



Moderate exercise and insulin in combination protect against brain growth retardation and weight loss by modulation of glucose metabolism in rat model of Alzheimer disease

Forough Radfar¹, Mehdi Shahbazi^{1*}, Shahzad Tahmasebi Boroujeni¹, Elahe Arabameri¹, Maryam Farahmandfar²

1. Department of Behavioral and Cognitive Sciences in Sports, Faculty of Sport Sciences and Health, University of Tehran, Tehran, Iran. (*Corresponding author: ✉ shahbazimehdi@ut.ac.ir,  <https://orcid.org/0000-0002-0628-3166>)
2. Department of Neuroscience and Addiction Studies, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Article Info	Abstract
<p>Original Article</p> <p>Article history: Received: 02 November 2023 Revised: 15 December 2023 Accepted: 19 December 2023 Published: 1 January 2024</p> <p>Keywords: Alzheimer's disease, Insulin, Spatial memory, Treadmill exercise.</p>	<p>Background: Alzheimer's disease (AD), the most prevalent neurodegenerative disorder, is characterized by amyloid-beta (Aβ) plaque accumulation, neurofibrillary tangles, neuronal death, inflammation, and oxidative stress.</p> <p>Aim: We investigated the effects of treadmill exercise and intranasal insulin on spatial memory, blood glucose level, and Physical growth indicators including weight, head circumference, and tail length.</p> <p>Materials and Methods: Seventy-two male Wistar rats, aged 8 weeks were into 9 groups at random (control, Sham, Aβ, Aβ + EX, Aβ + PINS, Aβ + INST, Aβ + EX + PINS, Aβ + EX + INST, and Aβ + EX + PINS + INST). We discovered that rats receiving Aβ₂₅₋₃₅ had impaired spatial memory, which was associated with weight loss, brain growth retardation, Tail Length, and elevated blood glucose levels. Data from each trial was statistically analyzed using IBM SPSS Statistics 22 software, Two-way ANOVA, and post-hoc analysis Tukey test. The cut-off for statistical significance was $P \leq 0.05$.</p> <p>Results: Our results show that the improvement of spatial memory due to the improvement of metabolism and growth indicators can be affected by pre-treatment exercise and intranasal insulin. Also, exercise training and intranasal insulin improved spatial memory and prevented brain growth retardation, increased blood glucose, weight loss, and tail length in animals treated with Aβ₂₅₋₃₅.</p> <p>Conclusion: Exercise can amplify the positive benefits of intranasal insulin treatment on memory. The results of our research showed that exercise and insulin can prevent brain growth retardation and prevent spatial memory disorders by improving glucose metabolism.</p>

Cite this article: Radfar F, Shahbazi M, Tahmasebi Boroujeni Sh, Arabameri E, Farahmandfar M. "Moderate exercise and insulin in combination protect against brain growth retardation and weight loss by modulation of glucose metabolism in rat model of Alzheimer disease". *Sport Sciences and Health Research*. 2024; 16(1): 1-13.

doi: <https://doi.org/10.22059/SSHR.2024.369689.1116>.

EISSN: 2717-2422 | Web site: <https://sshr.ut.ac.ir/> | Email: sshr@ut.ac.ir

© The Author(s). Publisher: University of Tehran, Faculty of Sport Sciences and Health



1. Introduction

Alzheimer's disease (AD) is a neurodegenerative condition that is associated with aging and is considered to be one of the most common problems to public health all over the world [1]. AD is typified by the build-up of hyperphosphorylated tau (p-tau) protein and β -amyloid (A β) peptide in the brain, which eventually results in synaptic loss and cell death. Progressive cognitive decline, including memory loss, brain growth retardation, and impairment of higher executive functions, is a hallmark of AD clinical manifestations. However, there are currently no viable pharmaceutical treatments for AD, despite a plethora of knowledge regarding the origin of the disease [2, 3, 4]. Unintended weight loss may indicate preclinical AD years before clinical symptoms appear. AD patients who lose weight have worse cognition signs and faster clinical deterioration [5].

There is still scant and inconsistent data to support a link between body weight and dementia risk. Numerous studies have demonstrated that dementia is preceded by weight loss. Additionally, weight loss has been observed both at the time of diagnosis and as the dementia worsens [6].

The studies showed that AD is connected with brain shrinkage and cognitive impairment, and it also has a tendency to hasten the loss of lean mass [7]. Obesity increases inflammation and insulin resistance. According to recent research, AD may be a metabolic illness linked to abnormalities in the brain's metabolism of glucose [8]. In actuality, brain growth retardation and cognitive impairments are linked to abnormalities of glucose metabolism [9].

Since AD research began, metabolic reasons have been a priority. In an

Alzheimer's disease model, a high-fat diet can produce metabolic dysfunction, which increases amyloid beta aggregation and cognitive dysfunction [10]. Metabolic illnesses including type 2 diabetic mellitus (T2DM) and AD share many similarities [11]. This condition is characterized by inflammation, oxidative damage, and abnormal insulin signaling. High-fat diets and obesity worsen peripheral inflammation in T2DM, which causes cellular stress and insulin resistance. Amyloid beta activates microglia and astrocytes in AD, causing central nervous system (CNS) inflammation and insulin resistance. This contributes to synaptic degeneration and cognitive decline in T2DM. Caloric restriction (CR) delays age-related disorders including Alzheimer's. This is likely related to enhanced metabolic function, including lower pro-inflammatory cytokines, insulin sensitivity, and belly fat mass [11, 12]. Any action that delays the start of this condition can be quite beneficial, given how common it is.

The current guidelines recommend that non-pharmacological interventions should be performed first, followed by the lowest drug dose and for a short period of time, pharmacological interventions should be on the agenda. Among the non-pharmacological interventions, physical activity is a promising option.

According to research conducted on cognitively normal older persons, maintaining a healthy level of physical fitness may be able to counteract the structural and functional changes that occur in the brain as a natural part of the aging process [13, 14]. Exercise seems to stimulate neurogenesis in the brains of both humans and animals [15]. Aerobic exercise, in particular, appears to inhibit both global and regional brain growth retardation,

which results in an increase in effectively increased brain volume [16]. In older individuals with great aerobic ability, there is relatively minimal evidence of brain structural atrophy [13]. Performing aerobic activity over six months results in an increase in the volume of the hippocampus, frontal lobe, and temporal lobe [17]. According to these results, there is a chance that aerobic exercise can prevent brain growth retardation [17].

Insulin has been suggested as a potential therapeutic treatment for AD due to the presence of insulin resistance in the symptoms of the disease. In late-onset Alzheimer's disease (LOAD), in addition to being related to brain atrophy and dementia, age-dependent concurrent decrease of insulin and IGF is also associated with these symptoms [8].

Considering the documented roles of insulin and exercise in the mechanisms of synaptic plasticity and memory formation, to identify therapeutic methods that can be used before the onset of AD, we decided to evaluate the effect of exercise and insulin on growth indicators, spatial memory, and glucose metabolism.

2. Materials and Methods

2.1. Animals

Seventy-two male Wistar rats, aged 8 weeks, were purchased from the Animals

Center of Royan Institute of Iran. The rats were housed in groups of three per cage in animal houses with a 12-hour light/dark cycle, with lights on at 7 a.m. and off at 7 p.m. They had free access to food and water. The animals were randomly divided into nine groups, each consisting of eight rats: (1) healthy control (control), (2) Sham, (3) Alzheimer's disease (A β), (4) Alzheimer's disease with treadmill exercise (A β + EX), (5) Alzheimer disease with pretreatment insulin (A β + PINS), (6) Alzheimer's disease with insulin treatment (A β + INST), (7) Alzheimer disease with treadmill exercise and pretreatment insulin (A β + EX + PINS), (8) Alzheimer disease with treadmill exercise and insulin treatment (A β + EX + INST), and (9) Alzheimer disease with treadmill exercise, pretreatment insulin, and insulin treatment (A β + EX + PINS + INST). In order to minimize the number of animals used and reduce their suffering, every possible effort was made.

The University of Tehran's Ethics Committee on the Use of Animals authorized all studies after ensuring that they were conducted in compliance with the National Institutes of Health's (NIH) Guide for the Care and Use of Laboratory Animals. Figure 1 shows a study design in our research.

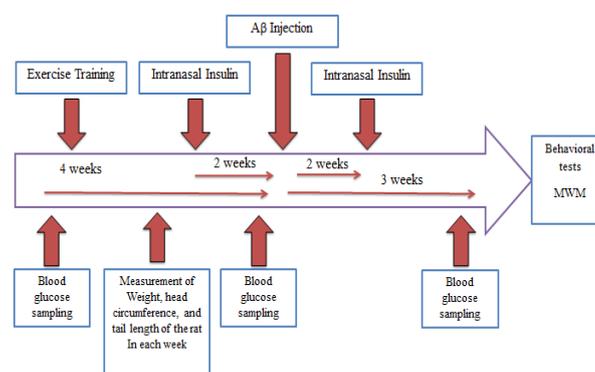


Figure 1. The study design of the experimental protocol. Bilateral intravenous administration of A β (10 μ g/rat)/saline was performed on the animals. Every group was provided with customized training and treatment plans.

2. 2. $A\beta$ (25-35) preparation and intra-cerebroventricular injection

To induce AD, $A\beta_{25-35}$ peptide was administered. To allow peptide fibrillization and aggregation associated with toxicity to occur, an $A\beta_{25-35}$ peptide (Tocris-131602-53-4, Bristol, UK) was incubated in sterile saline at a concentration of 5 $\mu\text{g/L}$ at 37°C for 72 hours. After incubation, the peptide was stored at a temperature of -20°C until it was used [18]. After being anesthetized with ketamine and xylazine (70 vs. 10 mg/kg body weight), the rats were placed on a stereotaxic frame. A stainless steel guide cannula (23G, 9 mm length) was bilaterally implanted in the intra-cerebroventricular region (ICV: AP: -0.08, ML: 1.5, DV: 3.5 mm) [18]. The open field work was completed one week later. Bilateral intracerebral injections of saline or $A\beta_{25-35}$ (10 $\mu\text{g/rat}$) were administered to both the sham-operated and the $A\beta$ -treated groups using a 25 mL syringe and an injection needle with a gauge of 27 [19].

2. 3. Treadmill exercise protocol

Before beginning treadmill training, the rats got used to their new environment on the treadmill by walking for 10 min a day, five days a week, at a pace of 5 m/min. The exercise regimen was performed five days a week on a 0% gradient from 9:00 AM to 16:00 PM for a duration of four weeks [20]. During the first and second weeks, the rats ran for 30 min per day (2x15-min sessions) at a pace of 10 m/min. To avoid muscle fatigue, 5 min of inactive rest were permitted in between sessions. Over the next few weeks, both the duration and the intensity of the exercise grew steadily. For instance, the rats ran for 45 min a day (3x15-min sessions), at a pace of 15 m/min during the third week. In the fourth week, the rats ran for 60 min a day at a pace of 15 m/min (4x15-min sessions). The rats

received a little shock during training, with an intensity of 0.5 milliamperes, which did not put the animal through excessive stress. This was done to incentivize the rats to run nonstop [21].

2. 4. Intranasal insulin administration

Sigma brand human insulin with number 1342106 manufactured in the United States of America was prepared for intranasal use. The intranasal insulin dosage that markedly enhanced memory functions was determined to be (2 IU) in a prior investigation [22]. The rats were held by the backs of their necks and had their heads pulled back with a pad while an Eppendorf pipette was used to inject insulin into each nostril. Rats in the $A\beta$ +PINS and $A\beta$ +EX+PINS groups were given insulin for 14 days prior to beta injection, 14 days following beta injection in the $A\beta$ +INST and $A\beta$ +EX+INST groups, and 28 days total (14 days prior to and 14 days following) after the 2 IU beta injection in the $A\beta$ +EX+PINS+INST group.

2. 5. Measurement of weight, head circumference, and tail length of the rat

To evaluate and measure physical indicators, weight, and tail length and head circumference of rats were measured and recorded at the beginning of each week. A scale was used to measure the weight index, and a caliper was used to evaluate the tail length and head circumference of rats.

2. 6. Behavioral assessment

The researchers used the Morris water maze to assess spatial learning and memory in rats. The width of the pool was 150 cm and its height was 90 cm, and the water temperature was between 25 and 35°C. A 10 cm Plexiglas platform was 1 cm below the water in the center of the south-east quadrant (target quadrant) of this pool. Four

days of four-trial blocks assessed spatial learning. Each experiment started with a random animal implanted in the pool wall at one of the four quadrants. Animals had 60 sec to find concealed platforms. Rats that found the platform within 60 sec were permitted to relax for 20 sec, while those that didn't were escorted there. The probing test was 24 hours after acquisition. Each rat was given 60 sec to swim freely in the quadrant opposite the objective once the platform was removed from the water. Time in the target quadrant determined spatial memory. One hour following the last probing test, rats' visual-motor coordination was tested with a visible platform task with four trials. Bright aluminum foil covered the platform, which was 1.5 cm above the water and situated in the southwest quadrant of the map opposite the target. In all trials, rat swimming speed and platform search time and distance were measured. In probing testing, the Ethovision tracking device (version 11.5, Noldus, Netherlands) measured the target zone traveled distance [23].

2. 7. Glucose level measurement

EasyGluco blood glucose meter made in South Korea was used to evaluate blood glucose level. A nocturnal fasting glucose level was determined using blood samples drawn from the tail between 8:00 and 8:30 a.m. on three separate occasions (one week following adaptation, one week following the conclusion of the intervention program, and one week following the A β injection). The blood's glucose content was determined using a glucose meter.

2. 8. Statistical analysis

Data from each trial was statistically analyzed using IBM SPSS Statistics 22 software, Two-way ANOVA, and post-hoc analysis Tukey test. The cut-off for

statistical significance was $P \leq 0.05$. Data was analyzed using two-way repeated measures ANOVA with time as the within-subjects component and treatment (different groups) as the between-subjects factor to ascertain the differences between each week. One-way analysis of variance was used to evaluate spatial memory performance on the probe day.

3. Results

3. 1. Weight

Statistical analysis showed a significant difference between the groups in the seventh week of measurement, 21 days after A β injection ($F(48,378) = 6.91, P = 0.0001$). Tukey's multiple comparison tests showed that the group that received A β injection had less weight after injection compared to treatment groups ($P < 0.0001$). Also, groups that received exercise, exercise with insulin pretreatment, or a combination of both did not experience weight loss compared to the A β group [A β +EX ($F(48,378) = 6.91, P = 0.0007$), A β +EX+PINS ($F(48,378) = 6.91, P = 0.004$), and A β +EX+PINS+INST ($F(48,378) = 6.91, P = 0.002$). Figure 2].

3. 2. Rat head circumference

Statistical analysis showed a significant difference between the groups in the seventh week of measurement, 21 days after A β injection ($F(48,378) = 3.07, P = 0.0001$). Tukey's multiple comparison tests showed that the group that received A β had a significant decrease in the distance between the two ears, which is an indicator of rat head circumference measurement ($P < 0.0001$). The difference between A β +EX ($F(48,378) = 3.07, P = 0.0007$), A β +EX+PINS ($F(48,378) = 3.07, P = 0.004$), and A β +EX+PINS+INST ($F(48,378) = 3.07, P = 0.002$) and A β groups was evident. The results were shown in Figure.3.

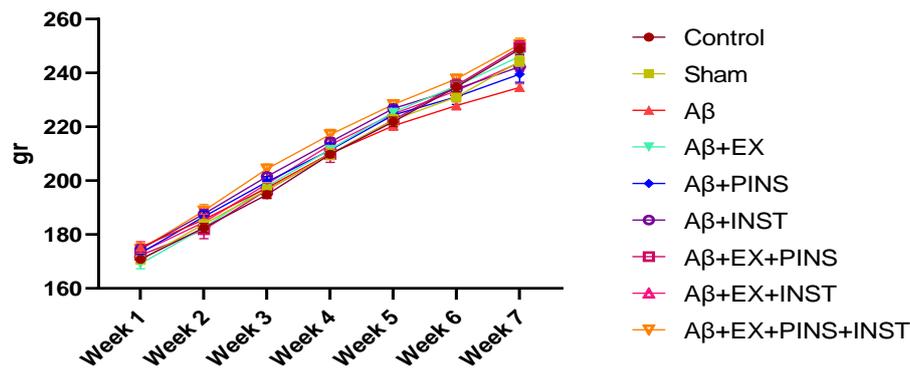


Figure 2. Weight measurement. According to the collected data, there were significant differences between the groups in the weight. Results are shown as mean \pm SEM (N= 8 in each group).

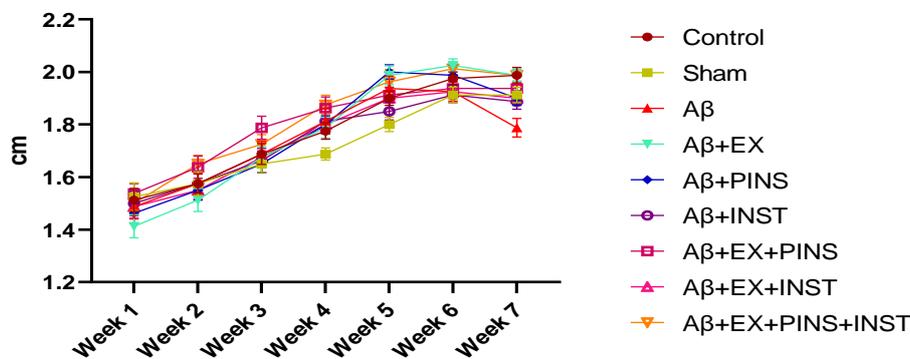


Figure 3. Rat head circumference measurement. According to the collected data, there were significant differences between the groups in the weight. Results are shown as mean \pm SEM (N= 8 in each group).

3. 3. Rat tail

Statistical analysis showed a significant difference between the groups in the fourth, fifth, sixth, and seventh weeks of measurement ($F(48,441)= 1.5, P=0.02$). Tukey's multiple comparison tests showed that in the fourth week, the groups Aβ+EX, Aβ+EX+PINS, and Aβ+EX+PINS+INST significantly had longer tail lengths than the Aβ group [Aβ+EX ($F(48,441)= 1.5, P= 0.04$), Aβ+EX+PINS ($F(48,441)= 1.5, P= 0.01$), and Aβ+EX+PINS+INST ($F(48,441)= 1.5, P= 0.004$)]. In the fifth week, the groups Aβ+EX, Aβ+EX+PINS, Aβ+EX+INST, and Aβ+EX+PINS+INST significantly had longer tail lengths than the Aβ group [Aβ+EX ($F(48,441)= 1.5, P=0.005$), Aβ+EX+PINS ($F(48,441)= 1.5,$

$P= 0.0004$), Aβ+EX+INST ($F(48,441)= 1.5, P=0.003$), and Aβ+EX+PINS+INST ($F(48,441)= 1.5, P<0.0001$)]. In the sixth week, the groups Aβ+EX, Aβ+EX+PINS, Aβ+EX+INST, and Aβ+EX+PINS+INST significantly had longer tail lengths than the Aβ group [Aβ+EX ($P=0.0001$), Aβ+EX+PINS ($P<0.0001$), Aβ+EX+INST ($P=0.0001$), and Aβ+EX+PINS+INST ($P<0.0001$)]. In the seventh week, the groups Aβ+EX, Aβ+EX+PINS, Aβ+EX+INST, and Aβ+EX+PINS+INST significantly had longer tail lengths than the Aβ group [Aβ+EX ($F(48,441)= 1.5, P<0.0001$), Aβ+EX+PINS ($F(48,441)= 1.5, P<0.0001$), Aβ+EX+INST ($F(48,441)= 1.5, P=0.0002$), and Aβ+EX+PINS+INST ($F(48,441)= 1.5, P<0.0001$), Figure 4].

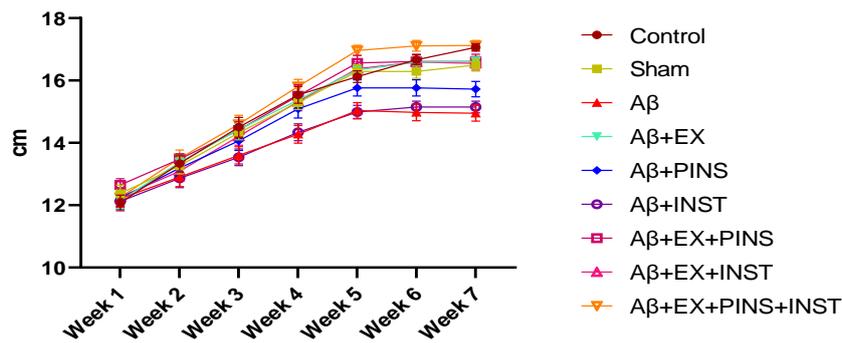


Figure 4. Rat tail measurement. According to the collected data, there were significant differences between the groups in the weight. Results are shown as mean \pm SEM (N 8 in each group).

3. 4. Glucose

The statistical analysis revealed significant differences between the groups in the level of glucose in different time ($F(8, 126)=4.86, P=0.0001$). Post hoc Tukey multiple comparison tests were performed at times 3 and revealed that the group that received A β injections had significantly greater level of glucose ($P<0.0001$). Also, the groups that received exercise, insulin treatment or a combination of both methods showed lower blood glucose levels than the A β group [A β +EX ($F(8, 126)=4.86, P=0.02$), A β +INST ($F(8, 126)=4.86, P=0.01$), A β +EX+PINS ($F(8, 126)=4.86, P=0.043$), A β +EX+INST ($F(8, 126)=4.86, P=0.04$), and A β +EX+PINS+INST ($F(8, 126)=4.86, P=0.007$, Table 1].

3. 5. Spatial memory

On day five of the probing test, the capacity for spatial memory was evaluated. The Traveled distance in the targeted quadrant varied significantly between the groups, according to one-way ANOVA results ($F(8, 63)=18.19, P<0.0001$). The distance traveled in the target quadrant was greater among the intervention groups than the group that received A β [A β +EX ($F(8, 63)=18.19, P=0.0004$), A β +EX+PINS ($F(8, 63)=18.19, P=0.01$), and A β +EX+PINS+INST ($F(8, 63)=18.19, P=0.000$, Figure 5].

4. Discussion

Results show impaired spatial memory in rats three weeks post-A β_{25-35} injection compared to vehicle or control injections. MWM test results showed a decrease in targeted zone traveled distance. AD causes a steady deterioration in cognitive functions, particularly recollection and learning [24]. This work utilized an established model using intra-cerebroventricular injection of A β_{25-35} fibrils. This impacted the rats' spatial memory in the MWM test 21 days following the treatment, confirming and expanding prior observations [25]. Memory impairment mediated by A β_{25-35} is related with aberrant cerebral glucose metabolism, mitochondrial dysfunction and oxidative stress [26]. In animal models of AD, exercise and insulin increases neurotrophic factors, which promote neurogenesis, synaptic plasticity, antioxidant capacity, and angiogenesis. This may help AD sufferers' cognition and memory [27, 28].

Additionally, it appears that insulin signaling affects memory through other signaling pathways in the brain, specifically in the hippocampus. Therefore, insulin may act through different signaling pathways in addition to its effects on boosting metabolism and enhancing glucose absorption [29].

Table 1. Blood glucose levels (mg/DL). According to the collected data, there were significant differences between the groups in the level of glucose in time 3. Results are shown as mean \pm SEM (N= 8 in each group).

Groups	Time 1	Time 2	Time 3
Control	70.37 \pm 2.86	73.37 \pm 2.3	75.37\pm2.59
Sham	70.87 \pm 2.16	72.37 \pm 1.61	76.87\pm0.93
A β	71.37 \pm 2.2	72.34 \pm 1.59	101.01\pm2.52
A β +EX	71.12 \pm 1.81	75.75 \pm 1.44	86.87\pm2.61 #
A β +PINS	72.5 \pm 1.89	71.62 \pm 1.67	88.62\pm2.62
A β +INST	69.87 \pm 2.4	73.12 \pm 1.64	87.12\pm2.08 #
A β +EX+PINS	71.5 \pm 2.77	77.12 \pm 1.1	87.87\pm2.45 #
A β +EX+INST	73.75 \pm 2.49	72.62 \pm 2.44	88\pm2.57 #
A β +EX+PINS+INST	76.5 \pm 1.59	70.75 \pm 1.34	84.87\pm2.45 ##

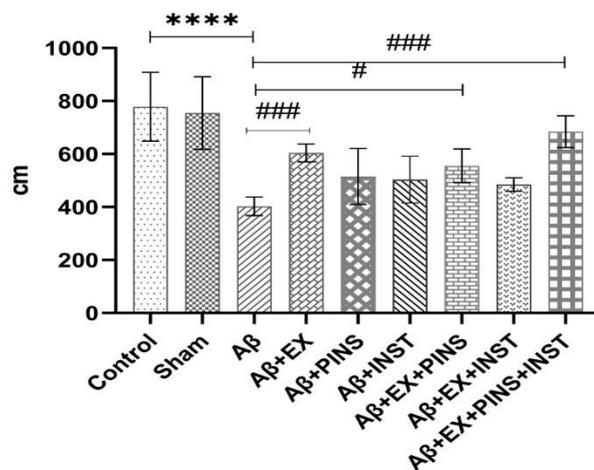


Figure 5. Test of MWM. Nine groups of adult Wistar rats were created at random. Spatial memory effects of ICV-A β /Saline dosing The MWM parameters for the several groups on the probing day were displayed. Probe trial: In comparison to the group serving as the control, the A β group's performance was subpar. Despite this, traveled distance in the target zone significantly increased in the A β +EX, A β +EX+PINS, and A β +EX+PINS+INST groups ($P < 0.0001$). The A β impact appears to have been reversed by EX and intranasal INS therapy. Results are shown as mean \pm SEM (N= 8 in each group).

Changes in glucose metabolism could be one of the possible mechanisms explaining the combined effects of insulin and exercise [30]. A β aggregation is associated with hypometabolism, and intranasal insulin therapy enhances glucose uptake in the hippocampus, according to previous reports. These findings suggest that insulin influences memory by changing glucose metabolism [31]. Running has also been shown to increase hippocampus glucose metabolism in AD-modeling APP/PS1 mice, which in turn improves the mice's cognitive performance.

Additionally, it has been demonstrated that physical activity increases the amount of insulin receptors in the hippocampal synaptic membrane, hence enhancing hippocampal insulin signaling [32].

Also, the tail length and head circumference of the animals with exercise and insulin intervention were higher than in the amyloid group.

In this regard, our findings demonstrated that receiving amyloid raises blood glucose levels, and exercise and insulin prevent this rise in blood glucose by improving glucose metabolism. While

inactivity increases the risk of developing neurodegenerative diseases including Parkinson's and Alzheimer's, exercise improves cognitive function, attention processing, learning, and speed memory [33]. Exercises focus on particular regions of the brain, including the hippocampus, medial temporal cortex, and prefrontal cortex. Regular exercisers in their senior years have larger brain sizes in these neural networks [34]. Proteins involved in mitochondrial biogenesis, antioxidant enzymes, antiapoptotic proteins, and neurotrophic factors are just a few of the proteins whose expression is increased by regular exercise.

Crucially, GLUT3 expression is upregulated by the neurotrophic factor BDNF, which stimulates energy metabolism [35]. Similar to the signaling pathway triggered by insulin, exercise also activates the intracellular signaling pathway, which results in the translocation of proteins like GLUT4 from the intracellular compartment into the plasma membrane [36].

Brain growth retardation appears to be linked not just to the energy used during physical exercise. but also to the body's overall energy metabolic rate. A correlation was found between the volume of the brain and the basal metabolism in a study that included prosimians, anthropoid apes, and humans [37]. The brain growth retardation progression might be influenced by basal metabolic rate (BMR).

It is often recognized that as people age, their basal metabolism lowers. As a result of a decrease in basal metabolism, the loss of skeletal muscle that occurs with advancing age may be a risk factor for the progression of brain growth retardation. When it comes to the aged, a decrease in basal metabolism may be compensated for

by engaging in physical activity [38]. Obesity and metabolic syndrome contribute to brain growth retardation. Obesity and metabolic syndrome improve with exercise. A cross-sectional study reveals that physical activity prevents obesity and causes the brain volume-activity association [39, 40]. Exercise and insulin boost cognitive function, although the mechanism is unknown. Because metabolic alterations occur before amyloid plaques and tangles-enriched neurodegeneration, AD may be a metabolic condition. AD goes beyond neurodegeneration [41]. Increased metabolic rate may be accompanied by higher calorie consumption and prevent compensatory increase in fat tissue. This suggests that metabolic problems are present in the 3xtg AD mice model before AD symptoms such brain amyloid plaques. These metabolic abnormalities may cause AD [41]. The improvement of spatial memory and the appropriate use of glucose are significantly influenced by insulin signaling.

One of the main features of AD pathogenesis is oxidative damage; from the very beginning of the disease, patients exhibit high levels of oxidative stress indicators and reduced antioxidant capacity. Despite the fact that the production of reactive oxygen species (ROS) is essential for optimal cell function, it has been established that excessively high levels of ROS have been associated with DNA damage and neurodegeneration [42]. This is because they lead to an increase in the production and accumulation of A β , as well as the induction of hyperphosphorylated and aggregated Tau proteins. This, in turn, leads to mitochondrial dysfunction and further ROS production, creating a vicious cycle. One of the cues that have been shown to either up- or down-regulate the amounts

of microRNAs implicated in the pathophysiology of AD is oxidative stress.

Furthermore, brain A β levels have been linked to a higher risk of cognitive deterioration from preclinical stages, according to a recent study. Hence, a crucial factor in the development of AD is mitochondrial malfunction and the associated generation of ROS, which aid in the buildup of A β .

Research indicates that exercise training improves redox status, resulting in enhanced antioxidant status and reduced pro-inflammatory markers. In pre-clinical studies, a mouse model (3 \times Tg-AD) with the Psen1 mutation and co-injected APPS we and tauP301L transgenes showed that oxidative stress levels are linked to AD severity and physical exercise improves reversal of symptoms [43]. Additionally, mice who have been trained to swim have better cognitive function, more robust mitochondria, higher expression levels of PGC-1, Mfn1/2, and Drp1 proteins, and lower levels of oxidative stress than mice that have not received any training [44].

Further research by Rajasekar et al. (2016) showed that intranasal insulin delivery improved memory impairment in rats treated with STZ by restoring CBF, brain energy metabolism, BDNF level, CREB activation, cholinergic function, and mitochondrial activity [22]. Lack of insulin reduces mitochondrial oxidative enzymes in the cerebrum, hypothalamus, and hippocampus, which have many insulin receptors. Insulin deficiency decreased mitochondrial fusion proteins (MFN1, MFN2, and OPA1) and increased mitochondrial fission protein (DRP1), suggesting that mitochondrial dynamics are negatively affected by insulin deficiency [45].

Inflammation is another possible

mechanism that insulin and exercise could be responsible for when it comes to influencing memory [46].

It is believed that neuroinflammation is a significant component in Alzheimer's disease [47]. Additionally, it has been demonstrated that running on a treadmill can help improve memory deficiencies [48].

In AD, proinflammatory cytokines are elevated, which results in the hippocampal downregulation of BDNF expression [49]. Changes in the metabolism of glucose could be one of the possible mechanisms explaining the synergistic effects of insulin and exercise. Due to the fact that A β aggregation is associated with hypometabolism and intranasal insulin therapy enhances glucose uptake in the hippocampus, it has previously been reported that insulin influences memory by changing glucose metabolism [50].

Running has also been shown to increase hippocampus glucose metabolism in AD-modeling APP/PS1 mice, which in turn improves the mice's cognitive performance [32].

Furthermore, it has been demonstrated that physical activity increases the amounts of insulin receptors in the hippocampal synaptic membrane, hence enhancing hippocampal insulin signaling [31].

5. Conclusion

Our findings suggest that exercise can amplify the positive benefits of intranasal insulin treatment on memory. The results of our research showed that exercise and insulin can prevent brain growth retardation and prevent spatial memory disorders by improving glucose metabolism.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The authors have completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. This study was approved by the Ethics Committee of Yazd University (Ethics Code: IR.YAZD.REC.1402.014). All participants have signed informed consent prior to enrolment in the study. This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

References

- [1] Abubakar MB, Sanusi KO, Ugusman A, Mohamed W, Kamal H, Ibrahim NH, et al. "Alzheimer's disease: An update and insights into pathophysiology". *Front Aging Neurosci.* 2022; 14: 1-16. doi: [10.3389/fnagi.2022.742408](https://doi.org/10.3389/fnagi.2022.742408)
- [2] Ledig C, Schuh A, Guerrero R, Heckemann RA, Rueckert D. "Structural brain imaging in Alzheimer's disease and mild cognitive impairment: Biomarker analysis and shared morphometry database". *Sci Rep.* 2018; 8(1): 1-17. doi: [10.1038/s41598-018-29295-9](https://doi.org/10.1038/s41598-018-29295-9)
- [3] Mrdjen D, Fox EJ, Bukhari SA, Montine KS, Bendall SC, Montine TJ. "The basis of cellular and regional vulnerability in Alzheimer's disease". *Acta Neuropathol.* 2019; 138(5): 729-49. <https://doi.org/10.1007/s00401-019-02054-4>
- [4] Huang X, Zhao X, Cai Y, Wan Q. "The cerebral changes induced by exercise interventions in people with mild cognitive impairment and Alzheimer's disease: A systematic review". *Arch Gerontol Geriatr.* 2022; 98: 1-9. doi: [10.1016/j.archger.2021.104547](https://doi.org/10.1016/j.archger.2021.104547)
- [5] White H, Pieper C, Schmader K DM. "The association of weight change in Alzheimer's disease with severity of disease and mortality : A longitudinal analysis. *J Am Geriatr Soc.* 1998; 46(6): 1223-7. doi: [10.1111/j.1532-5415.1998.tb04537.x](https://doi.org/10.1111/j.1532-5415.1998.tb04537.x)
- [6] Johnson DK, Wilkins CH. "Accelerated weight loss may precede diagnosis in alzheimer disease". *Arch Neurol.* 2014; 63(9): 1312-7. doi: [10.1001/archneur.63.9.1312](https://doi.org/10.1001/archneur.63.9.1312)
- [7] Burns JM, Johnson DK, Watts A, Swerdlow RH, Brooks WM. "Lean mass is reduced in early alzheimer's disease and associated with brain atrophy". *Arch Neurol.* 2011; 67(4): 428-33. doi: [10.1001/archneurol.2010.38](https://doi.org/10.1001/archneurol.2010.38)
- [8] Šerbedžija P, Ishii DN. "Insulin and insulin-like growth factor prevent brain atrophy and cognitive impairment in diabetic rats". *Indian J Endocrinol Metab.* 2012; 16(3): 601-10. doi: [0.4103/2230-8210.105578](https://doi.org/0.4103/2230-8210.105578)
- [9] Zhao N, Xu B. "The beneficial effect of exercise against Alzheimer's disease may result from improved brain glucose metabolism". *Neurosci Lett.* 2021; 763(June): 18-21. doi: [10.1186/s40035-023-00364-y](https://doi.org/10.1186/s40035-023-00364-y)
- [10] Do K, Laing BT, Landry T, Bunner W, Mersaud N, Matsubara T, et al. "The effects of exercise on hypothalamic neurodegeneration of Alzheimer's disease mouse model". *PLoS One.* 2018; 3: 1-17. doi: [10.1371/journal.pone.0190205](https://doi.org/10.1371/journal.pone.0190205)
- [11] Mendez MF. "Early-onset alzheimer's disease : Nonamnesic subtypes and type 2 AD". *Arch Med Res.* 2012; 43(8): 677-85. <http://dx.doi.org/10.1016/j.arcmed.2012.11.009>. doi: [10.1016/j.arcmed.2012.11.009](https://doi.org/10.1016/j.arcmed.2012.11.009)
- [12] Tobia C, Ebersole JL, Novak MJ. "Caloric restriction and chronic inflammatory diseases". *Oral Dis.* 2012; 18: 16-31. doi: [10.1111/j.1601-0825.2011.01830.x](https://doi.org/10.1111/j.1601-0825.2011.01830.x)
- [13] Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, et al. "Aerobic fitness reduces brain tissue loss in aging humans". *J Gerontol.* 2003; 58(2): 176-80. doi: [10.1093/gerona/58.2.m176](https://doi.org/10.1093/gerona/58.2.m176)
- [14] Colcombe SJ, Kramer AF, Erickson KI, Scalf P, McAuley E, Cohen NJ, et al. "Cardiovascular

- fitness , cortical plasticity, and aging". *PNAS*. 2004; 101(9): 3316-21. doi: [10.1073/pnas.0400266101](https://doi.org/10.1073/pnas.0400266101).
- [15] Draganski B, May A. "Training-induced structural changes in the adult human brain". *Behav Brain Res J*. 2008; 192: 137-42. doi: [10.1016/j.bbr.2008.02.015](https://doi.org/10.1016/j.bbr.2008.02.015).
- [16] Erickson KI, Kramer AF. "Aerobic exercise effects on cognitive and neural plasticity in older adults". *Br J Sport Med*. 2008; 43: 22-4. doi: [10.1016/j.bbr.2008.02.015](https://doi.org/10.1016/j.bbr.2008.02.015).
- [17] Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, et al. "Aerobic exercise training increases brain volume in aging humans". *J Gerontol*. 2006; 61(11): 1166-70. doi: [10.1093/geron/61.11.1166](https://doi.org/10.1093/geron/61.11.1166).
- [18] Karimi-Zandi L, Zahmatkesh M, Hassanzadeh G, Hosseinzadeh S. "Increment of CSF fractalkine-positive microvesicles preceded the spatial memory impairment in amyloid beta neurotoxicity". *Cytokine*. 2022; 160: 156050. <https://doi.org/10.1016/j.cyto.2022.156050>.
- [19] Song Y, Li P, Liu L, Bortolini C, Dong M. "Nanostructural differentiation and toxicity of Amyloid- β 25-35 aggregates ensue from distinct secondary conformation". *Sci Rep*. 2018; 8(1): 2-10. <http://dx.doi.org/10.1038/s41598-017-19106-y>.
- [20] Khodadadi D, Gharakhanlou R, Naghdi N, Salimi M, Azimi M, Shahed A. "Treadmill exercise ameliorates spatial learning and memory deficits through improving the clearance of peripheral and central amyloid-Beta Levels 1". *Neurochem Res*. 2018. <http://dx.doi.org/10.1007/s11064-018-2571-2>.
- [21] Azimi M, Gharakhanlou R, Naghdi N, Peptides DK. "Moderate treadmill exercise ameliorates amyloid- β -induced learning and memory impairment, possibly via increasing AMPK activity and up-regulation of the PGC-1 α ". *Elsevier*. 2017 Apr; 102: 78-88. doi: [10.1016/j.peptides.2017.12.027](https://doi.org/10.1016/j.peptides.2017.12.027).
- [22] Rajasekar N, Nath C, Hanif K, Shukla R. "Intranasal insulin exerts beneficial effects by improving cerebral blood flow, Nrf-2 expression and BDNF in STZ (ICV) induced memory-impaired rats". *Life Sci*. 2016; 173: 1-10. <http://dx.doi.org/10.1016/j.lfs.2016.09.020>.
- [23] Wang R, Zhang Y, Li J, Zhang C. "Resveratrol ameliorates spatial learning memory impairment induced by A β 1-42 in rats". *Neuroscience*. 2017; 344: 39-47. <http://dx.doi.org/10.1016/j.neuroscience.2016.08.051>.
- [24] Palop JJ, Mucke L. "Amyloid- β -induced neuronal dysfunction in Alzheimer's disease: From synapses toward neural networks". *Nat Neurosci*. 2010; 13(7): 812-8. <http://dx.doi.org/10.1038/nn.2583>.
- [25] Li B, Liang F, Ding X, Yan Q, Zhao Y, Zhang X, et al. "Interval and continuous exercise overcome memory deficits related to β -Amyloid accumulation through modulating mitochondrial dynamics". *Behav Brain Res*. 2019; 376(800): 1-10. doi: [10.1016/j.bbr.2019.112171](https://doi.org/10.1016/j.bbr.2019.112171).
- [26] Kumar V, Kim S, Bishayee K. "Dysfunctional glucose metabolism in Alzheimer's disease onset and potential pharmacological interventions". *Int J Mol Sci Rev*. 2022; 23(9540): 1-16. doi: [10.3390/ijms23179540](https://doi.org/10.3390/ijms23179540).
- [27] Dao AT, Zagaar MA, Alkadhi KA. "Moderate treadmill exercise protects synaptic plasticity of the dentate gyrus and related signaling cascade in a rat model of Alzheimer's disease". *Mol Neurobiological*. 2014; 52(3): 14-6. doi: [10.1007/s12035-014-8916-1](https://doi.org/10.1007/s12035-014-8916-1).
- [28] Nojoki F, Ebrahimi-Hosseinzadeh B, Hatamian-Zarmi A, Khodagholi F, Khezri K. "Design and development of chitosan-insulin-transfersomes (Transfersulin) as effective intranasal nanovesicles for the treatment of Alzheimer's disease: In vitro, in vivo, and ex vivo evaluations". *Biomed Pharmacother*. 2022; 153: 1-11. doi: [10.1016/j.biopha.2022.113450](https://doi.org/10.1016/j.biopha.2022.113450).
- [29] Zhao W, Chen H, Xu H, Moore E, Meiri N, Quon MJ, et al. "Brain insulin receptors and spatial memory". *J Biol Chem*. 1999; 274(49): 34893-902. doi: [10.1074/jbc.274.49.34893](https://doi.org/10.1074/jbc.274.49.34893).
- [30] Pascoal TA, Mathotaarachchi S, Kang MS, Mohaddes S, Shin M, Park AY, et al. "A β -induced vulnerability propagates via the brain's default mode network". *Nat Commun*. 2019; 10(2353): 1-13. <http://dx.doi.org/10.1038/s41467-019-10217-w>.
- [31] Moreira JD, Zimmer ER, Muller AP, Haas CB, Lulhier F, Perry MLS, et al. "Exercise increases insulin signaling in the hippocampus: Physiological effects and pharmacological impact of intracerebroventricular insulin administration in mice". *Hippocampus*. 2011; 1092(2011): 1082-92. doi: [10.1002/hipo.20822](https://doi.org/10.1002/hipo.20822).
- [32] Zhang S, Zhu L, Peng Y, Zhang L, Chao F, Jiang L, et al. "Long-term running exercise improves cognitive function and promotes microglial glucose metabolism and

- morphological plasticity in the hippocampus of APP/ PS1 mice". *J Neuroinflammation*. 2022; 19(34): 1-21. doi: [10.1186/s12974-022-02401-5](https://doi.org/10.1186/s12974-022-02401-5).
- [33] Camandola S. "Brain metabolism in health, aging , and neurodegeneration". *EMBO J*. 2017; 24: 1-19. doi: [10.15252/embj.201695810](https://doi.org/10.15252/embj.201695810).
- [34] Berchicci M, Lucci G, Russo F Di. "Benefits of physical exercise on the aging brain : The role of the prefrontal cortex". *Journals Gerontol*. 2013; 68(11): 1337-41. doi: [10.1186/s12974-022-02401-5](https://doi.org/10.1186/s12974-022-02401-5).
- [35] Mattson MP. "Energy intake and exercise as determinants of brain health and vulnerability to injury and disease". *Cell Metab*. 2012; 16(6): 706-22. <http://dx.doi.org/10.1016/j.cmet.2012.08.012>.
- [36] Rui QP, Xiao FW, Fei Fei P, Weizhe Zh, Jiaming Sh, Xiaoqun G. "Regular exercise enhances cognitive function and intracephalic GLUT expression in Alzheimer's Disease model mice". *J Alzheimer's Dis*. 2019; 20: 1-14. doi: [10.3233/JAD-190328](https://doi.org/10.3233/JAD-190328).
- [37] Leonard WR, Marcia RNU. "Evolutionary perspectives on human nutrition : The influence of brain and body size on diet and metabolism". *Am J Hum Biol*. 1994; 6(3): 77-88. doi: [10.1002/ajhb.1310060111](https://doi.org/10.1002/ajhb.1310060111).
- [38] Ravussin E. "A neat way to control weight ? How to create a spin current". *Science*. 2005; 307(10): 2004-6. doi: [10.1126/science.1108597](https://doi.org/10.1126/science.1108597).
- [39] Bruehl H, Wolf OT, Sweat V, Tirsi A, Richardson S, Convit A. "Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with Type 2 Diabetes Mellitus". *Brain Res* 2009; 64(3): 186-94. <http://dx.doi.org/10.1016/j.brainres.2009.05.032>.
- [40] Enzinger C, Fazekas F, Matthews PM, Ropele S, Schmidt H, Smith S, et al. "Risk factors for progression of brain atrophy in aging Six-year follow-up of normal subjects". *Neurology*. 2005; 64: 1704-11. doi: [10.1212/01.WNL.0000161871.83614.BB](https://doi.org/10.1212/01.WNL.0000161871.83614.BB).
- [41] LaFerla FM, Green KN, Oddo S. "Intracellular amyloid- β in Alzheimer's disease". *Nat Rev Neurosci*. 2007; 8(7): 499-509. doi: [10.1038/nrn2168](https://doi.org/10.1038/nrn2168).
- [42] Simioni C, Zauli G, Martelli AM, Vitale M, Gonelli A, Neri LM. "Oxidative stress : role of physical exercise and antioxidant nutraceuticals in adulthood and aging". *Oncotarget*. 2018; 9(24): 17181-98. doi: [10.18632/oncotarget.24729](https://doi.org/10.18632/oncotarget.24729).
- [43] Pathological A, Aging B, García-mesa Y, Colie S, Corpas R, Cristòfol R, et al. "Oxidative stress is a central target for physical exercise neuroprotection against pathological brain aging". *Biol Sci*. 2015; 71: 1-11. doi: [10.1093/gerona/glv005](https://doi.org/10.1093/gerona/glv005).
- [44] Luo L, Dai J, Guo S, Lu A, Gao X, Gu Y, et al. "Lysosomal proteolysis is associated with exercise- induced improvement of mitochondrial quality control in aged hippocampus". *Journals Gerontol Biol Sci*. 2017; 1-10. doi: [10.1093/gerona/glw242](https://doi.org/10.1093/gerona/glw242).
- [45] Endocrinology D, Statistics B, Clinic M, Ruegsegger GN, Manjunatha S, Summer P, et al. "Insulin deficiency and intranasal insulin alter brain mitochondrial function : A potential factor for dementia in diabetes". *The FASEB Journal*. 2019; 1-15. <http://dx.doi.org/10.1096/fj.201802043R>.
- [46] Heppner FL, Ransohoff RM, Becher B. "Immune attack : The role of inflammation in Alzheimer disease". *Nat Publ Gr*. 2015; 16(6): 358-72. <http://dx.doi.org/10.1038/nrn3880>.
- [47] Chang Y, Hung L, Chen Y, Wang W. "Insulin reduces in fl ammation by regulating the activation of the NLRP3 In fl ammasome". *Front Immunol*. 2021; 11: 1-11. doi: [10.3389/fimmu.2020.587229](https://doi.org/10.3389/fimmu.2020.587229).
- [48] Park S, Kim T, Sung Y, Park Y, Kim M, Shin M. "Treadmill exercise ameliorates short-term memory impairment by suppressing hippocampal neuroinflammation in poloxamer-407-induced hyperlipidemia rats". *Int Neurol J*. 2021; 25(Suppl 2): 81-9. doi: [10.5213/inj.2142342.171](https://doi.org/10.5213/inj.2142342.171).
- [49] Giacobbo BL, Doorduyn J, Klein HC, Dierckx RAJO, Bromberg E, Vries EFJ De. "Brain-derived neurotrophic factor in brain disorders : Focus on neuroinflammation". *Mol Neurobiol*. 2019; 56: 3295-312. doi: [10.1007/s12035-018-1283-6](https://doi.org/10.1007/s12035-018-1283-6).
- [50] Brabazon F, Wilson CM, Jaiswal S, Reed J, Frey WH, Byrnes KR. "Intranasal insulin treatment of an experimental model of moderate traumatic brain injury". *J Cereb Blood Flow Metab*. 2017; 37(9): 3203-18. doi: [10.1177/0271678X16685106](https://doi.org/10.1177/0271678X16685106).