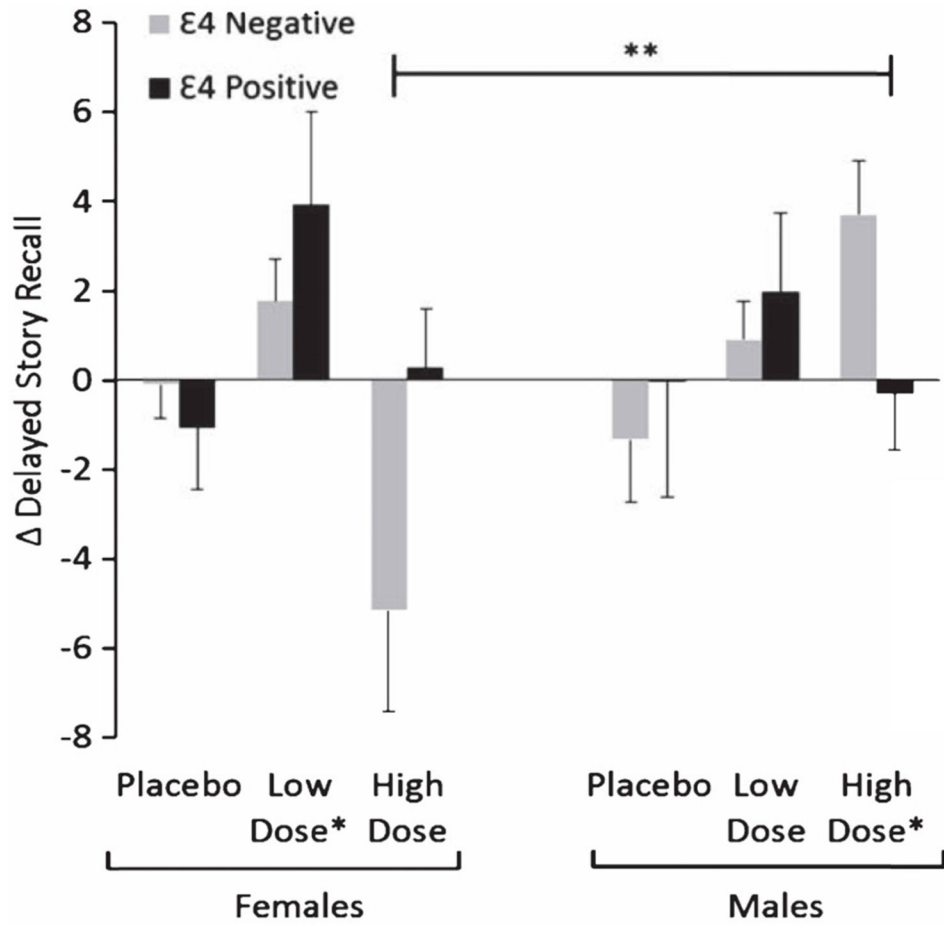
Mean change scores for delayed story recall from baseline to month 4, with standard errors of the mean (error bars), by sex and treatment group. Placebo indicates saline condition, Low Dose indicates 20 IU intranasal insulin, High Dose indicates 40 IU intranasal insulin. Note: A higher score represents cognitive improvement over time. \*Significant treatment effect,  $p = 0.02$ . \*\*Significant sex  $\times$  treatment Interaction,  $p = 0.05$ .



**Figure 2.**

Mean change scores for ADCS-ADL from baseline to month 4, with standard errors of the mean (error bars), by sex and treatment group. Placebo indicates saline condition, Low Dose indicates 20 IU intranasal insulin, High Dose indicates 40 IU intranasal insulin. Note: A higher score represents functional improvement over time; scores are adjusted for age.

\*Significant sex×treatment interaction,  $p = 0.01$ . \*\*Significant sex×treatment interaction,  $p < 0.01$ .



**Figure 3.**

Mean change scores for delayed story recall from baseline to month 4, with standard errors of the mean (error bars), by sex, treatment group, and ApoE 4 carriage. Placebo indicates saline condition, Low Dose indicates 20 IU intranasal insulin, High Dose indicates 40 IU intranasal insulin. Note: A higher score represents improvement over time. \*Significantly improved over placebo (treatment effect). \*\*Significant treatment $\times$ sex $\times$ ApoE status interaction,  $p < 0.01$ .

Table 1

Mean and standard deviation of demographic variables and weight by sex and treatment

|                                     | Placebo          |                | Low dose         |                | High dose        |                |
|-------------------------------------|------------------|----------------|------------------|----------------|------------------|----------------|
|                                     | Females (n = 13) | Males (n = 17) | Females (n = 14) | Males (n = 22) | Females (n = 18) | Males (n = 20) |
| Age <sup>1</sup>                    | 72.8 (9.7)       | 76.4 (10.4)    | 71.1 (8.1)       | 73.9 (7.1)     | 67.3 (10.0)      | 72.2 (7.8)     |
| Education                           | 13.9 (3.0)       | 16.3 (3.1)     | 14.9 (2.4)       | 16.0 (4.1)     | 16.9 (2.8)       | 15.6 (2.8)     |
| 3MSE                                | 77.8 (19.9)      | 89.0 (10.3)    | 88.6 (10.2)      | 80.6 (15.9)    | 80.3 (17.3)      | 87.9 (11.4)    |
| ApoE ε4 (%yes)                      | 41.7%            | 47.1%          | 50.0%            | 50.0%          | 44.4%            | 40.0%          |
| BMI <sup>1</sup>                    | 26.2 (3.0)       | 28.3 (5.0)     | 24.7 (3.1)       | 28.0 (4.2)     | 26.0 (4.9)       | 27.7 (5.0)     |
| Plasma insulin <sup>1</sup> (ng/ml) | 14.46 (4.10)     | 15.59 (7.09)   | 15.00 (4.80)     | 14.41 (4.92)   | 15.00 (5.98)     | 16.47 (5.54)   |
| Weight: Baseline <sup>1</sup>       | 146.8 (8.50)     | 199.9 (7.81)   | 148.7 (8.44)     | 185.4 (6.58)   | 138.1 (7.36)     | 186.0 (6.93)   |
| Weight: 4 months <sup>1</sup>       | 147.0 (8.53)     | 201.53 (7.83)  | 148.1 (8.47)     | 184.8 (6.60)   | 141.0 (7.38)     | 187.4 (6.95)   |

<sup>1</sup> Sex difference, males greater than females,  $p < 0.05$ ;

BMI, body mass index; 3MSE, Modified Mini-Mental State Exam.