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REVIEW ARTICLE

Safety and efficacy of intranasal insulin in patients with Alzheimer's disease: a systematic review and meta-analysis

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ABSTRACT

Background and Aim: We performed this meta-analysis to evaluate the safety and efficacy of intranasal insulin in Alzheimer's disease (AD) patients.

Methods: A literature search was conducted for PubMed, Scopus, and Web of Science from inception until August 2022. Documents were screened for qualified articles, and all concerned outcomes were pooled as risk ratios or mean difference (MD) in the meta-analysis models using Review Manager (RevMan version 5.4).

Results: Our results from 12 studies favored intranasal insulin over placebo in terms of Alzheimer Disease's Assessment Scale-cognitive subscale (ADAS-cog) 20 IU, (MD = −0.13, 95% CI [−0.22, −0.05], $P = 0.003$). The overall effect did not favor either of the two groups for ADAS-cog 40 IU, memory composite 20 IU and 40 IU, and adverse events (MD = −0.08, 95% CI [−0.16, 0.01], $P = 0.08$), (MD = 0.65, 95% CI [−0.08, 1.39], $P = 0.08$), (MD = 0.25, 95% CI [−0.09, 0.6], $P = 0.15$), and (MD = 1.28, 95% CI [0.75, 2.21], $P = 0.36$), respectively.

Conclusion: Ultimately, this meta-analysis showed that intranasal insulin in small doses (20 IU) significantly affects patients with AD. Further studies are recommended on reliable insulin delivery devices to increase insulin in the central nervous system.

Relevance for Patients: Intranasal insulin has shown promising results in treating patients with AD. The lower doses (20 IU) can play a positive role in improving the disease. As research continues, it is likely that this treatment will become more widely accepted and utilized in clinical practice.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative illness characterized by changes in behavior and personality, cognitive impairment, and memory loss [1]. Major neuropathological features of AD are thought to be the buildup of extracellular amyloid/senile plaques made of intracellular neurofibrillary tangles and amyloid-(A). It is worth mentioning that AD-sensitive brain areas exhibit substantial abnormalities in glucose metabolism and reduced neuron use of glucose as a result of disruptions in the insulin signaling pathway [2].

Several studies recently suggested that insulin may be essential in preserving the brain's mitochondrial balance and cerebral bioenergetics [3]. In addition, it could have an impact on the clearance of the significant factors in the path mechanism of AD, including

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amyloid peptide and tau protein phosphorylation [3]. Low insulin levels in the central nervous system (CNS) may be caused by impaired insulin transport through the blood-brain barrier (BBB). Therefore, raising brain insulin may stop the degenerative processes associated with AD [3]. Based on that, a wide range of pharmacological substances and delivery strategies has been developed and studied.

The olfactory bulb, cerebral cortex, hippocampus, hypothalamus, cerebellum, and choroid plexus have the highest insulin receptor density [4]. Accordingly, through the roof of the nose, insulin can cross through the BBB and systemic circulation, entering the brain through the olfactory, trigeminal, and nerve fiber pathways [5].

Binding insulin with its receptor will lead to autophosphorylation of the insulin receptor and induction of insulin receptor substrate (IRS). Activation of AKT, which is one of the signaling routes that insulin activates, through IRS phosphorylation has been linked to improvements in neuronal protection, learning, and memory functions among AD patients [6].

However, several previous studies have shown conflicting findings regarding the influence of intranasal insulin on dementia in AD patients. Therefore, in this study, we aimed to fill the gap in detecting the real effect of intranasal insulin on these patients.

2. Materials and Methods

This systematic review and meta-analysis were reported following the PRISMA declaration requirements [7]. The protocol of this study was registered on the PROSPERO (CRD42022355827).

2.1. Eligibility criteria

The following conditions were considered for the study:

(i) Population: Studies on patients who have AD or mild cognitive impairment.
(ii) Intervention: Studies where the exposed group was intranasal insulin.
(iii) Comparator: Studies where the control group received a placebo.
(iv) Outcome: Studies stated one or more of the following outcomes: Alzheimer Disease's Assessment Scale-cognitive subscale (ADAS-cog) either 40 IU or 20 IU, and adverse effects (headache, fall, and rhinitis/upper respiratory infection [URI]). In addition, memory composite (delayed story recall) 40 IU and 20 IU, dementia severity rating scale (DSRS), AD cooperative study-activities of daily living (ADCS-ADL), clinical dimension rating-sum of boxes (CDR-SOB), and cerebral spinal fluid (CSF) biomarkers of AD.
(v) Study design: Studies that were designated as randomized clinical trials (RCTs).
(vi) Studies excluded: Not published in the English language, comments, review articles, case reports, observational studies, abstracts, and letters to the editor.

2.2. Search strategy

Three electronic databases (PubMed, Scopus, and Web of Science) were searched from their inception until August 2022 using the following query: (Alzheimer OR [Senile Dementia] OR [Dementia Presenile]) AND [Insulin OR Novolin OR Iletin]).

2.3. Selection process

The titles and abstracts of all citations considered for inclusion were reviewed by three authors independently. Then, we extracted the full text of the selected studies to evaluate their applicability and validated them according to our systematic review and meta-analysis standards. Discrepancies were resolved by consensus.

2.4. Data extraction

Data were extracted from an online data extraction sheet by four independent authors. The extracted data included: (1) A summary of the included studies, (2) baseline characteristics for the included population, (3) risk of bias domains, and (4) outcome measures. Any disagreements were solved by a fifth author.

2.5. Quality appraisal

We used the Cochrane assessment tool 2 (ROB2) for randomized controlled trials [8]. Using that tool, each study was assessed for the possibility of bias in the following domains: (1) Random sequence generation, (2) allocation concealment, (3) blinding of participants, personnel, and outcome assessors, (4) incomplete outcome data, (5) selective outcome data reporting, and (6) other sources of bias. The degree of bias in the authors' conclusions is classified as "low risk," "some concerns," or "high risk."

2.6. Synthesis methods

Continuous were pooled as mean difference (MD) between the two groups from baseline to the endpoint in the meta-analysis models utilizing the inverse variance (IV) method. We assumed a fixed-effect model of the MD as the main analysis model. Nevertheless, relative risk (RR) was used to pool dichotomous data in a fixed-effect model using the Mantel-Haenszel (M-H) method. RevMan software (version 5.4 for Windows) was applied to run the statistical analysis. In addition, we used the Chi-square test (Cochrane Q test) to assess the statistical heterogeneity of the included studies. Significant heterogeneity was reflected by $I^2 > 50\%$ with $P < 0.1$.

3. Results

3.1. Results of study selection and characteristics

Our literature search process retrieved 9119 records. After removing duplicates, 6391 abstracts were evaluated, and 19 articles were eligible for full-text screening. Of them, 12 studies were included in this study. Due to the heterogeneity in some included studies, we conducted a meta-analysis of seven studies. The PRISMA flow diagram of the study selection process is shown in Figure 1.

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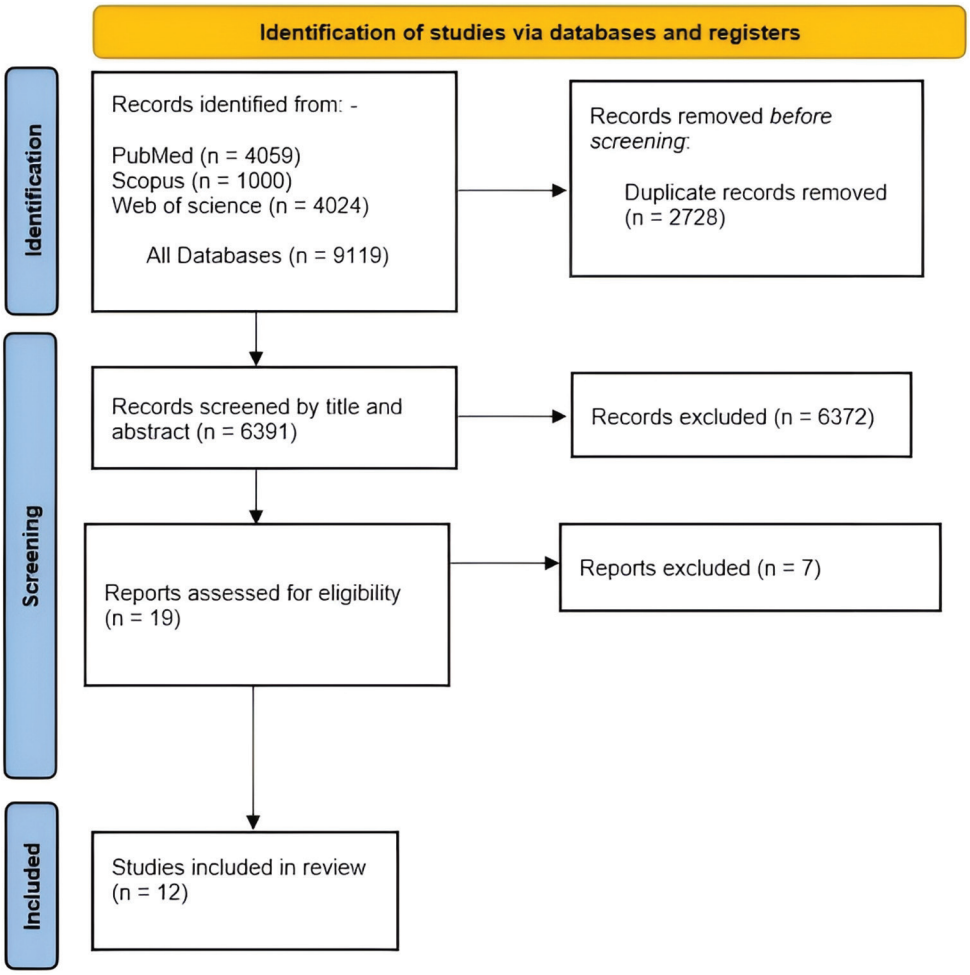

Figure 1. PRISMA flow diagram of studies' screening and selection.

The number of patients who were included in the meta-analysis was 620, including 382 who were treated with intranasal insulin and 238 who received a placebo. A summary of the eligible studies and the characteristics of their patients are presented in Tables 1 and 2.

3.2. Quality assessment

According to RoB2, we found four studies with an overall low risk of bias [9-12] and eight had some concerns. The reasons for some concerns are that four studies had some concerns in the randomization process [13-16] and four had some concerns in the selection of the reported results [17-20] (Figure 2**)**.

3.3. Outcomes

3.3.1. ADAS-cog 40 IU

Six studies were reported for ADAS-cog 40 IU with a total of 486 participants. The findings presented that there was no significant difference between the intranasal insulin and the placebo according to ADAS-cog 40 IU. The MD was −0.08 (95% CI: −0.16–0.01, $P = 0.08$). The pooled studies were homogenous ($I^2 = 0\%$, $P = 0.92$) (Figure 3A).

3.3.2. ADAS-cog 20 IU

Three studies represented ADAS-cog 20 IU with a total of 191 participants. Pooled studies favored the insulin effect over the placebo. The MD was −0.13 (95% CI: −0.22 – −0.05, $P = 0.003$). The results were homogenous ($I^2 = 0\%$, $P = 0.86$) (Figure 3B).

3.3.3. Memory composite (delayed story recall) 40 IU

We pooled the four studies that provided relevant data for memory composite 40 IU involving 400 participants. The overall effect did not favor either of the two groups in terms of memory composite 40 IU. The MD was 0.25 (95% CI: −0.09 – 0.6, $P = 0.15$). The results were homogenous ($I^2 = 0\%$, $P = 0.99$) (Figure 3C).

3.3.4. Memory composite (delayed story recall) 20 IU

Two studies reported relevant data for memory composite 20 IU involving a total of 132 participants. There was no significant difference between the intranasal insulin and the placebo. The MD was 0.65 (95% CI: −0.08 – 1.39, $P = 0.08$). The pooled studies were homogenous ($I^2 = 0\%$, $P = 0.51$) (Figure 3D).

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Table 1. Summary of the included studies

Study ID	Design	Country	Duration (months)	Patient's eligibility	Type of insulin	Outcomes measured	Main finding
Claxton *et al.* [9]	RCT	United States	48	• Patients with mild cognitive impairment or Alzheimer's disease	- Novolin R; Novo Nordisk, Princeton, New Jersey	- Primary: • Delayed story recall • DSRS - Secondary: • ADAS-Cog • ADCS-ADL	Intranasal insulin improves MCI and AD
Claxton *et al.* [15]	RCT	United States	24	• Patients with mild cognitive impairment or AD	- Levemir; Novo Nordisk, Princeton, New Jersey	- Primary • A verbal memory composite score - Secondary • Executive function • Visuospatial working memory • Caregiver-rated functional ability - Metabolic outcomes • Homeostatic model assessment for insulin resistance (HOMA-IR)	Intranasal insulin improves AD
Craft *et al.* [11]	RCT	United States	6	• Patients who scored between 0.5 and 1 on the Clinical Dementia Rating • Who >15 on the Mini-Mental State Examination	- Novolin R; Novo Nordisk, Princeton, New Jersey	Primary: • Delayed story recall • DSRS	Intranasal insulin improves MCI and AD
Craft *et al.* [14]	RCT	United States	4	• Patients who were probably AD • Who >15 on the Mini-Mental State Examination	- Humulin R U-100, Eli Lilly and Co. - Levemir®; Novo Nordisk, Princeton, New Jersey	Primary: • Delayed story recall Secondary: • ADAS-Cog • DSRS • MRI volume changes in AD-related regions of interest • Cerebrospinal fluid AD markers	Intranasal insulin improves AD
Craft *et al.* [10]	RCT	United States	48	• Adults between the ages of 55 and 85 • Patients with mild cognitive impairment or AD • MMSE scores of 20 or higher • Global clinical dementia ratings of 0.5 or 1.0 • Logical memory-delayed scores falling within a predetermined education-adjusted range.	- Humulin-RU-100; Lilly	Primary: • ADAS-Cog Secondary: • ADCS-ADL • CDR-SB • Immediate and delayed story recall.	Intranasal insulin does not improve MCI and AD

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Table 1. *(Continued)*

Study ID	Design	Country	Duration (months)	Patient's eligibility	Type of insulin	Outcomes measured	Main finding
Rosenbloom *et al.* [16]	Randomized, cross-over	United States	6	• Mild-moderate AD • Who are >65 years old and 85 years old. • The Clinical Dementia Rating (CDR) for each individual ranged from 1 to 2 • Mini-Mental State Examination (MMSE) scores ranged from 18 to 26	- Rapid-acting insulin	• Peripheral glucose levels • Verbal memory, safety, and efficacy • Serum insulin levels acutely	Intranasal insulin does not improve MCI and AD
Rosenbloom *et al.* [12]	RCT	United States	6	• Patients with mild cognitive impairment or AD • Montreal Cognitive Assessment (MoCA) scores of 18–27	NR	Primary: • ADAS-Cog • CDR-SB Secondary: • (COWAT) • (WMS)-IV • Blood glucose and insulin level • Adverse effects (AEs) and severe adverse effects (SAEs)	Intranasal insulin does not improve MCI and AD
Mustapic *et al.* [13]	RCT	United States	4	• Patients aged 55 or greater • Patients with mild cognitive impairment or AD • Who is on stable doses of memantine (Namenda) or cholinesterase inhibit	NR	• ADAS-Cog • Mini-Mental State Examination • AD Assessment Scale for cognition and insulin signaling mediators as biomarkers, especially EV biomarkers of insulin resistance as (pS312-IRS-1 and pY-IRS-1)	Intranasal insulin improves MCI and AD
Kellar *et al.* [20]	RCT	United States	18	• Adults between the ages of 55 and 85 • Patients with mild cognitive impairment or AD • MMSE scores of 20 or higher • Global clinical dementia ratings of 0.5 or 1.0 • Logical memory-delayed scores falling within a predetermined education-adjusted range	- Humulin-R U100	• CSF macrophage-derived chemokine • CSF interferon-γ, CSF immune/inflammatory/vascular markers • Changes in cognition, brain volume, and amyloid and tau concentrations • CSF markers of inflammation, immune function, and vascular integrity and assessed their relationship with changes in cognition, brain volume, and CSF amyloid and tau concentrations, reduced interleukin-6, cerebral spinal fluid (CSF) biomarker profiles and slower symptom progression	Intranasal insulin improves MCI and AD
Reger *et al.* [18]	RCT	United States	NR	• Patients with mild cognitive impairment or AD	- Novolin R, Novo Nordisk, Princeton, NJ, USA	• Verbal memory • Verbal Memory • Plasma β-Amyloid • Plasma insulin and glucose levels	Intranasal insulin improves MCI and AD
Reger *et al.* [17]	RCT	United States	NR	• Patients with mild cognitive impairment or AD	- Novolin R, Novo Nordisk	Primary: • Intended to be verbal memory after a delay • Attention • Caregiver assessments of functional state Secondary: • Plasma levels of insulin, glucose, beta-amyloid, and cortisol	Intranasal insulin improves MCI and AD

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Table 1. *(Continued)*

Study ID	Design	Country	Duration (months)	Patient's eligibility	Type of insulin	Outcomes measured	Main finding
Reger *et al.* [19]	RCT	United States	NR	• There were no neurological disorders (other than AD)	- Novolin R containing cresol, Novo Nordisk, Princeton, NJ, USA	Primary: • Cognitive data, such as (verbal memory for story recall) Secondary: • Plasma insulin • Blood glucose levels • Attention, • Working memory • Negative effects such as (nosebleed – nose soreness)	Intranasal insulin improves AD

Abbreviations: Apo e4: Apolipoprotein E4; DSRS: Dementia Severity Rating Scale; ADAS-cog: Alzheimer Disease's Assessment Scale-cognitive subscale; ADCS-ADL: Alzheimer's Disease Cooperative Study–activities of daily living; CDR-SOB: Clinical Dimension Rating–Sum of Boxes; MMSE: Mini-Mental State Examination; NR: Not reported; AD: Alzheimer's disease; MCI: Mild cognitive impairment; CSF: Cerebral spinal fluid; COWAT: Controlled Oral Word Association Test; WMS-IV: Wechsler Memory Scale

3.3.5. Memory composite (delayed story recall) long-acting

Two studies provided adequate data for memory composite long-acting involving 63 participants. We found no significant difference between the intranasal insulin and the placebo. The MD was 0.58 (95% CI: −0.04 – 1.19, $P = 0.07$). The results were homogenous ($I^2 = 0\%$, $P = 0.71$) (Figure 3E).

3.3.6. DSRS 40 IU

Three studies reported relevant data for DSRS 40 IU with a total of 160 participants. The overall effect did not favor either of the two groups in terms of DSRS 40 IU. The MD was −0.15 (95% CI: −0.88 – 0.57, $P = 0.68$). The findings of the studies were homogenous ($I^2 = 0\%$, $P = 0.6$) (Figure 3F).

3.3.7. DSRS 20 IU

Regarding DSRS 20 IU, we identified two relevant studies with a total of 132 participants. There was no significant difference between the two groups. The MD was −0.11 (95% CI: −0.82 – 0.6, $P = 0.76$). The pooled articles were homogenous ($I^2 = 0\%$, $P = 0.59$) (Figure 4A).

3.3.8. DSRS-LA

Two studies provided relevant data for DSRS-LA with a total of 63 participants. The overall effect did not favor either of the two groups in terms of DSRS-LA. The MD was 0.16 (95% CI: −3.98 – 4.29, $P = 0.94$). The results were homogenous ($I^2 = 0\%$, $P = 0.85$) (Figure 4B)

3.3.9. ADCS-ADL 40 IU

We identified three studies that reported relevant data for ADCS-ADL 40 IU involving a total of 376 participants. The overall effect did not favor either of the two groups in terms of ADCS-ADL 40 IU. The MD was 0.04 (95% CI: −0.07 – 0.15, $P = 0.49$). The pooled studies were homogenous ($I^2 = 0\%$, $P = 0.58$) (Figure 4C).

3.3.10. ADCS-ADL 20 IU

Two studies provided adequate data for ADCS-ADL 20 IU with a total of 132 participants. The overall effect did not favor either of the two groups in terms of ADCS-ADL 20 IU. The MD was 0.02 (95% CI: −0.09 – 0.13, $P = 0.72$). The results were homogenous ($I^2 = 0\%$, $P = 0.59$) (Figure 4D).

3.3.11. Clinical Dementia Rating-Sum of Boxes score

Two studies provided relevant data for clinical dimension rating – the sum of boxes involving 268 participants. We did not find a significant difference between the two groups. The MD was 0.36 (95% CI: −0.19 – 0.92, $P = 0.2$). The results were homogenous ($I^2 = 0\%$, $P = 0.54$) (Figure 4E).

3.3.12. CSF biomarkers of AD

We found a non-significance difference between the intranasal insulin and the placebo in the case of CSF biomarkers of AD. The MD was −3.23 (95% CI: −9.9 – 3.44, $P = 0.34$). In addition, the overall effect did not favor either of the two groups in terms of Abeta42, Tau, and Tau-P. More information is given in Figure 5.

3.3.13. Adverse effects

We categorized data into three subgroups (Headache, Rhinitis/URI, and Fall) involving 1001 participants. The findings of overall adverse events and the subgroups revealed no significant difference between the two groups. The RR of overall adverse events was 1.28 (95% CI: 0.75 – 2.21, $P = 0.36$). All details are in Figure 6.

4. Discussion

The introduction of effective medicine for several CNS-related disorders, including AD, by nose-to-brain drug administration, has been considered a revolutionary process [21]. Intranasal insulin is one of these treatments that have shown a beneficial impact on AD patients [11]. In the present study, 12 RCTs were included in the study. All the included studies retrieved from our literature search compared intranasal insulin with placebo in terms of safety and efficacy. The duration of treatment in the included studies ranged from 4 months to 4 years. The findings of our meta-analysis revealed that intranasal insulin might be significant in improving cognition in Alzheimer's disease patients measured by ADAS-cog with lower doses being more effective. There was

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Table 2. Baseline characteristics for the population of the included studies

Study ID	Groups	No of participants	Age, mean (SD)	Sex (m/f)	BMI, mean (SD)	Education, mean (SD)	Apo e4 status, N (%)	Diagnosis MCI/AD	DSRS, mean (SD)	ADAS-cog, mean (SD)	ADCS-AD, mean (SD)	Delayed story recall score, mean (SD)	MMSE, mean (SD)	CDR-SOB, mean (SD)
Craft *et al.* [11]	Placebo	30	74.9 (8.7)	17/13	27.4 (4.3)	15.3 (3.2)	13 (44.8)	21/9	1.64 (1.04)	1.93 (0.76)	3.75 (0.16)	2.25 (1.04)	NR	NR
	20 IU of Insulin	36	72.8 (9)	22/14	26.7 (4.8)	15.5 (3.0)	18 (50)	20/16	1.72 (0.96)	2.21 (0.72)	3.79 (0.18)	1.86 (1.02)	NR	NR
	40 IU of Insulin	38	69.9 (8.6)	20/18	26.9 (4.3)	16.2 (3.03)	16 (42.1)	23/15	1.78 (1.04)	2.26 (0.73)	3.77 (0.18)	1.99 (1.04)	NR	NR
Craft *et al.* [14]	Placebo	12	68.4 (8.9)	6|6	26.7 (3.3)	16.5 (2.0)	8 (66.66)	8/4	7.3 (6.9)	20 (11.7)	NR	NR	24.8 (4.2)	NR
	Regular insulin	12	70.5 (9.1)	7|5	28.8 (6.1)	15.6 (2.8)	8 (66.66)	7/5	7.7 (6.8)	19.8 (12.8)	NR	NR	26 (3.7)	NR
	detemir	12	67.3 (7.8)	6|6	29.4 (6.6)	14.8 (2.4)	6 (50)	7/5	8.7 (6.7)	21.6 (13.7)	NR	NR	25.2 (4.1)	NR
Craft *et al.* [10]	Placebo(blind)	119	71.1 (6.8)	61/58	NR	16.3 (2.9)	77 (64.7)	46/73	NR	24.73 (7.56)	NR	NR	24.84 (2.72)	3.35 (1.51)
	Placebo (open-label)	104	71 (7.0)	53/51	NR	16.4 (2.8)	65 (62.5)	41/63	NR	24.07 (7.3)	NR	NR	24.93 (2.73)	3.31 (1.53)
	Insulin (blind)	121	70.5 (7.4)	62/59	NR	16.1 (2.6)	79 (65.3)	41/80	NR	25.91 (8.28)	NR	NR	24.79 (2.75)	3.59 (1.51)
	Insulin (open-label)	106	70.3 (7.3)	57/49	NR	16.1 (2.7)	72 (67.9)	35/71	NR	25.34 (8.25)	NR	NR	24.95 (2.7)	3.56 (1.45)
Claxton *et al.* [9]	Placebo	30	74.84 (10.09)	17/13	27.39 (4.3)	15.26 (3.23)	13 (43.3)	64/40	7.17 (5.59)	2.05 (0.97)	43.7(6.8)	11.27 (7.6)	NR	NR
	20 IU of Insulin	36	72.8 (7.5)	22/14	26.7 (4.1)	15.5 (3.5)	18 (50)	64/40	7.16 (4.28)	2.21 (0.63)	44.17 (5.38)	9.3 (8.36)	NR	NR
	40 IU of Insulin	18	69.8 (9.12)	20/18	26.8 (4.9)	16.2 (2.8)	16 (42.1)	64/40	7.57 (5.36)	2.22 (0.65)	43.5 (6.61)	10.55 (8.23)	NR	NR
Claxton *et al.* [15]	Placebo	20	NR	NR	NR	NR	NR	39/21	NR	NR	NR	NR	NR	NR
	20 IU of Insulin	21	NR	NR	NR	NR	NR	39/21	NR	NR	NR	NR	NR	NR
	40 IU of Insulin	19	NR	NR	NR	NR	NR	39/21	NR	NR	NR	NR	NR	NR
Rosenbloom *et al.* [16]		12	72	9/3	NR	NR	NR		NR	NR	NR	NR	NR	NR
Rosenbloom *et al.* [12]	Insulin	19	68.4 (8.1)	10|9	25 (4.4)	NR	NR	14/21	NR	23.2 (5.4)	NR	NR	NR	NR
	Saline	16	74.4 (6.4)	10|6	24.5 (3.6)	NR	NR	9/10	NR	23.3 (6.1)	NR	NR	NR	NR
Mustapic *et al.* [13]	Placebo	26	76.2 (9.06)	11 |15	NR	NR	23 (50)	69/31	NR	1.93 (0.13)	NR	NR	85 (13.56)	NR
	20 IU Insulin	33	70.9 (6.83)	12/21	NR	NR	15 (45)	55/45	NR	2.21 (0.12)	NR	NR	83.64 (14.15)	NR
	40 IU Insulin	32	69.6 (9.13)	12 |20	NR	NR	14 (44)	63/38	NR	2.2 (0.12)	NR	NR	84.31 (15.44)	NR

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Table 2. *(Continued)*

Study ID	Groups	No of participants	Age, mean (SD)	Sex (m/f)	BMI, mean (SD)	Education, mean (SD)	Apo e4 status, N (%)	Diagnosis MCI/AD	DSRS, mean (SD)	ADAS-cog, mean (SD)	ADCS-AD, mean (SD)	Delayed story recall score, mean (SD)	MMSE, mean (SD)	CDR-SOB, mean (SD)
Kellar et al, [20]	Placebo	20	71.58 (7.7)	13|7	NR	17.2 (2.48)	15 (75)	5/15	NR	24.13 (6.46)	43.25 (7.89)	NR	NR	2.69 (1.42)
	Insulin	18	69.94 (6.12)	10|8	NR	16.05 (2.87)	14 (78)	8/10	NR	25.11 (9.17)	38.89 (7.36)	NR	NR	3.25 (1.72)
Reger *et al.* [18]	Normal E4−	48	73.8 (1)		26.1 (0.6)	14.6 (0.4)	48 (52)	20/13	NR	NR	NR	NR	NR	NR
	Normal E4+	11	72.5 (2)		25.6 (1.2)	15.2 (0.8)	11 (12)	20/13	NR	NR	NR	NR	NR	NR
	Memory-impaired E4−	11	76.3 (2)		26.4 (1.2)	14.5 (0.8)	11 (12)	20/13	NR	NR	NR	NR	NR	NR
	Memory-impaired E4+	22	76.8 (1.4)		26.5 (0.8)	14.7 (0.6)	22 (24)	20/13	NR	NR	NR	NR	NR	NR
Reger *et al.* [19]	Normal E4−	27	75.4 (6.4)	12|15	25.6 (3)	15.3 (2.1)	NR	NR	NR	NR	NR	NR	NR	NR
	Normal E4+	8	73 (5.2)	3|5	25.9 (4.3)	15.4 (2.3)	NR	NR	NR	NR	NR	NR	NR	NR
	MCI E4−	8	76.8 (5.4)	4|4	24 (3.8)	14 (3.2)	NR	13/13	NR	NR	NR	NR	NR	NR
	MCI E4+	5	76.6 (3.7)	4|1	24.3 (2.3)	13.8 (3.6)	NR	13/13	NR	NR	NR	NR	NR	NR
	AD E4−	6	76.7 (7.4)	2|4	25.2 (1.7)	13.3 (4.0)	NR	13/13	NR	NR	NR	NR	NR	NR
	AD E4+	7	76.6 (5.3)	3|4	25.4 (2.8)	15.7 (2.1)	NR	13/13	NR	NR	NR	NR	NR	NR
Reger *et al.* [18]	Placebo	12	79.3 (1.7)		26 (1.3)	15.5 (0.9)	NR	14/11	NR	NR	NR	NR	NR	NR
	Insulin	13	77.1 (1.6)		26.9 (1.2)	14.9 (0.8)		14/11	NR	NR	NR	NR	NR	NR

Abbreviations: Apo e4: Apolipoprotein E4; DSRS: Dementia Severity Rating Scale; ADAS-cog: Alzheimer Disease's Assessment Scale-cognitive subscale; ADCS-ADL: Alzheimer's Disease Cooperative Study–activities of daily living; CDR-SOB: Clinical Dimension Rating–Sum of Boxes; MMSE: Mini-Mental State Examination; NR: Not reported; AD: Alzheimer's disease; MCI: Mild cognitive impairment

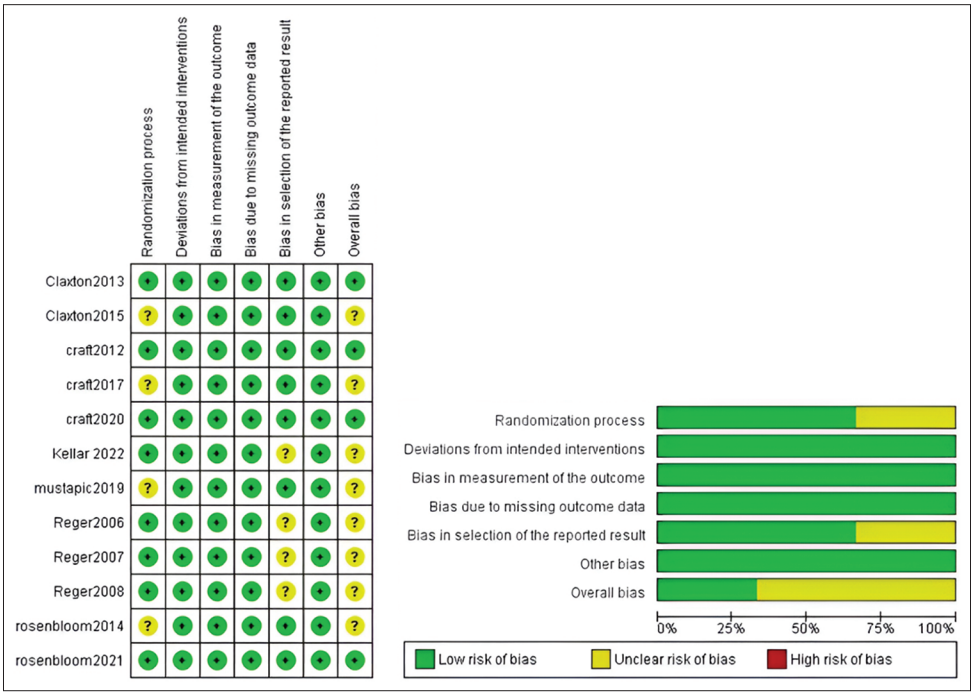

Figure 2. The risk of bias summary and risk of bias graph according to the Cochrane risk of bias assessment tool.

no difference in the incidence of adverse effects between both arms which suggests that short-term intranasal insulin could be safe in treating Alzheimer's disease patients. In addition, CSF biomarkers, clinical dementia rating score, dementia severity rating scale, and memory composite showed no significance in either dose of insulin compared to the placebo.

In general, ADAS-Cog is a reliable assessment tool for Alzheimer's disease. It contains items regarding language, memory, praxis, and orientation with higher scores representing greater impairment. These items are beneficial in not only diagnosing Alzheimer's disease patients from healthy people but also helping in determining the severity of the disease through the items in the orientation section [22]. Furthermore, we need to differentiate between MCI and dementia. MCI is a period between dementia and normal cognitive impairment associated with getting older. There are amnestic and non-amnestic types of it [23]. Dementia is a generic term for a deterioration in cognitive functions that makes it difficult to carry out daily tasks [24]. Once the patient is in the MCI stage, it is regarded as a major risk of being a dementia patient [23].

Our meta-analysis showed significantly less decline in ADAS-cog score from the baseline in the insulin 20 IU group when compared to the placebo. This is consistent with individual study results which showed that the insulin group had less decline in cognitive function over time when compared to the placebo [9,11,13].

The results did not reach a significant cutoff point when we compared insulin 40 IU and placebo regarding ADAS-cog scores. The findings of the individual studies involved in the analysis varied. Some studies supported our results that 40 IU insulin loses its effect on cognition. A possible explanation of this finding could be attributed to the small sample size, the short duration of the trials which make it difficult to detect significant differences, and the use of unreliable devices or insulin formulations that are not proven to be effective on memory [12,25,26]. Claxton 2013 [9] and Craft 2017 [14] showed a possible correlation between ApoE4 and the treatment response specific to the 40 IU insulin. In addition, Claxton 2013 [9] demonstrated a gender/ApoE4 interaction with a better improvement of cognitive function in ApoE4 negative males and more decline in ApoE4 negative females [9,14]. Given our p-value (0.08) and our confidence interval, most of it was in the direction of favoring 40 IU insulin. Further studies with larger sample sizes and longer durations are needed to confirm this association and the efficacy of using the 40 IU insulin in the treatment of Alzheimer's disease patients.

On the contrary, some studies showed that ADAS-cog score is significantly improved with the administration of 40 IU insulin compared to placebo. Craft 2012 [11] showed a significant difference in ADAS-cog score between 40 IU insulin and placebo [11]. Reger *et al*. demonstrated that the effect of insulin on cognition is dose-dependent and the curve shows a U-shaped pattern, meaning that enhanced cognition can be achieved by optimal dose while the extremes of doses will have less effect. The 40 IU might have exceeded the optimal dose of memory but not for other items of the ADAS-cog score and this explains the significant difference between the placebo in the ADAS-cog score and not in delayed story recall [18]. Another study showed that the insulin signaling pathway is better activated in smaller doses compared to higher doses which can cause insulin resistance and worsen the condition of already existing memory impairment [27]. Claxton's 2013 results support this theory as they showed similar results regarding delayed story recall and ADAS-cog score [9].

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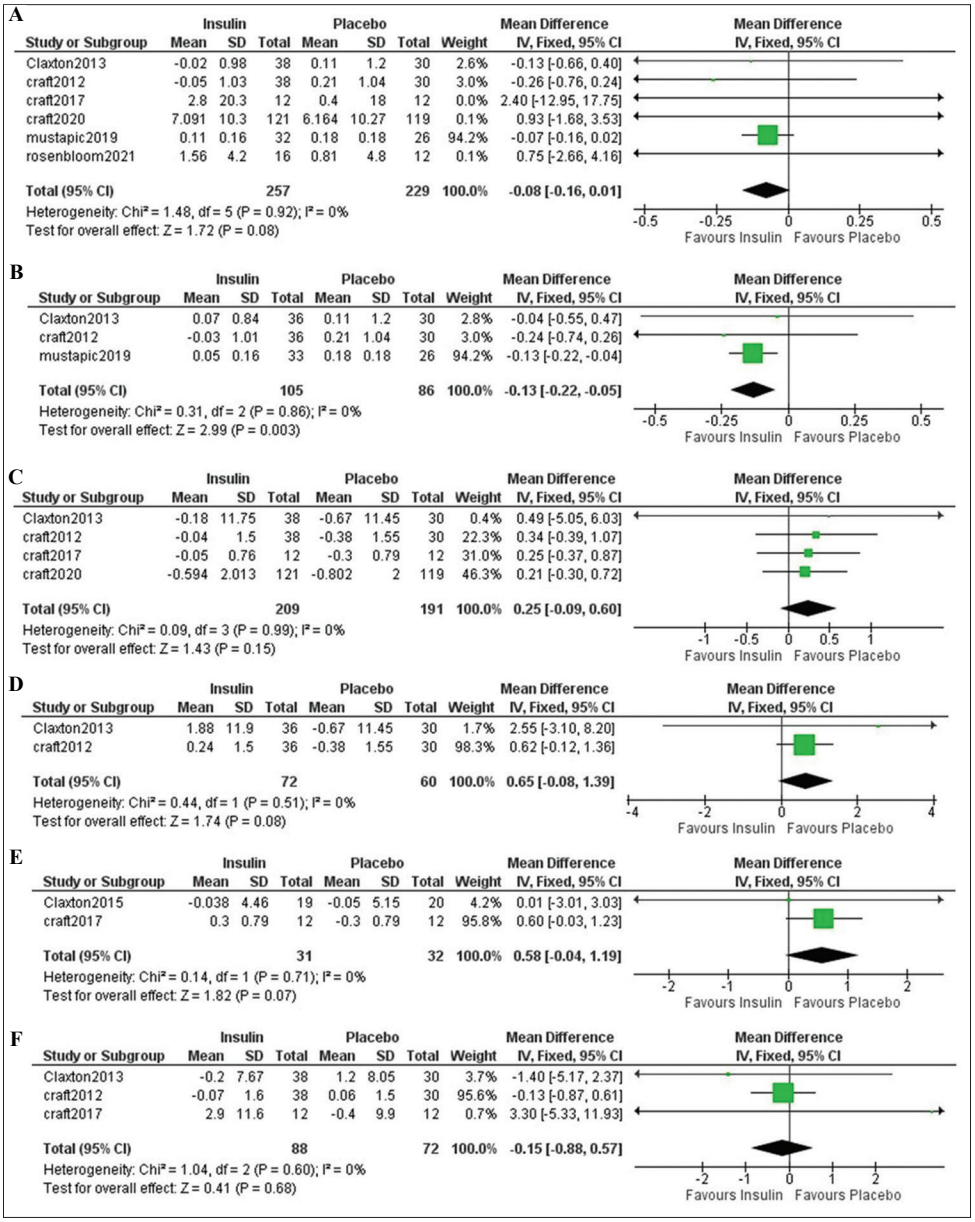

Figure 3. Forest plots of mean difference in (A) ADAS-cog 40 IU, (B) ADAS-cog 20 IU, (C) memory composite 40 IU, (D) memory composite 20 IU, (E) memory composite long-acting, and (F) DSRS 40 IU. Abbreviations: ADAS-cog: Alzheimer Disease's Assessment Scale-cognitive subscale; DSRS: Dementia Severity Rating Scale.

We found that there was a non-significant difference between 20 IU insulin and placebo regarding delayed story recall. Craft 2012 reported a significantly better story recall compared to a placebo [11]. This could be attributed to the imprecision and wide confidence interval observed in Claxton 2013 [9]. When we compared 40 IU insulin with a placebo, there was no significant difference in story recall between both groups. This is consistent with the results of individual studies and could be explained by the U-shaped dose-dependent theory that was mentioned above. This means that 40 IU insulin might have exceeded the optimal dose for memory composite.

The ADCS-ADL is a scale used to measure the capability of AD patients to perform daily activities with higher scores indicating better preservation of functional capacity [9]. Our results showed that there is no difference in ADCS-ADL between both insulin groups and the placebo. This is consistent with the findings of two of the included studies which reported this outcome. However, Claxton 2013 showed a difference in ADCS-ADL between males and females in favor of females [9]. Moreover, Craft 2012 showed that there is a significant difference between the insulin and placebo group for Alzheimer's disease but not for amnestic mild cognitive impairment (aMCI) [11].

The DSRS is a similar scale to ADCS-ADL determined by a questionnaire that contains questions about the cognitive, functional, and social status of the patient. Higher scores indicate greater impairment. Our results found no significant difference

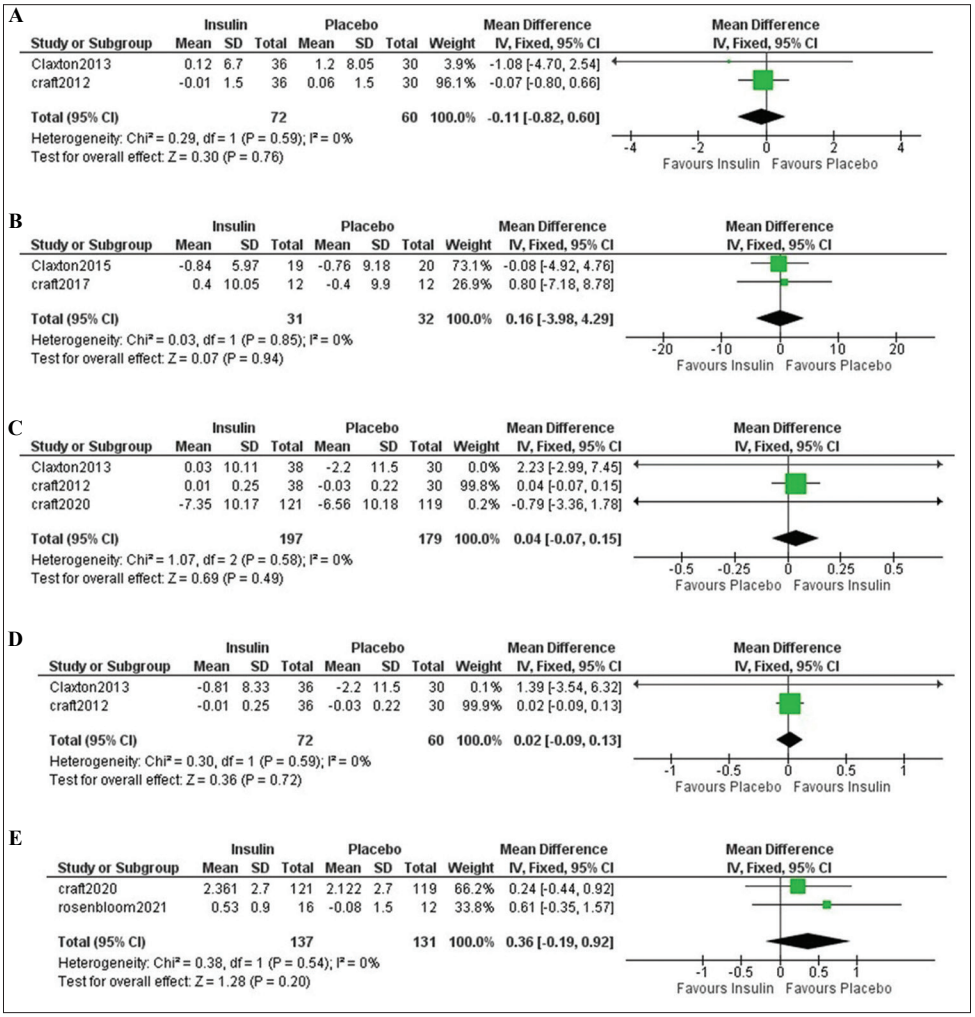

Figure 4. Forest plots of mean difference in (A) DSRS 20 IU, (B) DSRS-LA, (C) ADCS-ADL 40 IU, (D) ADCS-ADL 20 IU, and (E) Clinical Dementia Rating-Sum of Boxes score. Abbreviations: DSRS: Dementia Severity Rating Scale; ADCS-ADL: Alzheimer's disease Cooperative Study-activities of daily living.

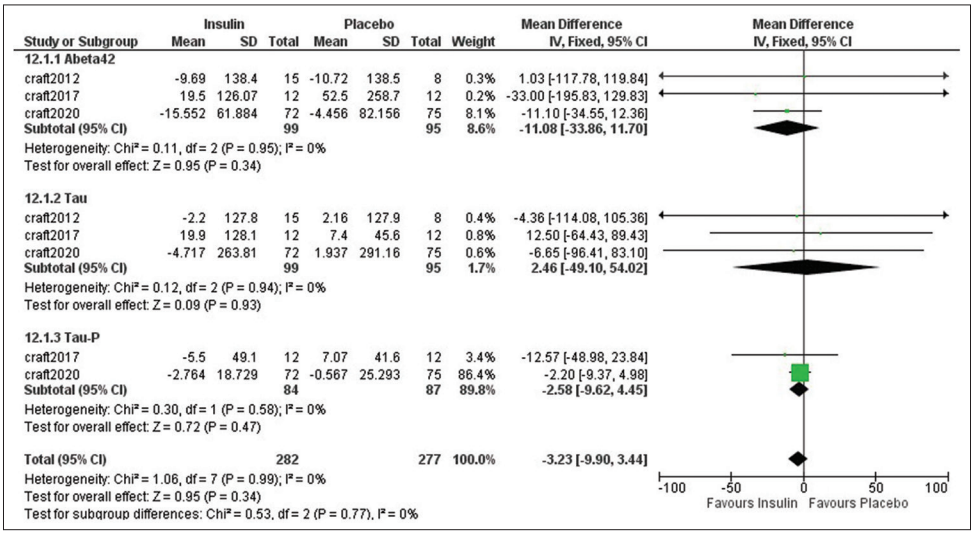

Figure 5. Forest plots of mean difference in CSF Biomarkers of AD. Abbreviations: CSF: Cerebral Spinal Fluid; AD: Alzheimer's disease.

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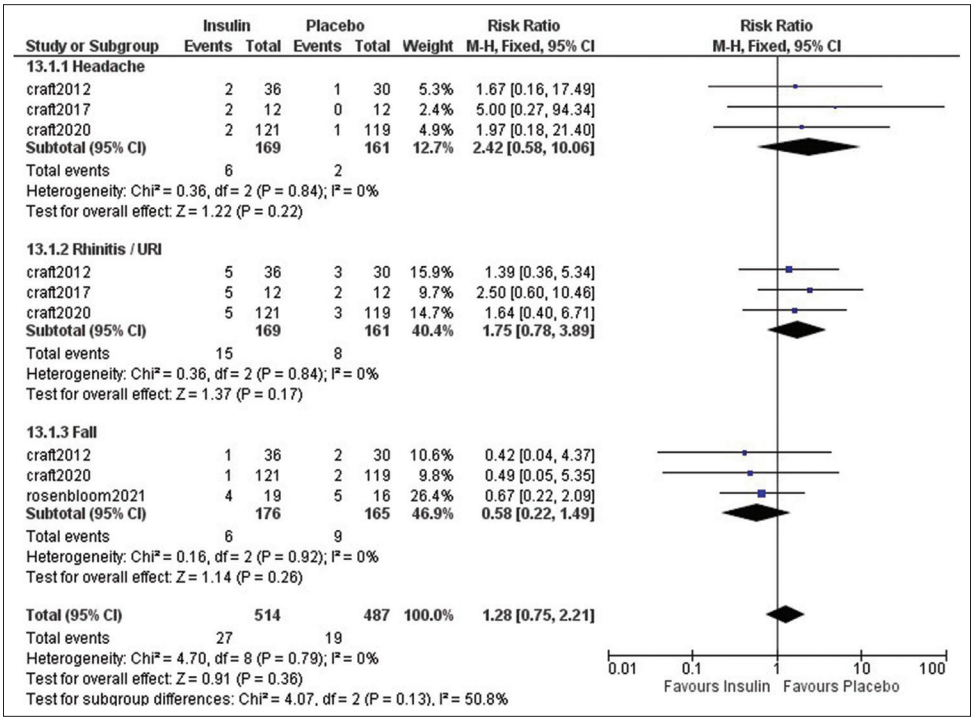

Figure 6. Forest plots of risk ration in adverse events (Headache, Rhinitis/URI, and Fall). Abbreviations: URI: Upper respiratory infection.

between the low dose of insulin and the placebo group. Craft 2012 reported similar results at 6 months of evaluation [11]. However, significant improvement in DSRS at 2 months was observed. Moreover, Claxton 2013 endpoint at 4 months revealed marginal significance in DSRS between placebo and 20 IU insulin [9]. This could be due to a time-dependent relation which suggests that 20 IU insulin might be beneficial in the short-term and relatively loses its benefits onward. Regarding 40 IU or long-acting insulin, our results as well as the results of the individual studies showed no difference with the placebo irrespective of the time of assessment.

In the context of the main pathophysiological changes in AD patients, Beta peptides, Tau protein, and Tau-p protein are known to play the main role in AD pathology. Insulin was thought to protect against amyloid-beta peptides and reduce tau phosphorylation [28-30]. However, the limited insulin transport across the BBB reduces this protective effect [31]. The intranasal administration of insulin was a new route to bypass the BBB as insulin travels along perivascular pathways following olfactory and trigeminal nerves [32].

In our study, we assessed the effect of intranasal insulin administration on the levels of the three biomarkers A beta, tau, and tau-p in the CSF. We found no significant difference in any of the three biomarkers between insulin and placebo. Although this is consistent with the individual study results, exploratory analysis of one study showed that increased levels of amyloid-beta concentration and decreased tau protein–to–AB 42 ratio were associated with improved delayed story recall and daily function. This association was only found in the insulin group. Thus, such results could not be attributed to disease progression status. Moreover, selection bias could have happened since not all participants underwent lumbar punctures [11]. However, additional studies with larger sample sizes are needed to further examine the effect of intranasal insulin on Alzheimer's disease pathology.

In the assessment of intranasal insulin safety, our meta-analysis showed no significant difference in the incidence of complications in the insulin group compared to the placebo group. The included studies reported no serious adverse effects, and the complications were limited to minor complications such as upper respiratory symptoms and rhinitis. Apart from a higher rate of nasal irritation reported in Rosenbloom and a higher total number of minor adverse events reported in Craft 2012, the overall results showed no significant difference in the incidence of complications between insulin and placebo groups [11,12]. Moreover, these studies reported good compliance which was not different between the two arms [11,12,14]. Thus, weighing the risk-benefit ratio of this treatment, intranasal insulin could be a safe therapy for Alzheimer's disease patients. Rosenbloom included non-insulin-dependent diabetic patients who reported that they well tolerated the treatment with no major adverse effects or hypoglycemia. However, these studies were done over a short duration, making their long-term safety and efficacy inconclusive. Thus, larger sample sizes and longer-duration clinical trials are needed to assess the long-term benefits of intranasal insulin and the correlation between patients' characteristics and their response to treatment.

In the latest network meta-analysis [33], the efficacy of six different antidiabetic drugs was evaluated, including intranasal insulin 40 IU and 20 IU. No discernable difference was found when assessing the acceptability of the agents (defined by all-

cause discontinuation). In addition, Cognitive assessment using ADAS-Cog showed no significant improvement in either dose (20 IU or 40 IU) compared with the placebo. Nonetheless, our study found that intranasal insulin delivered at 20 IU improved the ADAS-Cog, but not at 40 IU. This cognitive change in response to low-dosage intranasal insulin was related to neuronal extracellular vesicles (EV) biomarkers of insulin resistance (pS312-IRS-1, pY-IRS-1), suggesting activation of the insulin signaling cascade at the IRS-1 level.

4.1. Limitations and strengths of the study

The major limitation of this study included: The inability to perform a meta-analysis of five of the included studies due to several variations between these articles, such as reporting different outcomes utilizing various scores and some discrepancies in the duration of intervention. Future research is warranted to explore the efficacy of intranasal insulin in a larger sample with longer follow-ups, taking into consideration the apoE4 status and the progressive neurodegeneration that occurs over many years and needed longer duration studies.

Nevertheless, the strengths of our study are as follows: (1) Our meta-analysis represented the last updated evidence assessing the efficacy and safety of intranasal insulin in patients with AD, (2) we provided a more comprehensive analysis in an attempt to solve the previous conflicting findings, and (3) we complied the PRISMA checklist when representing this manuscript and conducted all steps as stated in the Cochrane Handbook in our review.

5. Conclusion

Ultimately, the current results of intranasal insulin are encouraging in terms of safety and efficacy. Our findings demonstrate that the administration of lower doses (20 IU) has distinctly more efficacy than higher doses (40 IU) as revealed by the ADAS-cog scale. To learn about the variations (sex, age, and ApoE4 carriage) in treatment responses and make the most of this intervention, further trials are required. In addition, a future investigation should require reliable insulin delivery devices with proven capacity to increase insulin in the CNS.

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Conflicts of Interest

All authors have no conflicts of interest.

References

[1] Michailidis M, Tata DA, Moraitou D, Kavvadas D, Karachrysafi S, Papamitsou T, *et al.* Antidiabetic Drugs in the Treatment of Alzheimer's Disease. Int J Mol Sci 2022;23:4641.

[2] Kazkayasi I, Telli G, Nemutlu E, Uma S. Intranasal Metformin Treatment Ameliorates Cognitive Functions Via Insulin Signaling Pathway in ICV-STZ-induced Mice Model of Alzheimer's Disease. Life Sci 2022;299:120538.

[3] Akel H, Csóka I, Ambrus R, Bocsik A, Gróf I, Mészáros M, *et al. In Vitro* Comparative Study of Solid Lipid and PLGA Nanoparticles Designed to Facilitate Nose-to-brain Delivery of Insulin. Int J Mol Sci 2021;22:13258.

[4] Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the Brain: Sources, Localization and Functions. Mol Neurobiol 2013;47:145-71.

[5] Maher MA, Kandeel WA, Hammam OA, Attia YM, Mahmoud S, Salah M. Histopathological Evaluation of Insulin-DMSO Formula Designed for Direct Nose-to-brain Delivery. Histol Histopathol 2021;37:431-9.

[6] Bazrgar M, Khodabakhsh P, Dargahi L, Mohagheghi F, Ahmadiani A. MicroRNA Modulation is a Potential Molecular Mechanism for Neuroprotective Effects of Intranasal Insulin Administration in Amyloid βeta Oligomer Induced Alzheimer's Like Rat Model. Exp Gerontol 2022;164:111812.

[7] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al.* The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. BMJ 2021;372:n71.

[8] Sterne JA, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, *et al.* RoB 2: A Revised Tool for Assessing Risk of Bias in Randomised Trials. BMJ 2019;366:l4898.

[9] Claxton A, Baker LD, Wilkinson CW, Trittschuh EH, Chapman D, Watson GS, *et al.* Sex and ApoE Genotype Differences in Treatment Response to Two Doses of Intranasal Insulin in Adults with Mild Cognitive Impairment or Alzheimer's Disease. J Alzheimers Dis 2013;35:789-97.

[10] Craft S, Raman R, Chow TW, Rafii MS, Sun CK, Rissman RA, *et al.* Safety, Efficacy, and Feasibility of Intranasal Insulin for the Treatment of Mild Cognitive Impairment and Alzheimer Disease Dementia: A Randomized Clinical Trial. JAMA Neurol 2020;77:1099-109.

[11] Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, *et al.* Intranasal Insulin Therapy for Alzheimer Disease and Amnestic Mild Cognitive Impairment: A Pilot Clinical Trial. Arch Neurol 2012;69:29-38.

[12] Rosenbloom M, Barclay TR, Kashyap B, Hage L, O'Keefe LR, Svitak A, *et al.* A Phase II, Single-center, Randomized, Double-blind, Placebo-controlled Study of the Safety and Therapeutic Efficacy of Intranasal Glulisine in Amnestic Mild Cognitive Impairment and Probable Mild Alzheimer's Disease. Drugs Aging 2021;38:407-15.

[13] Mustapic M, Tran J, Craft S, Kapogiannis D. Extracellular Vesicle Biomarkers Track Cognitive Changes Following Intranasal Insulin in Alzheimer's Disease. J Alzheimers Dis 2019;69:489-98.

[14] Craft S, Claxton A, Baker LD, Hanson AJ, Cholerton B,

Trittschuh EH, *et al.* Effects of Regular and Long-acting Insulin on Cognition and Alzheimer's Disease Biomarkers: A Pilot Clinical Trial. J Alzheimers Dis 2017;57:1325-34.

[15] Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, *et al.* Long-Acting Intranasal Insulin Detemir Improves Cognition for Adults with Mild Cognitive Impairment or Early-stage Alzheimer's Disease Dementia. J Alzheimers Dis 2015;44:897-906.

[16] Rosenbloom MH, Barclay TR, Pyle M, Owens BL, Cagan AB, Anderson CP, *et al.* A Single-dose Pilot Trial of Intranasal Rapid-acting Insulin in Apolipoprotein E4 Carriers with Mild-moderate Alzheimer's Disease. CNS Drugs 2014;28:1185-9.

[17] Reger MA, Watson GS, Green PS, Wilkinson CW, Baker LD, Cholerton B, *et al.* Intranasal Insulin Improves Cognition and Modulates Beta-amyloid in Early AD. Neurology 2008;70:440-8.

[18] Reger MA, Watson GS, Green PS, Baker LD, Cholerton B, Fishel MA, *et al.* Intranasal Insulin Administration Dose-dependently Modulates Verbal Memory and Plasma Amyloid-beta in Memory-impaired Older Adults. J Alzheimers Dis 2008;13:323-31.

[19] Reger MA, Watson GS, Frey WH 2nd, Baker LD, Cholerton B, Keeling ML, *et al.* Effects of Intranasal Insulin on Cognition in Memory-impaired Older Adults: Modulation by APOE Genotype. Neurobiol Aging 2006;27:451-8.

[20] Kellar D, Register T, Lockhart SN, Aisen P, Raman R, Rissman RA, *et al.* Intranasal Insulin Modulates Cerebrospinal Fluid Markers of Neuroinflammation in Mild Cognitive Impairment and Alzheimer's Disease: A Randomized Trial. Sci Rep 2022;12:1346.

[21] Prabakaran A, Agrawal M, Dethe MR, Ahmed H, Yadav A, Gupta U, *et al.* Nose-to-brain Drug Delivery for the Treatment of Alzheimer's Disease: Current Advancements and Challenges. Expert Opin Drug Deliv 2022;19:87-102.

[22] Anderson NH, Woodburn K. Old-age psychiatry. In: Companion to Psychiatric Studies. Amsterdam: Elsevier; 2010. p. 635-92.

[23] Chen YX, Liang N, Li XL, Yang SH, Wang YP, Shi NN. Diagnosis and Treatment for Mild Cognitive Impairment: A Systematic Review of Clinical Practice Guidelines and Consensus Statements. Front Neurol 2021;12:719849.

[24] Gale SA, Acar D, Daffner KR. Dementia. Am J Med 2018;131:1161-9.

[25] Ito K, Hutmacher MM. Predicting the Time to Clinically Worsening in Mild Cognitive Impairment Patients and its Utility in Clinical Trial Design by Modeling a Longitudinal Clinical Dementia Rating Sum of Boxes from the ADNI Database. J Alzheimers Dis 2014;40:967-79.

[26] Evans S, McRae-McKee K, Wong MM, Hadjichrysanthou C, De Wolf F, Anderson R. The Importance of Endpoint Selection: How Effective does a Drug Need to be for Success in a Clinical Trial of a Possible Alzheimer's Disease Treatment? Eur J Epidemiol 2018;33:635-44.

[27] Sciacca L, Cassarino MF, Genua M, Pandini G, Le Moli R, Squatrito S, *et al.* Insulin Analogues Differently Activate Insulin Receptor Isoforms and Post-receptor Signalling. Diabetologia 2010;53:1743-53.

[28] Gasparini L, Gouras GK, Wang R, Gross RS, Beal MF, Greengard P, *et al.* Stimulation of Beta-amyloid Precursor Protein Trafficking by Insulin Reduces Intraneuronal Beta-amyloid and Requires Mitogen-activated Protein Kinase Signaling. J Neurosci 2001;21:2561-70.

[29] Lee CC, Kuo YM, Huang CC, Hsu KS. Insulin Rescues Amyloid Beta-induced Impairment of Hippocampal Long-term Potentiation. Neurobiol Aging 2009;30:377-87.

[30] De Felice FG, Vieira MN, Bomfim TR, Decker H, Velasco PT, Lambert MP, *et al.* Protection of Synapses Against Alzheimer's-linked Toxins: Insulin Signaling Prevents the Pathogenic Binding of Abeta Oligomers. Proc Natl Acad Sci U S A 2009;106:1971-6.

[31] Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D Jr. Cerebrospinal Fluid and Plasma Insulin Levels in Alzheimer's Disease: Relationship to Severity of Dementia and Apolipoprotein E Genotype. Neurology 1998;50:164-8.

[32] Lochhead JJ, Wolak DJ, Pizzo ME, Thorne RG. Rapid Transport within Cerebral Perivascular Spaces Underlies Widespread Tracer Distribution in the Brain After Intranasal Administration. J Cereb Blood Flow Metab 2015;35:371-81.

[33] Cao B, Rosenblat JD, Brietzke E, Park C, Lee Y, Musial N, *et al.* Comparative Efficacy and Acceptability of Antidiabetic Agents for Alzheimer's Disease and Mild Cognitive Impairment: A Systematic Review and Network Meta-analysis. Diabetes Obes Metab 2018;20:2467-71.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013