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Effect of Intranasal Insulin on Cognitive Function: A Systematic Review

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Aim: Epidemiological and mechanistic studies raised the possibility that cognitive function may be affected by brain responses to insulin. We systematically reviewed and analyzed existing clinical trials that assessed the potential beneficial effects of intranasal insulin administration on cognitive functions.

Methods: Interventional studies measuring changes in cognitive functions in response to intranasal insulin were retrieved and included if they were in English and assessed cognitive functions before and after treatment. Cohen's effect size was calculated to allow comparison between studies.

Results: Eight studies (328 participants) were analyzed. No significant side effects of intranasal insulin administration were reported. Seven studies included healthy subjects' response to intranasal insulin, and three evaluated the cognitive effect among patients with minimal cognitive impairment or overt Alzheimer's disease. In healthy people, Cohen's effect size calculations suggest that only 160 IU/d intranasal insulin induced potential beneficial effects. Although females, when compared head-to-head, exhibited greater improvements in cognitive tests than men, the composite analysis of all included studies did not support this trend. Among cognitively impaired patients, only lower doses of insulin were assessed, and 20 IU revealed potential beneficial effects on cognitive functions. This was significant in a single study assessing long-term intranasal insulin administration, whereas acute administration of 20 IU intranasal insulin tended to show a beneficial effect on immediate recall in Apo ε4(−), but not Apo ε4(+), patients.

Conclusions: The current limited clinical experience suggests potential beneficial cognitive effects of intranasal insulin. Analyses provide clinical considerations for future research aimed at elucidating whether intranasal insulin may be used to improve cognitive functions. **(*J Clin Endocrinol Metab* 97: 366–376, 2012)**

Cognitive dysfunction denotes a wide spectrum of conditions from minimal cognitive impairment (MCI) to dementia. MCI is in the milder range of this spectrum (1–4), whereas dementia is an end-stage form of cognitive dysfunction that affects the ability to perform activities of daily living. Its occurrence increases over a lifetime, implying that the rise in life expectancy and the aging of the world's population will result in increased prevalence of cognitive dysfunction, turning it into a leading cause of loss of function worldwide. A study conducted in 2005 reported a worldwide prevalence of 24.3 million people suffering from dementia, with 4.6 million new cases of dementia diagnosed every year, and it was estimated that the number of patients will double every 20 yr, reaching 81.1 million by 2040 (5).

In recent years, it was conclusively demonstrated that an association exists between diabetes mellitus and cognitive impairment (6–12). Diabetic patients have a greater

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; CI, confidence interval; CNS, central nervous system; MCI, minimal cognitive impairment.

rate of decline in cognitive function, and exhibit approximately a 1.5-fold greater risk of accelerated cognitive decline and a 1.6-fold greater risk for the development of future dementia (13). The diabetes pandemic may therefore also contribute to the increasing prevalence of MCI and overt dementia. Parallel to these epidemiological data, accumulating mechanistic evidence emerged suggesting that the brain is a previously unrecognized target of insulin, the hormone deficient in type 1 diabetes and whose secretion and/or actions are impaired in type 2 diabetes. Insulin participates in neuronal maintenance, neurogenesis (14), and in the central regulation of energy balance (15) and food intake. Complementarily, persons with Alzheimer's disease (AD) were reported by some (16), although not all (17), studies to have lower cerebrospinal fluid levels of insulin compared with healthy people, even if they were nondiabetic. Moreover, the expression of insulin receptors throughout the brain is reduced in this disease (18, 19).

Intranasal delivery of insulin has emerged as a potentially effective means of introducing this hormone to the brain (much like MSH/ACTH and vasopressin) without a significant rise in its circulating levels (20). Cerebrospinal fluid levels of insulin peaked 30 min after administration and remained above levels measured in placebo-treated volunteers 80 min after administration (21). This mode of delivery has several advantages. Intranasal delivery is a noninvasive means of bypassing the blood-brain barrier. Drugs that normally do not cross the blood-brain barrier can be delivered in this manner to the central nervous system (CNS) within minutes. Moreover, this route of delivery can assist in minimizing the systemic effects of the drug, which in the case of insulin are obviously very significant (21). Intriguingly, one of the first reports on effects of intranasal insulin administration demonstrated a measurable effect on auditory evoked potential patterns during task performance in healthy volunteers (22). The exact route by which therapeutics, like intranasal insulin, reach the CNS remains controversial, but some pathways have been suggested, such as the olfactory pathway. Olfactory sensory neuron cell bodies are located in the distal epithelium of the nasal cavity, thus exposing their dendrites directly to the external environment. The axons travel through the perforated cribriform plate, reaching the synaptic glomeruli in the olfactory bulb (23, 24). An additional theory emphasizes the trigeminal route that uses the trigeminal nerve, which innervates the nasal cavity (like the olfactory nerve) and provides a direct route into the CNS (25, 26).

Several studies have addressed the clinical utility of intranasal insulin in improving cognitive function. These studies used an array of cognitive tests in different populations, making a valid comparison between results difficult. In the present study, our objective was to analyze existing reports of clinical studies that assessed the use of intranasal insulin for improving cognitive functions. Using systematic review paradigms, we assessed whether current literature provides sufficient support for beneficial effects of intranasal insulin administration on cognitive function and/or whether current experience can help guide future research in this area.

Materials and Methods

Search strategy and study inclusion criteria

Reports of studies assessing the influence of intranasal insulin on cognitive function were retrieved by systematically searching medical databases, discussion with experts, and examination of bibliographies of relevant review articles. Searches were conducted using Medline, EMBASE, the Cochrane library, and web-based clinical trial registries (www.clinicaltrials.gov) by one of the authors (E.S.) with the aid of a librarian. In Medline and EMBASE, the searches combined intranasal insulin, cognition or memory AND/OR central nervous system. Results of our search were confirmed in an additional independent database (Google Scholar). The titles and abstracts were then screened to identify articles meeting the inclusion criteria. Finally, full texts of these articles were obtained and reviewed. Studies were included in this review if they: 1) were human studies; 2) were written in English; 3) used intranasal insulin as the intervention; and 4) assessed cognitive functions before and after treatment, allowing extrapolation of the change in cognitive function.

Validity assessment

Validity was assessed by one of the authors according to the following criteria: 1) study design (parallel or crossover); 2) blinding of intervention providers and participants; and 3) degree of loss to follow-up.

Data abstractions

Prespecified data were extracted. Items extracted included: 1) population characteristics; 2) intervention (dose of insulin given); 3) duration of intervention (acute or long-term); and 4) cognitive assessment tools used (types, and whether immediate or delayed recall test was used). For each arm of the study, the number of participants and cognitive test scores (mean and SD) with and without intranasal insulin were collected.

Statistical analysis

For all studies, Cohen's effect size (27, 28) was calculated by dividing the difference of means by the calculated pooled SD. This measure allows comparing studies using different methodologies. A positive effect size denotes a beneficial effect of the intervention, whereas a negative value denotes the opposite. Generally, the larger the effect size, the greater is the impact of the intervention. A value of at least 0.8 is considered to be a large (positive) effect, 0.5 is medium, and 0.2 is small (27). The 95%

confidence intervals (CI) for effect size were calculated by using Hedges and Olkin's formula:

$$\sigma[d] = \sqrt{\frac{N1 + N2}{N1 \times N2} + \frac{d^2}{2(N1 + N2)}}$$

When numerical data were not obtainable from the publication, we contacted the corresponding authors and obtained such data for the most accurate calculations. Where this was not fully available to us, means and SD values were extracted from the figures (29, 30). All data from studies included in this systematic review were considered and presented. Insulin doses investigated in a single study (10 and 60 IU) were mentioned only in the legends, as were results of acute insulin administration in studies that also assessed long-term administration. In all cases, results of both acute and delayed recall are presented.

Results

Literature search outcome and validity assessment

The initial search yielded 43 items. Eight met the inclusion criteria and were fully reviewed and analyzed. Excluded items were typically reviews, papers describing hypotheses, and studies in animal and cellular model systems. Table 1 summarizes the validity assessment of the eight studies included in this systematic review. As can be noted, three of the studies were double-blinded, with only one study (30) describing the randomization procedure. The four remaining studies were based on a crossover study design. Only minimal loss to follow-up was reported in all studies, ranging from 0 to 1.63% (Table 1). Among the randomized studies, three studies (31–33) included an 8-wk intervention period, whereas another used a 3-wk intervention (30). Three crossover studies evaluated the effect of a single dose (insulin *vs.* placebo) treatment.

Measures of cognitive function

Cognitive function was assessed using a variety of tests measuring a range of cognitive domains, with the most common tests used being the word list recall (five studies), story recall, digit span, and object location (two studies each). Briefly, Word List Memory is part of a larger battery (by the Consortium to Establish a Registry for Alzheimer Disease), which as a whole possesses good reliability (a test retest coefficient of 0.62 after 1 month). Its aim is to test the ability to remember newly learned information in the short term. Three trials of a 10-item word list are presented. Immediately after each trial, the subject is asked to recall as many items as possible (immediate recall) or is asked after a defined time-lapse to repeat as many words from the list as possible (delayed recall). Of note, the battery has been shown to discriminate between cognitively intact individuals and those with dementia, but it exhibits a diminished ability to discriminate between those with mild cognitive impairment and cognitively intact individuals (35–37). The Digit Span Forward & Backwards (DSFB) assesses memory and concentration. The participant is read a sequence of numbers starting from three digits up to nine digits. The participant is then required to repeat the digits first in the sequence they were read and then in reverse order (38). The reported average reliability coefficient in people 45–55 yr of age was 0.66 (39). The DSFB is considered a poor measure of intelligence with poor correlations to other tests of general intelligence. However, it has good discriminative value in the lower level; *i.e.* most cognitively intact adults will be able to retain five digits forward and three backward. A marked fall in the score on the DSFB is often a first sign of cognitive impairment (39). Recall of a story assesses memory, learning, and recall of complex unfamiliar information. The examiner reads two paragraphs, stopping after each reading for the participant to give his/her immediate free recall. Each paragraph contains a number of "ideas" that the participant is supposed to recall (38). The test has an immediate recall component and a delayed one. The mirror tracing task requires the subject to trace a nongeometrical figure that is reflected in a mirror. The score on the test is an average of speed and accuracy of test performance (40). Object location task is a spatial learning task that relies on temporal lobe structures, including the hippocampus. The task is a computerized version of the game "concentration." It consists of 15 card pairs showing pictures of animals and objects. The subject has to memorize the respective location during two runs of presentation and to recall them (40–42).

TABLE 1. Validity assessment of eight trials assessing intranasal insulin for cognitive function included in the present systematic review

First author, year (Ref.)	Design of study	Blinding indicated?	Percentage loss to follow-up
Benedict, 2008 (40)	Crossover	No	0
Hallschmid, 2008 (32)	Randomized	Yes[a]	0
Benedict, 2004 (31)	Randomized	Yes[a]	0
Reger, 2006 (34)	Crossover	No	0
Reger, 2008 (30)	Randomized	Yes[a]	1.63%
Reger, 2008 (29)	Crossover	No	0
Krug, 2010 (41)	Crossover	Yes[a]	0
Benedict, 2007 (33)	Randomized	Yes[a]	0

[a] Double-blind study design.

Cognitive effects of intranasal insulin treatment

Table 2 summarizes outcomes reported in the eight studies that were included in this systematic review. Seven

TABLE 2. Summary of intranasal insulin intervention studies

First author, year (Ref.)	Population characteristics	Intervention	Cognitive assessment tool	n	Mean ± SD	SE	*P* value for comparison	Cohen's effect size
Benedict, 2008 (40)	Healthy, normal weight, with no medications[a]	Intranasal insulin 160 IU *vs.* placebo (acute)	Digit span (immediate recall)	Females, treated = 18	20.00 ± 4.71	1.11	$P < 0.05$	0.26
				Females, placebo = 18	18.64 ± 5.43	1.28		
				Males, treated = 14	15.70 ± 4.37	1.17	$P > 0.2$	−0.31
				Males, placebo = 14	17.20 ± 5.01	1.34		
			Object location (immediate recall)	Females, treated = 18	51.90 ± 24.18	5.7	$P < 0.01$	0.67
				Females, placebo = 18	39.00 ± 12.30	2.9		
				Males, treated = 14	44.60 ± 22.07	5.9	$P > 0.17$	−0.44
				Males, placebo = 14	52.30 ± 11.60	3.1		
			Mirror tracing (immediate recall)	Treated = 32	68.81 ± 12.90	2.28	$P > 0.56$	−0.74
				Placebo = 32	70.84 ± 17.08	3.02		
Hallschmid, 2008 (32)[f]	Obese men	Intranasal insulin 160 IU *vs.* placebo (long-term)	Word list (delayed recall)[g]	Treated = 15	2.87 ± 2.17	0.56	$P = 0.05$	0.94
				Placebo = 15	1.13 ± 1.47	0.38		
Benedict, 2004 (31)[f]	Healthy	Intranasal insulin 160 IU *vs.* placebo (long-term)	Word list (immediate recall)	Treated = 19	13.82 ± 3.70	0.85	$P > 0.05$	0.09
				Placebo = 19	13.48 ± 3.53	0.81		
			Word list (delayed recall)	Treated = 19	6.20 ± 4.49	1.03	$P = 0.04$	0.74
				Placebo = 19	2.92 ± 4.36	1		
Reger, 2006 (34)	Probable AD or MCI *vs.* healthy	Intranasal insulin 20 or 40 IU *vs.* placebo (acute)	Story recall (sum of immediate + delayed recall)	Healthy, 20 IU = 35	39.70 ± 10.65	1.8		0.02
				Healthy, placebo = 35	39.90 ± 10.06	1.7		
				Cognitive impaired, Apo ϵ4 (−), 20 IU = 14	17.80 ± 10.10	2.7	$P < 0.05$	0.39
				Cognitive impaired, Apo ϵ4 (−), placebo = 14	13.90 ± 10.10	2.7		
				Cognitive impaired, Apo ϵ4 (+), 20IU = 12	12.80 ± 10.39	3		−0.42
				Cognitive impaired, Apo ϵ4 (+), placebo = 12	16.50 ± 6.92	2		
				Healthy, 40 IU = 35	39.20 ± 11.24	1.9		−0.06
				Healthy, placebo = 35	39.90 ± 10.05	1.7		
				Cognitive impaired, Apo ϵ4 (−), 40IU = 14	17.10 ± 11.22	3	$P < 0.05$	0.30
				Cognitive impaired, Apo ϵ4 (−), placebo = 14	13.90 ± 10.10	2.7		
				Cognitive impaired, Apo ϵ4 (+), 40 IU = 12	15.00 ± 11.08	3.2		−0.16
				Cognitive impaired, Apo ϵ4 (+), placebo = 12	16.50 ± 6.92	2		
			Word list (sum of immediate + delayed recall)	Healthy, 20 IU = 35	46.20 ± 11.24	1.9		−0.25
				Healthy, placebo = 35	48.90 ± 10.64	1.8		
				Cognitive impaired, Apo ϵ4 (−), 20 IU = 14	30.20 ± 10.85	2.9		−0.09
				Cognitive impaired, Apo ϵ4 (−), placebo = 14	31.20 ± 10.47	2.8		
				Cognitive impaired, Apo ϵ4 (+), 20 IU = 12	31.10 ± 10.73	3		−0.02
				Cognitive impaired, Apo ϵ4 (+), placebo = 12	31.30 ± 10.39	3.1		
				Healthy, 40 IU = 35	49.50 ± 13.01	2.2		0.05
				Healthy, placebo = 35	48.90 ± 10.64	1.8		
				Cognitive impaired, Apo ϵ4 (−), 40 IU = 14	33.30 ± 12.34	3.3	$P = 0.03$	0.18
				Cognitive impaired, Apo ϵ4 (−), placebo = 14	31.20 ± 10.47	2.8		
				Cognitive impaired, Apo ϵ4 (+), 40 IU = 12	27.80 ± 12.47	3.6	$P < 0.05$	−0.30
				Cognitive impaired, Apo ϵ4 (+), placebo = 12	31.30 ± 10.39	3		
Reger, 2008 (30)	AD or MCI	Intranasal insulin 20 IU *vs.* placebo (long term)	Memory saving score (immediate recall/ 20-min delayed recall ratio)	Treated = 13	0.49 ± 0.18	0.05	$P = 0.04$	1.13 (0.22)[d]
				Placebo = 12	0.30 ± 0.17	0.05		
			Voice onset time (immediate recall/ 20-min delayed recall ratio)	Treated = 13	1620.42 ± 449.93	124.79	$P = 0.04$	0.74[b] (0.43)[d]
				Placebo = 12	1325.39 ± 373.08	107.70		
Reger, 2008 (29)[g]	AD or MCI (n = 33) *vs.* healthy (n = 59)	10, 20, 40, 60 IU intranasal insulin or placebo (acute)	Story recall (immediate recall)	Apo ϵ4 (−) placebo = 11	32 ± 9.94[c]	3		
				Apo ϵ 4 (−) 20 IU = 11	36.5 ± 9.94[c]	3	$P = 0.03$	0.47[e]
				Apo ϵ 4 (−) 40 IU = 11	33.5 ± 9.94[c]	3	$P = 0.02$	0.16[e]
				Apo ϵ 4 (+) placebo = 22	31.5 ± 9.38[c]	2		
				Apo ϵ 4 (+) 20 IU = 22	29 ± 9.38[c]	2		−0.27[e]
				Apo ϵ 4 (+) 40 IU = 22	26 ± 9.38[c]	2		−0.6[e]
			Word list learning (immediate recall)	Apo ϵ4 (−) placebo = 11	17 ± 3.97[c]	1.2		
				Apo ϵ4 (−) 20 IU = 11	19.3 ± 3.31[c]	1.1		0.66[e]
				Apo ϵ4 (−) 40 IU = 11	16.4 ± 3.97[c]	1.2		−0.15[e]
				Apo ϵ4 (+) placebo = 22	16 ± 3.75[c]	0.8		
				Apo ϵ4 (+) 20 IU = 22	16.2 ± 3.75[c]	0.8		0.05[e]
				Apo ϵ4 (+) 40 IU = 22	15.4 ± 3.75[c]	0.8		−0.16[e]
			Story recall (delayed recall)	Apo ϵ4 (−) placebo = 11	33 ± 9.95[c]	3		
				Apo ϵ4 (−) 20 IU = 11	33 ± 13.26[c]	4		0[e]
				Apo ϵ4 (−) 40 IU = 11	28.33 ± 13.26[c]	4		−0.41[e]
				Apo ϵ4 (+) placebo = 22	27.5 ± 14.07[c]	3		
				Apo ϵ4 (+) 20 IU = 22	23 ± 14.07[c]	3		−0.32[e]
				Apo ϵ4 (+) 40 IU = 22	22 ± 11.72[c]	2.5		−0.43[e]

(*Continued*)

TABLE 2. Continued

First author, year (Ref.)	Population characteristics	Intervention	Cognitive assessment tool	n	Mean ± SD	SE	*P* value for comparison	Cohen's effect size
			Word list learning (delayed recall)	Apo ε4 (−) placebo = 11	4 ± 2.5[c]	0.75		
				Apo ε4 (−) 20 IU = 11	4.93 ± 2.5[c]	0.75	$P < 0.05$	0.39[e]
				Apo ε4 (−) 40 IU = 11	3.75 ± 2.5[c]	0.75		−0.1[e]
				Apo ε4 (+) placebo = 22	3.47 ± 2.34[c]	0.5		
				Apo ε4 (+) 20 IU = 22	3 ± 2.34[c]	0.5		−0.2[e]
				Apo ε4 (+) 40 IU = 22	3.5 ± 2.34[c]	0.5		0.01[e]
Krug, 2010 (41)	Healthy postmenopausal women	Intranasal insulin 160 IU (acute)	Digit span (immediate recall)	Treated = 14	15.78 ± 1.07	0.28	$P < 0.03$	1.73
				Placebo = 14	14.07 ± 0.97	0.25		
			Object location (immediate recall)	Treated = 14	40.85 ± 4.96	1.32	$P > 0.34$	1.24
				Placebo = 14	35.28 ± 4.29	1.14		
Benedict, 2007 (33)[f]	Healthy men	Intranasal ASP-I *vs.* RH-I *vs.* placebo (long term)	Word list (immediate recall)	RH-I = 12	13.3 ± 5.19	1.5	$P > 0.05$ (*vs.* placebo)	0.02 (*vs.* placebo)
				ASP-I = 12	13.4 ± 4.84	1.4	$P > 0.05$ (*vs.* placebo)	0.04 (*vs.* placebo)
							$P > 0.05$ (*vs.* RH-I)	0.02 (*vs.* RH-I)
				Placebo = 12	13.2 ± 5.88	1.7		
			Word list (delayed recall)	RH-I = 12	5.85 ± 5.33	1.54	$P < 0.05$ (*vs.* placebo)	0.85 (*vs.* placebo)
				ASP-I = 12	7.91 ± 3.11	0.9	$P < 0.01$ (*vs.* placebo)	2 (*vs.* placebo)
							$P < 0.05$ (*vs.* RH-I)	0.49 (*vs.* RH-I)
				Placebo = 12	2.41 ± 2.59	0.75		

ASP-I, aspart insulin; RH-I, regular human insulin.

[a] Except for oral contraceptives, which all females took.

[b] Cohen's effect size is given here in absolute value because lower voice onset time indicates improvement (insulin *vs.* placebo).

[c] Means, SD and SE were calculated according to relevant figure because they were not included in original article.

[d] Effect size in original article, referring to the improvement seen in insulin treatment group, between d 0 and 21.

[e] Calculated Cohen's effect for specific dose of intranasal insulin *vs.* placebo.

[f] The study also assesses the effects of acute administration on immediate and/or delayed recall.

[g] The study also assesses the effect of 10 and 60 U insulin.

studies evaluated the cognitive effect of intranasal insulin administration in healthy individuals (31–34, 40, 41), and three studies evaluated the cognitive effect in AD or MCI (29, 30, 34). Collectively, 328 individuals were cognitively assessed. Table 2 lists the population characteristics of each trial, the dose and duration (acute or long-term) of the intervention, cognitive assessment tool group sizes, means, SD value, SE value, *P* value, and the calculated Cohen's effect size.

The effect of intranasal insulin on cognitive functions in healthy (noncognitively impaired) subjects

Four of the seven studies assessing healthy persons demonstrated improved cognitive functions when treated with intranasal insulin. This effect was observed with high doses of intranasal insulin (160 IU), but not with lower doses (60 IU or less), suggesting a possible dose effect (Fig. 1). Beneficial effects of intranasal insulin on some of the cognitive function tests were reported with 160 IU insulin in studies assessing both long-term (Fig. 1A) and acute insulin administration (one of two studies; Fig. 1B). Obese subjects did not seem to be resistant to the cognitive effect of this dose of intranasal insulin (effect size of 0.94 in obese men *vs.* 0.74 in healthy individuals; Fig. 1A), although a resistance to the weight-regulatory effect of intranasal insulin was reported (32). Interestingly, although a study that compared head-to-head the effect of acute intranasal insulin on cognitive functions in males *vs.* females concluded that only females benefited from such effects (40), other studies that used long-term intervention seemed to show similar beneficial effects in both sexes (Fig. 2).

Effect of intranasal insulin on cognitive functions in the cognitively impaired

Cohen's effect size calculations suggest a clear beneficial effect of 20 IU of intranasal insulin on cognitive functions in cognitively impaired (MCI or AD) patients in one study (30), only a trend in a second study (29), and no clear effect in a third report (34). Importantly, the clear positive effect was observed with long-term 20 IU insulin and when the voice onset time and memory saving score

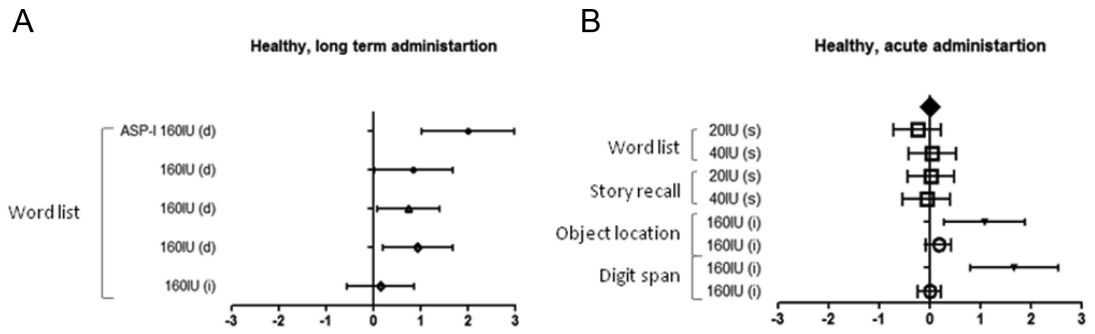

FIG. 1. Effect of intranasal insulin in healthy persons. Cohen's effect size and 95% CI were calculated, as detailed in *Materials and Methods*, for studies included in the systematic review, which assessed healthy persons, and long-term intranasal insulin administration (A) or acute insulin administration (B). Insulin doses used are indicated (IU/d), as well as whether cognitive functions were immediate recall (i), delayed recall (d), or sum of immediate + delayed (s). Symbol sizes are proportional for the relative group size. Studies depicted in the figure and their symbols are: Benedict *et al.* (33), *black circles*; Benedict *et al.* (31), *white triangle*; Hallshmid *et al.* (32) (study is in obese males), *white diamonds*; Krug *et al.* (41), *inverted black triangle*; Benedict *et al.* (40), *white circles*; Reger *et al.* (34), *white rectangles*; and Reger *et al.* (29), *black diamond*: Information on healthy controls from this paper is reported as "data not shown" and includes 10–60 IU insulin.

were used as the cognitive tests (Fig. 3A). The other studies with nonsignificant results (per the 95% CI calculations of the Cohen's effect size) relied on verbal memory after acute intranasal insulin administration. Notably, two studies stratified the cohort based on Apo ε genotyping and found that only Apo ε4-negative patients might benefit from intranasal insulin, whereas the Apo ε4-positive genotype may even exhibit deterioration in cognitive function tests (29, 34). These propositions could be supported only to a limited degree by effect size analyses (Fig. 3, B and C, respectively), with a medium-strength trend (Cohen effect size of 0.66) observed in Apo ε4-negative patients after acute administration of 20 IU intranasal insulin and assessing immediate recall (Table 2 and Fig. 3B).

Discussion

This systematic review summarizes the current literature addressing intervention studies aimed at elucidating the effect of intranasal insulin on cognitive functions. Whereas current literature clearly is insufficient to support clinical recommendations, this analysis can offer certain insights and directions for future research, highlighting potential subgroups, doses, and cognitive function tests that perhaps can more likely detect beneficial effects of this putative novel intervention (Table 3). The surprisingly low rate of reported adverse effects of this intervention, even in high doses, and its potential benefits are major incentives to continue efforts in this direction. Indeed, 15 clinical trials are currently listed in the NIH registry (www.clinicaltrials.gov) assessing the potential benefits of intranasal insulin, three of which include cognitive function tests as an outcome.

Strengths and limitations

Several major limitations of our analysis warrant consideration. The most important bias in a systematic review is the publication bias. To avoid such bias, an extensive search and double checking of the results were conducted,

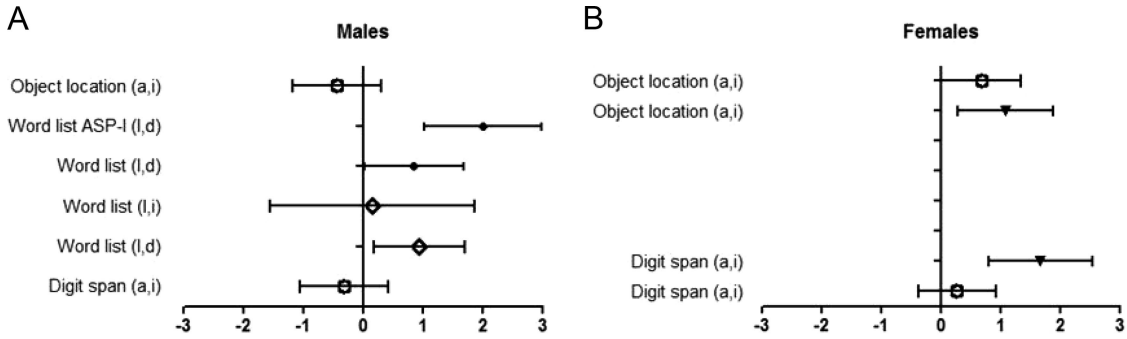

FIG. 2. Effect size of intranasal insulin treatment on scores of cognitive function tests in males (A) and females (B). Cohen's effect size and 95% CI were calculated (as detailed in *Materials and Methods*) from studies that indicated the effect of intranasal insulin in healthy males and/or females. All studies used an insulin dose of 160 IU/d delivered either acutely (a) or long-term (l), and the cognitive tests specified were conducted to determine immediate recall (i) or delayed recall (d). Symbol sizes are proportional for the relative group size. Studies depicted in the figure and their symbols are: Benedict *et al.* (33), *black circles*; Hallshmid *et al.* (32) (study is in obese males), *white diamonds*; Krug *et al.* (41), *inverted triangle*; and Benedict *et al.* (40), *white circles*.

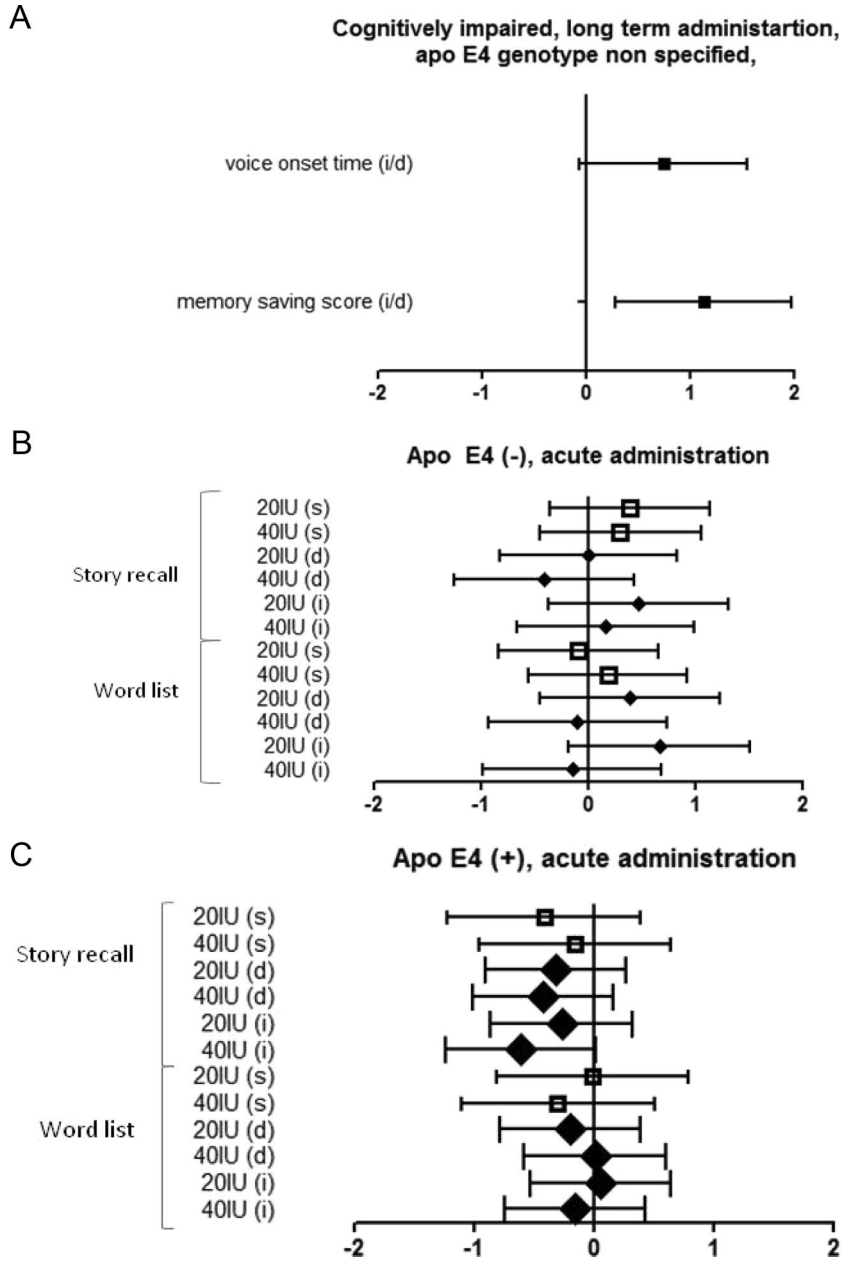

FIG. 3. Effect size of intranasal insulin in cognitive impaired patients. Cohen's effect size and 95% CI were calculated, as detailed in *Materials and Methods*, for studies included in the systematic review, which assessed cognitively impaired persons (persons with minimal cognitive impairment or AD). Patients were either not stratified based on Apo E genotyping (A), or stratified for being Apo ε4 negative or positive (B and C, respectively). Insulin doses used are indicated (IU/d), as well as whether cognitive functions were immediate recall (i), delayed recall (d), or the sum of immediate + delayed (s). Symbol sizes are proportional for the relative group size. Studies depicted in the figure and their symbols are: Benedict *et al.* (33), *black rectangles*; Reger *et al.* (34), *white rectangles*; and Reger *et al.* (29), *black diamond*.

as well as consultation with experts in this field and approaching the authors of the original publications for clarifications. Being a novel experimental therapy, the use of intranasal insulin has so far been assessed in only a limited number of people (less than 350) and has been conducted by only two research groups, one in Seattle, Washington, and one in Lübeck, Germany. As stated in *Materials and Methods*, this paucity in existing information prompted us not to overly restrict the inclusion criteria, thereby including in the analysis studies that were not double-blinded and in several cases not randomized. Yet, most studies that were not randomized were of crossover design, thus reducing biases that may have been introduced by the experimental design. Another major limitation was the minimal overlap between the studies that were reviewed—in the characteristics of the participants, in the cognitive tests performed to assess outcomes of intervention, and in the overall results—thus preventing us from meta-analyzing the data. Finally, all studies assessed relatively short-term effects (single dose to 8-wk intervention), precluding our ability to assess whether more chronic interventions may yield clearer outcomes. Despite these limitations, the strengths of this review are in the provision of a timely summary of existing (albeit limited) information by using an integrating measure (the Cohen effect size) to assess an experimental therapeutic approach for treating a highly prevalent and debilitating condition. Moreover, the analysis may guide investigators to consider selection of specific groups, doses, and cognitive function tests when designing future studies, as detailed below.

Dose and duration of intranasal insulin

The doses used in the eight studies varied from 20 to 160 IU/d, with only two (29, 34) of the studies comparing different doses head-to-head. In healthy persons, only a dose of 160 IU intranasal insulin was associated with positive Cohen effect size (Fig. 1), and a single study suggested that an insulin analog may be superior to regular insulin with this dose (33). Of note, this conclusion is not deduced from studies directly comparing different doses and is supported by the integrated comparison of different studies provided herein. In contrast, in cognitively impaired persons there was no clear association between higher intranasal insulin doses and results in cognitive function tests, and the most effective dose seemed to be 20

TABLE 3. Summary of insights gained from this systematic review

What was already known on this topic:
- Intranasal insulin is a proposed novel therapy to elicit cognitive improvement.
- Different studies show the possible effects of intranasal insulin on cognition.

What this study adds:
- Evidences of intranasal insulin effects on cognitive functions are summarized and categorized, utilizing data analysis allowing for comparison of different tests by the various studies.
- Different subgroups may exhibit different responses to intranasal insulin:
  - Intranasal insulin may be effective in healthy people.
  - This effect may require high insulin doses (160 IU) and can be observed with both acute and long-term administration.
  - Although females have been proposed to gain more benefit than men from intranasal insulin, this is not supported by the composite analysis of four studies.
  - Cognitive effects of intranasal insulin in patients with AD or MCI vary among studies.
  - A positive effect was observed in one study that utilized long-term insulin administration and specific cognitive function tests.
  - Apo ϵ4 genotype could have some effect on the response to intranasal insulin, but further studies are needed to confirm this trend association.
- Cognitive impairment "resistance" to intranasal insulin therapy: it is possible that AD or MCI patients need higher doses and/or long-term intranasal insulin administration in order to achieve cognitive improvement.
- Neither of the studies so far used high doses (160 IU) in AD or MCI patients, which were shown to be with minimal side effects in healthy volunteers.
- Brain insulin gradient of response: it is possible that different areas in the brain respond differently to intranasal insulin, explaining differences in the results of the various cognitive assessment tools used.

IU insulin. Of note, a dose of 160 IU, which was the only dose potentially beneficial in healthy persons, was not used in any of the studies assessing persons with cognitive impairment or AD (29, 30, 34). Brain insulin resistance has been proposed as a contributing factor to cognitive deterioration in AD and MCI (43). If indeed this is the case, higher rather than lower doses of insulin compared with those effective in noncognitively impaired persons would be predicted to have an impact on cognitive function in MCI/AD. Importantly, reported adverse effects to intranasal insulin, even at 160 IU/d, were minimal, with no severe adverse effects, suggesting high tolerability to this unique route of insulin administration. Interestingly, beneficial effects were noted by some studies irrespective of whether they used a single dose of intranasal insulin (acute), or a more extended treatment period, and positive effects were reported in studies using assessment of immediate or delayed recall.

Sex difference

A single study that compared males and females head-to-head (40) suggested that females may exhibit a more readily demonstrable cognitive benefit from acute intranasal insulin because they had more tests with statistically significant positive effect size than males (Fig. 2). Interestingly, a sex difference in response to intranasal insulin has also been noted in studies assessing intranasal insulin on the regulation of food intake in humans (40, 44) and in animal models (45). The underlying mechanism leading to this difference is still not identified, although brain estrogen signaling pathways were proposed to have a role. Consistently, estrogen modulates the structure and function of the hippocampus, which is related to memory functions and behavior (46). However, postmenopausal women responded similarly to a previous report in younger females (41). Furthermore, healthy men are not resistant to the putative positive effects of intranasal insulin on cognitive function, particularly when long-term administration and delayed recall were assessed (32). Overall, a systematic view of existing literature does not support the proposition that females are more likely than males to enjoy the putative beneficial effect of intranasal insulin on cognitive function, although additional head-to-head comparisons may be warranted.

Cognitively impaired *vs*. healthy population

As mentioned above, an intranasal insulin dose of 160 IU/d was associated with better performance in cognitive function tests among healthy persons. In persons with MCI or AD, two studies (29, 30) reported cognitive improvements that resulted in a medium to large calculated Cohen effect size. Of note, positive effects were found with different cognitive function tests—the Voice onset time and Memory saving score—whereas Story recall and Word list test showed a trend for positive effect only in a subgroup of patients who are Apo ε4 negative. As will be discussed below, it is possible that different tests have a different sensitivity to detect change and/or that they represent different cognitive functions more sensitive to insulin and/or anatomically more accessible to intranasal insulin. In addition, these two studies varied in insulin administration—a clear positive effect was noted in the study assessing long-term insulin administration only. It is therefore difficult to discern whether the clear beneficial effect of intranasal insulin in cognitively impaired patients could be attributed to the long-term insulin administration regimen and/or to the specific cognitive function test used. Yet, somewhat supporting the for-

mer possibility is a study on long-term administration of intranasal insulin to children with Phelan-McDermid syndrome, who exhibited improved cognitive functions as reported by their parents (47).

Apo ε4 genotype

It is well-established that inheritance of the Apo ε4 allele is associated with a higher likelihood of developing AD. Two of the studies stratified participants based on being either Apo ε4 (−) or (+) (29, 34). Based on the calculated Cohen effect size, only a moderate strength, nonsignificant Cohen effect size was evident in one study, but only in the Apo ε4-negative patients (29). Moreover, a possible detrimental effect was suggested by one of the studies using the Story recall test only among the Apo ε4(+) subgroup (Fig. 3). Future studies will have to consider whether addressing the Apo ε4 carrier state should be a prerequisite before inclusion in studies aimed at assessing effects of intranasal insulin in cognitively impaired patients.

Which cognitive function test should be used?

In addition to considerations mentioned above, this systematic review includes data suggesting that intranasal insulin improves verbal working memory in humans. The human verbal working memory is based on activation of the frontal cortex (48)—a brain region that is characterized by relative high density of insulin receptors in rodents (49). In contrast, the object location test assesses a cognitive function thought to be predominantly hippocampus-dependent. Mirror tracing was used in one study as a hippocampus-independent function (40), and this test did not show improvement with intranasal insulin administered to healthy volunteers. To the best of our knowledge, the insulin concentrations achieved by intranasal administration and insulin's dispersion in the various brain regions involved in memory functions are unclear. Thus, future studies in this field should consider inclusion of advanced imaging approaches to better determine brain regions affected by intranasal insulin. Such information may guide a better choice of cognitive function tests that assess brain regions more likely to be reached by intranasal insulin.

Continued interest in the possibility of delivering insulin to the CNS is fueled by mechanistic studies suggesting that brain insulin activity might have a protective effect against neurodegenerative processes and cognitive decline as their early manifestation. An impaired CNS insulin input can be tied to the pathogenesis of neurodegeneration in AD, deposition of amyloid plaques and neurofibrillary tangles, which are aggregates of hyperphosphorylated Tau. Insulin initiates a signaling cascade that inactivates glycogen synthase kinase-3, an enzyme that can phosphorylate the microtubule-associated protein Tau in cultured human neurons (50). Tau-Hyperphosphorylation, which can be induced in transgenic mice by overexpression of constitutively active glycogen synthase kinase-3 (51), decreases its affinity for microtubules and is thought to be a crucial event in the pathogenesis of AD and several other neurodegenerative diseases, collectively called "tauopathies." In addition to this putative connection between impaired insulin action and AD, amyloid precursor protein (APP), the primary component of the amyloid plaques, is affected by insulin as well; insulin and IGF-I receptors regulate APP secretion (52), and insulin resistance provoked by diet-induced obesity results in a marked increase in β-amyloid levels and age-dependent memory impairment in Tg2576 mice, an animal model of AD that expresses a mutant APP (53). Finally, by inducing protein kinase B phosphorylation, insulin can promote neuronal survival (54), suggesting that this hormone exerts direct neuroprotective effects. The complexity and still limited understanding of insulin signaling in the brain is highlighted by studies demonstrating that decreased, rather than increased, insulin signaling through insulin receptor substrate 2 in the brain may promote healthier aging and extend life span in mice (55). Following this rationale, it may be questioned whether the potentially effective dose of 160 IU/d used in clinical studies in fact activates insulin signaling in the brain, or conversely, induces a state of insulin resistance of insulin receptor substrate 2-mediated signaling, which manifests as improved cognitive function.

In conclusion, only limited data currently support the potential beneficial effects of intranasal insulin on cognitive functions. Nevertheless, this systematic review can offer several leads for considerations of patient subpopulations and stratification, doses and types of insulin, baseline cognitive state, and assessment tools that should be taken into account when designing future studies aimed at considering the therapeutic potential of this intervention.

Note Added in Proof. During production of this article, the following publication became available online: **Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B** 12 September 2011 Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Arch Neurol 10.1001/archneurol.2011.233.

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